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Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

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Original Research

Use of Controlled Medications in the Emergency Department: Narcotics and Psychotropics

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ABSTRACT

Objective: Although the trends and outcomes of controlled medications prescribed by emergency physicians especially opioids are well-defined in the literature, there is insufficient evidence regarding their parenteral use during emergency department (ED) visits. Thus, we aimed to determine the prevalence use of these drugs and the conditions under which they are ordered.

Methods: We conducted a retrospective study from January to June 2018 at a secondary care ED in Turkey. Narcotics and psychotropics, were administered parenterally (intravenous or intramuscular) during patients' ED visits. We obtained the following data from the registry and hospital records: time of use, age, sex, diagnosis, drug (active ingredient), and type of physician (general practitioner or attending).

Results: During the six-month study period, parenteral controlled medication was used in 1111 ED visits (1% of all ED visits). Tramadol and pethidine were the most commonly used narcotic drugs in the ED. They were often used for musculoskeletal pain (29.1% and 47.1%, respectively) and abdominal pain (22.5% and 18.6%, respectively). ED revisits of patients who took these drugs were related to cancer pain. Meanwhile, diazepam and biperiden were the predominantly used psychotropics. Anxiety/agitation was diagnosed in 69.1% of patients who received diazepam and acute exacerbation of psychiatric diseases in 70.6% of patients who received biperiden. However, revisits of these patients to the ED were related to acute exacerbation of psychiatric diseases.

Conclusion: The rate of controlled medication use in the studied hospital is much lower than that in developed countries. This finding can be attributed to different factors, such as physician attitude, patient demands, and possibly cultural differences. Finally, revisits of these patients to the ED were mostly related to acute exacerbation of chronic diseases.

Keywords: Benzodiazepine, emergency department, narcotic, opioid, psychotropic

INTRODUCTION

Narcotic drugs (opioids) can stop severe pain without loss of consciousness. Many narcotics are controlled, as they cause respiratory depression and have dangerous side effects (physical and psychological dependence). Psychotropic drugs, which have a stimulating effect on the central nervous system, and are used in the treatment of behavioral and psychiatric disorders by changing consciousness and emotions. Both drug groups are used in a controlled manner because they have addictive effects when used over long periods (1).

International conventions have been established to prevent the illegal use of these drugs. Turkey is a party to the 1961 United Nations Single Convention on Narcotic Drugs, the 1971 United Nations Convention on Psychotropic Substances, and the 1988 United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances (2-5). Prescribing controlled medications (especially narcotic drugs) is well-documented in research in western countries. According to the literature, opioid prescription rate is over 30% in EDs in the United States (US) (6). Studies also show that concern has been raised in developed countries (7). In Turkey, there are a few works regarding controlled prescriptions, and are not related to EDs (8, 9). In addition, parenteral use of these medications in EDs is unclear both in our country and in the literature.

In this study, we aimed at determining the prevalence of controlled medication use by patients in a secondary care ED in Turkey. We also identified the diagnoses and reasons for revisits of these patients.

METHODS

Study Design

We conducted a retrospective study from January to June 2018 in a secondary care ED in Turkey. This clinic is the largest and most crowded unit in its city. During the study period, the ED was visited 108,740 times. Ethics committee approval was received

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for this study from the ethics committee of the Nevşehir Hacı Bektaş Veli University (10.09.2018 – No:2018.10.119).

Study setting and population

In Turkey, controlled drugs are prescribed for outpatients by attending physicians, and for inpatients by all hospital physicians. Narcotic drugs classified by The Ministry of Health include methylphenidate and opioid derivations such as fentanyl, pethidine, hydromorphone, morphine, and codeine. Psychotropic medications include barbiturates and benzodiazepines such as alprazolam, lorazepam, diazepam, and midazolam. Tramadol, which is not a controlled drug in international settings, is under national control in Turkey. Biperiden is also under national control as a psychotropic drug, although it is an anticholinergic drug. Therefore, we included these drugs in this study.

In the hospital setting, narcotics and psychotropics are protected in pharmacies and sent to clinics in specific amounts by pharmacists when necessary. In EDs, these drugs are stored in locked cabinets for security. The responsibility of keeping records, counting, and returning of drugs belongs to the head nurse in the clinic. The drugs are used when ordered for patients by physicians. All these medications are administered parenterally (intravenous or intramuscular) in the ED.

We evaluated all ages in the study. We classified ED visits (where controlled drugs were administered) as single visits and revisits. Single visit means one patient had only one presentation to the ED, while revisit means one patient had more than one ED presentation. We obtained the following data from the registry and hospital records: time of use, gender, diagnosis, drug (active ingredient), and type of physician (general practitioner or attending). All controlled drugs are recorded in the registry when they are used. We included all records falling within the study period in the study.

Data Analysis

We performed statistical analyses using the Statistical Package for Social Sciences, IBM SPSS version 21.0 for Windows (IBM SPSS Corp.; Armonk, NY, USA). We presented continuous variables as median values and interquartile ranges. We described categorical variables as frequencies and percentages and compared using Pearson χ^2 or the Fisher exact test. A critical α value of 0.05 was accepted as statistically significant.

RESULTS

During the six-month study period, 1111 ED visits (1% of all ED visits) where any parenteral controlled medication was used were found. The median age was 43 years (IQR 31–59), and 51.9% of the patients were males. The rates of controlled drug use were 16.3% between 24:00 and 08:00 am, 37.2% between 08:00 and 16:00 hours, and 46.5% between 16:00 and 24:00 hours. Among these ED visits, 25.1% were revisits. These drugs were ordered by general practitioners in 62.2% of the patients and by attending emergency physicians in 37.8%. Two controlled medications were given in a single visit to 4.9% of the patients, while only one of such medication was given in the same visit to 95.1% of the patients.

Main Points:

- During the study period, parenteral controlled medication was used in 1% of all secondary care ED visits.
- This rate was much lower than that in developed countries.
- Tramadol and pethidine were the most commonly used narcotic drugs.
- Diazepam and biperiden were the predominantly used psychotropics.

Narcotics

Tramadol was ordered in 481 ED visits. The most prevalent diagnoses were musculoskeletal pain (29.1%), abdominal pain (22.5%), and renal colic (17.3%). The rate of revisit was 26.8%. The rates of musculoskeletal pain and abdominal pain were higher in the single visits than in the revisits, while that of cancer pain was higher in the revisits (Table 1).

Pethidine was used in 70 visits. The most common diagnoses were musculoskeletal pain (47.1%), abdominal pain (18.6%), and cancer pain (12.9%). The rate of revisit was 24.3%. The rate of musculoskeletal pain was higher in the single visits, while that of cancer pain was higher in the revisits than in the single visits (p=0.007) (Table 2).

Morphine was used in 18 visits. The most frequent diagnoses were myocardial infarction (77.8%), musculoskeletal pain (16.7%), and abdominal pain (5.6%). Fentanyl was used in only one visit, and it was ordered for musculoskeletal pain.

Psychotropics

Diazepam was used in 339 visits. Anxiety/agitation was the most common diagnosis in 69.1% of the patients who received this drug, followed by acute exacerbation of psychiatric diseases (13%) and convulsion (12.4%). Among these visits, 23.6% were revisits. Acute exacerbation of psychiatric diseases was more frequent in the revisits, whereas anxiety/agitation was more prevalent in the single visits (p<0.001) (Table 3). Rectal diazepam was used in 17 pediatric patient visits for convulsions.

Biperiden was ordered for 119 visits. Among all indications for biperiden, acute exacerbation of psychiatric diseases accounted for 70.6%, and anxiety/agitation comprised 29.4%. In total, 43.7% were revisits. The rate of revisits was higher in acute exacerbation of psychiatric diseases (51.2%) than in anxiety/agitation (25.7%) (p=0.011).

Midazolam was ordered for 114 visits. It was used mostly in sedation procedures (89.5%) and treatment for convulsions (10.5%). Thiopental and ketamine were used in three visits each for sedation procedures.

DISCUSSION

Between 2005 and 2007 in the US, the proportion of ED visits wherein controlled medications were prescribed was three-folds higher than that of ambulatory office visits for patients aged 15–29 years (10). Another study conducted in the US reported

Table 1. Clinical conditions in which Tramadol was used in the ED visits

	ED visits			
Clinical conditions, n (%)	**Total**	**Single**	**Multiple**	**p**
Musculoskeletal disease	140 (29.1)	114 (32.4)	26 (20.2)	<0.001
Nonspecific abdominal pain	108 (22.5)	95 (27.0)	13 (10.1)	
Renal colic	83 (17.3)	66 (18.8)	17 (13.2)	
Cancer pain	78 (16.2)	15 (4.3)	63 (48.8)	
Lumbalgia	42 (8.7)	35 (9.9)	7 (5.4)	
Headache	21 (4.4)	19 (5.4)	2 (1.6)	
Cholelithiasis	4 (0.8)	3 (0.9)	1 (0.8)	
Dysmenorrhea	2 (0.4)	2 (0.6)	0 (0)	
Arterial embolism	2 (0.4)	2 (0.6)	0 (0)	
Chest pain	1 (0.2)	1 (0.3)	0 (0)	
Total	481 (100)	352 (100)	129 (100)	

ED: emergency department

Table 2. Indications of Pethidine in the ED visits

	ED visits			
Clinical conditions, n (%)	**Total**	**Single**	**Multiple**	**p**
Musculoskeletal disease	33 (47.1)	29 (54.7)	4 (23.5)	0.007
Nonspecific abdominal pain	13 (18.6)	10 (18.9)	3 (17.6)	
Cancer pain	9 (12.9)	2 (3.8)	7 (41.2)	
Renal colic	8 (11.4)	6 (11.3)	2 (11.8)	
Headache	4 (5.7)	3 (5.7)	1 (5.9)	
Lumbar pain	2 (2.9)	2 (3.8)	0 (0)	
Myocardial infarction	1 (1.4)	1 (1.9)	0 (0)	
Total	70 (100)	53(100)	17(100)	

ED: emergency department

that the proportion of ED visits where any opioid medication was prescribed increased from 20.8% to 31.0% between 2001 and 2010 (6). In Turkey, the rate of controlled prescription use by healthcare centers in Istanbul for the city's population was 3.5% in 2009 (8). According to our study, controlled medication was used in 1% of all ED visits. Because the aforementioned previous studies reported data on prescriptions of controlled medications in healthcare settings, there is insufficient data regarding the use of parenteral medications during ED visits. Nevertheless, the rate obtained in the present work is much lower than that reported in western countries.

A study conducted in the US reported that the mean age of adult patients who are prescribed controlled substances during ED visits is 44 years (11). A research performed in a family medicine unit in Turkey showed that mean age of all aged patients who are prescribed psychotropic substances is between 32 and 39 years (9). This study found that the median ages of patients in national and international studies are similar.

Sutter et al. (12) showed that the most commonly used parenteral opioids in 2013 in the US were morphines (52.8%), hydromorphones (42.9%), and fentanyl (4.3%). A review performed by Patanwala et al. (13) showed the most commonly studied intravenous opioids in prehospital and ED settings as morphines, hydromorphones, fentanyl, and meperidine (pethidine). According to the International Narcotics Control Board (INCB) reports for 2017, buprenorphine, codeine, ethylmorphine, morphine, oxycodone,

Table 3. Indications of Diazepam in the ED visits

	ED visits			
Clinical conditions, n (%)	**Total**	**Single**	**Multiple**	**p**
Anxiety / agitation	231 (68.1%)	183 (70.7%)	48 (60%)	0.001
Acute exacerbation of psychiatric diseases	44 (13%)	22 (8.5%)	22 (27.5%)	
Convulsion	42 (12.4%)	34 (13.1%)	8 (10%)	
Vertigo	16 (4.7%)	14 (5.4%)	2 (2.5%)	
Headache	5 (1.5%)	5 (1.9%)	0 (0%)	
Sedation	1 (0.3%)	1 (0.4%)	0 (0%)	
Total	339 (100%)	259 (100%)	80 (100%)	

ED: emergency department

diphenoxylate, pethidine, fentanyl, and remifentanil were the principal narcotics consumed in 2016 in Turkey. Furthermore, the INCB reported levels of consumption of narcotics in Turkey for 2014–2016 (excluding preparations in Schedule III). The mean consumptions of fentanyl, buprenorphine, morphine, pethidine, oxycodone, hydromorphone, and others were 593, 53, 17, 16, 6, 2, and 18, respectively, in defined daily dose (DDD) per million inhabitants per day (14). In the present study, tramadol (Schedule IV), which is under national but not international control, was found to be the most commonly administered parenteral drug during ED visits, followed by pethidine, morphine, and fentanyl. These results differ from those in the INCB report because while INCB reported the use of all narcotic drugs in Turkey, only parenteral narcotics in EDs were evaluated in the present study.

Hoppe et al. (15) showed that the most prevalent diagnoses associated with opiate prescriptions in US EDs are back pain (10.2%), abdominal pain (10.1%), and extremity fracture (7.1%). O'Connor et al. (16) stated that the most common indications of intravenous morphine or hydromorphone are abdominal pain, trauma, and back pain. Similar to the literature, the present study found musculoskeletal pain and abdominal pain to be the most common indications.

Patel et al. (17) stated that nearly one-third of cancer patients receive any opioid during their ED visits. A study conducted by Ernst et al. (18) reported that 19% of patients on opioid medications for chronic pain revisit EDs within 90 days. In the current work, the rate of ED revisit was approximately 25%, of which more than 40% was related to cancer pain in the tramadol and pethidine groups.

According to a Centers for Disease Control and Prevention report regarding controlled substance prescription patterns, opioid analgesics were prescribed approximately twice as often as stimulants or benzodiazepines in 2013 in the US (19). Nevertheless, EDs have seen a sharp increase in benzodiazepine-related visits (20). Pharmacologic interventions (antipsychotics or benzodiazepines) are often used under certain conditions, such as acute agitation, which is becoming an increasingly common presentation to EDs. Benzodiazepines are not typically the first choice for these indications. However, because antipsychotics are not controlled medications, diazepam and biperiden were reported in this study.

In the literature, there is insufficient demographic data regarding the medical use of psychotropic drugs in EDs. According to an INCB report, the average consumption of benzodiazepines (group K - anxiolytics) in Turkey in 2015–2017 was 2.9 in DDD per thousand inhabitants per day (14). A study conducted in the United Kingdom reported that 26.1% of the population has taken a benzodiazepine or a Z-drug either under medical direction or misuse (21). Another study reported that among all patient visits from 1993 to 2010 in US ambulatory healthcare settings, the rate of benzodiazepine-related visits was 3.5%. In addition, the rate of anxiety and mood disorders is approximately 40% in patients with benzodiazepine prescriptions (22). In the current study, anxiety and agitation were the most common cause of ED visits (about 70%) among patients receiving diazepam, while acute exacerbation of psychiatric diseases was the most prevalent cause of ED visits (around 70%) in patients receiving biperiden. For both medications, the rate of revisits was more common for acute exacerbation of psychiatric diseases. Midazolam, which is a psychotropic drug, was ordered in up to 90% of visits for sedation procedures. Thiopental and ketamine were rarely used sedatives during the ED visits of patients.

CONCLUSION

Parenteral controlled medication was used in the studied clinic in 1% of all ED visits. Tramadol (under national control) and pethidine were the most typically preferred narcotics, and revisits to the ED were mainly related to cancer pain. Diazepam and biperiden (under national control) were the most common psychotropics used in the ED, and were frequently ordered for anxiety/agitation and acute exacerbation of psychiatric diseases, respectively. However, revisits to the ED were related to acute exacerbation of psychiatric diseases. Although there is insufficient data regarding the use of these controlled medications during ED visits, this rate seems to be much lower than that of developed countries' EDs. Nonetheless, regular follow-up regarding

chronic diseases may further reduce the revisits of these patients to the ED.

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REFERENCES

1. Von Kuenssberg Jehle D, Stiller G, Wagner D. Sensitivity in detecting free intraperitoneal fluid with the pelvic views of the FAST exam. Am J Emerg Med 2003; 21: 476-8. [Crossref]
2. Riddell J, Case A, Wopat R, Beckham S, Lucas M, McClung CD, et al. Sensitivity of Emergency Bedside Ultrasound to Detect Hydronephrosis in Patients with Computed Tomography-proven Stones. West J Emerg Med 2014; 15: 96-100. [Crossref]
3. Smith GD, Fry MM, Taylor D, Morgans A, Cantwell K. Effectiveness of the Valsalva Manoeuvre for reversion of supraventricular tachycardia. Cochrane Database Syst Rev 2015; 2: Cd009502. [Crossref]
4. Wen ZC, Chen SA, Tai CT, Chiang CE, Chiou CW, Chang MS. Electrophysiological mechanisms and determinants of vagal maneuvers for termination of paroxysmal supraventricular tachycardia. Circulation 1998; 98: 2716-23. [Crossref]
5. Quigley AJ, Stafrace S. Ultrasound assessment of acute appendicitis in paediatric patients: methodology and pictorial overview of findings seen. Insights Imaging 2013; 4: 741-51. [Crossref]
6. Mazer-Amirshahi M, Mullins PM, Rasooly I, van den Anker J, Pines JM. Rising opioid prescribing in adult U.S. emergency department visits: 2001-2010. Acad Emerg Med 2014; 21: 236-43. [Crossref]
7. Barnett ML, Olenski AR, Jena AB. Opioid-Prescribing Patterns of Emergency Physicians and Risk of Long-Term Use. N Engl J Med 2017; 376: 663-73. [Crossref]
8. Demircan D, Gulmez SE, Donertas B, Topcu I, Yilmaz H, Berkman K, et al. Use of drugs subject to controlled prescriptions: a retrospective analysis. Balkan Med J 2013; 30: 46-53. [Crossref]
9. Öztürk O, Öztürk G. Retrospective Analysis of Green Coloured Prescriptions Isuued in A Family Medicine Unit. Turkish Journal of Family Medicine and Primary Care 2017; 11: 38-42. [Crossref]
10. Fortuna RJ, Robbins BW, Caiola E, Joynt M, Halterman JS. Prescribing of controlled medications to adolescents and young adults in the United States. Pediatrics 2010; 126: 1108-16. [Crossref]
11. McAllister MW, Aaronson P, Spillane J, Schreiber M, Baroso G, Kraemer D, et al. Impact of prescription drug-monitoring program on controlled substance prescribing in the ED. Am J Emerg Med 2015; 33: 781-5. [Crossref]
12. Sutter ME, Wintemute GJ, Clarke SO, Roche BM, Chenoweth JA, Gutierrez R, et al. The Changing Use of Intravenous Opioids in an Emergency Department. West J Emerg Med 2015; 16: 1079-83. [Crossref]
13. Patanwala AE, Keim SM, Erstad BL. Intravenous opioids for severe acute pain in the emergency department. Ann Pharmacother 2010; 44: 1800-9. [Crossref]
14. Han X, Ouyang H, Chen X, Huang Y, Song Y, Zhang M, et al. Aberrant expression of Igf2/H19 in porcine parthenogenetic fetuses and placentas. Anim Reprod Sci 2013; 139: 101-8. [Crossref]
15. Hoppe JA, Nelson LS, Perrone J, Weiner SG, Prescribing Opioids Safely in the Emergency Department Study I, Prescribing Opioids Safely in the Emergency Department PSI. Opioid Prescribing in a Cross Section of US Emergency Departments. Ann Emerg Med 2015; 66: 253-9.e1.
16. O'Connor AB, Zwemer FL, Hays DP, Feng C. Outcomes After Intravenous Opioids in Emergency Patients: A Prospective Cohort Analysis. Acad Emerg Med 2009; 16: 477-87. [Crossref]
17. Patel PM, Goodman LF, Knepel SA, Miller CC, Azimi A, Phillips G, et al. Evaluation of Emergency Department Management of Opioid-Tolerant Cancer Patients With Acute Pain. J Pain Symptom Manage 2017; 54: 501-7. [Crossref]
18. Ernst FR, Mills JR, Berner T, House J, Herndon C. Opioid Medication Practices Observed in Chronic Pain Patients Presenting for All-Causes to Emergency Departments: Prevalence and Impact on Health Care Outcomes. J Manag Care Spec Pharm 2015; 21: 925-36. [Crossref]
19. Paulozzi LJ, Strickler GK, Kreiner PW, Koris CM, Centers for Disease C, Prevention. Controlled Substance Prescribing Patterns--Prescription Behavior Surveillance System, Eight States, 2013. MMWR Surveill Summ 2015; 64: 1-14. [Crossref]
20. Schmitz A. Benzodiazepine use, misuse, and abuse: A review. Ment Health Clin 2016; 6: 120-6. [Crossref]
21. Kapil V, Green JL, Lait CL, Wood DM, Dargan PI. Misuse of benzodiazepines and Z-drugs in the UK. Br J Psychiatry 2018; 205: 407-8. [Crossref]
22. Kaufmann CN, Spira AP, Alexander GC, Rutkow L, Mojtabai R. Trends in prescribing of sedative-hypnotic medications in the USA: 1993-2010. Pharmacoepidemiol Drug Saf 2016; 25: 637-45. [Crossref]

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Original Research

Effect of Statin Therapyin Tpe-Interval and Tpe/QTc Ratio in Patients with Familial Hypercholesterolemia

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ABSTRACT

Objective: Studies have shown that hypercholesterolemia can induce ventricular vulnerability to fibrillation, and statin therapy exerts antiarrhythmic effects.

In the present study, we investigated the relationship between arrhythmogenic substrate and familial hypercholesterolemia (FH) with regard to statin therapy.

Methods: We evaluated 46 statin-naive patients (41±12 years) with FH, and 46 healthy subjects (40±8 years) prospectively. Electrocardiography (ECG) of patients were compared before and after 6 months of intensive statin therapy and with control groups. The ECG parameters were calculated by two experienced cardiologists who blinded to each other's findings.

Results: There were no significant differences found between groups' baseline characteristics. Total cholesterol (343±49 mg/dL vs. 161±12 mg/dL; p<0.001) and low-density lipoprotein-cholesterol (LDL-C) (260±42 vs. 95±13; p<0.001) levels were significantly higher in FH group. Both total cholesterol (343±49 vs. 206±45; p<0.001) and LDL-C (260±42 vs. 138±40; p<0.001) levels were decreased after statin therapy. Both mean baseline Tpe-interval (90.7±9.3 ms vs. 77.6±7.3 ms; p<0.001) and Tpe/QTc ratio (0.219±0.02 vs. 0.193±0.01; p<0.001) were found to be significantly higher in FH group than control subjects. After statin therapy, Tpe-interval (90.7±9.3 ms vs. 81.3±8.3 ms; p<0.001) and Tpe/QTc ratio (0.219±0.02 vs. 0.201±0.02; p<0.001) were significantly decreased. Compared to the control group, Tpe-interval (81.3±8.3 ms vs. 77.6±7.3 ms; p=0.027) and Tpe/QTc (0.206±0.02 vs. 0.193±0.01; p=0.021) ratio remained higher in FH patients after statin therapy. There was a strong and positive correlation between basal LDL-C and Tpe-interval (r=0.740; p<0.001) and Tpe/QTc ratio (r=0.597; p<0.001)

Conclusion: This study showed that Tpe-interval and Tpe/QTc ratio on ECG were significantly prolonged in FH patients and improved with intense statin therapy by lowering LDL-C.

Keywords: Electrocardiography, familial hypercholesterolemia, Tpe/QTc ratio, Tpe-interval, ventricular arrhythmias

INTRODUCTION

Previous experimental studies have shown that hypercholesterolemia can induce proarrhythmic electrophysiological remodeling (1). Both clinical and experimental evidence have shown that the statin therapy provides antiarrhythmic effects. Statins reduce ventricular arrhythmias with their direct antiarrhythmic effects and through pleiotropic properties. This beneficial effect has been shown in nonischemic patients (2, 3). Various surface electrocardiogram (ECG) markers of dispersion of ventricular repolarization (DVR) including QT, QT_c, QT dispersion, QTc dispersion, and T_{peak}–T_{end} (Tpe-interval) have been proposed as predictors of risk for ventricular arrhythmia. The Tpe-interval and Tpe/QTc (QT interval corrected for heart rate) ratio are markers of DVR (4, 5).

Prolonged Tpe-interval reflects abnormal DVR and is related to increased risk of ventricular arrhythmogenesis (6-8). Moreover, previous studies have shown that the Tpe/QTc ratio, which is in-

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dependent of heart rate alteration, is a more accurate measurement of ventricular repolarization (6-8). Familial hypercholesterolemia (FH) is associated with elevated levels of low-density lipoprotein-cholesterol (LDL-C) (9). In FH patients, high cholesterol exposure begins in childhood and causes premature atherosclerosis. Most cases of sudden cardiac deaths occur because of ischemic heart disease that causes ventricular arrhythmia (10, 11). To our best knowledge, no study has evaluated the Tpe-interval and Tpe/QTc ratio as markers of ventricular arrhythmogenesis in patients with FH. Therefore, in the present study, we investigated the effect of intensive statin therapy on the Tpe-interval and Tpe/QTc ratio in patients with FH.

METHODS

Study Design and Population

A total of 49 newly diagnosed statin-naive FH patients were evaluated prospectively from January 2016 to October 2017. The diagnosis of FH was based on genetic analysis (pathogenic mutation in the LDL receptor, proprotein convertase subtilisin/kexin 9 [PCSK9] or apolipoprotein B-100 genes) in patients with LDL-C >190 mg/dL (5 mmol/L).

Forty-six age–gender-matched normolipidemic individuals without any medication were included in the study as the control group. All patients were followed-up for 6 months. Patients with coronary artery disease (CAD), history of myocardial infarction, moderate-to-severe valvular heart disease, hypertrophic or dilated cardiomyopathies, thyroid dysfunction, electrolyte disturbances, pulmonary, malignancy, renal dysfunction, hepatic disease, or connective tissue disease were all excluded from the study.

All patients had to undergone treadmill stress test with ECG according to the Bruce protocol or single-photon emission computed tomography to exclude subclinical CAD. Coronary angiography was performed in patients with positive or suspicious result in noninvasive stress testing. Thus, 3 patients were excluded with the diagnosis of CAD, and finally, 46 patients with FH constituted the study population.

The local institutional ethics committee of Gaziantep University Faculty of Medicine has approved the study protocol (2016/104). The study was in compliance with the principles of the Declaration of Helsinki. Informed consent was obtained from the patients included in the study.

Main Points:

- Previous studies have shown that hypercholesterolemia can induce ventricular vulnerability to fibrillation, and statin therapy exerts antiarrhythmic effects.
- In this study, we showed that Tpe-interval and Tpe/QTc ratios were increased in treatment-naive FH patients.
- These parameters were significantly decreased after an intensive statin therapy.
- These results support the possible effects of hypercholesterolemia and statin therapy on Tpe-interval and Tpe/QTc ratio.

Demographic characteristics, medical history, and medication and anthropometric measurements were recorded. Transthoracic echocardiography was performed with EPIQ (Philips Medical Systems, Bothell, WA, USA) in all patients.

Routine blood parameters included were total cholesterol, LDL-C, high-density lipoprotein-cholesterol, triglyceride, fasting blood glucose, and serum creatinine were assessed for all patients. However, for FH patients, these biochemical parameters were evaluated in the third and sixth month of statin therapy. Intensive statin therapy (atorvastatin 80 mg/day or rosuvastatin 40 mg/day) was prescribed at the time of diagnosis of FH and was kept constant throughout the study. Patients did not receive any non-statin lipid-lowering drug during the study period. All patients were followed-up for the side effects of statins, and no significant side effects were noticed during the study period.

Electrocardiography

The ECGs were evaluated both at the time of diagnosis of FH before statin treatment and 6 months after statin treatment. Baseline ECGs were compared between the FH population and control subjects, and also after intensive statin therapy in the FH group. Twelve-lead ECGs were obtained with 10 mm/mV amplitude, and 25 mm/s (Nihon Kohden Corp., Tokyo, Japan) in the supine position. All ECGs obtained in the first and last examination of patients were recorded in the computer. Adobe Photoshop software (400% magnification) was used to measure ECG parameters. Each ECG was evaluated by two experienced cardiologists who were blinded to each other's findings and status of each patient and control subject.

Patients presented with U waves, bundle branch block or evidence of any intraventricular conduction defect, left ventricular hypertrophy, left ventricular dysfunction, and atrial fibrillation in their ECG were excluded from the study. Also, patients who were taking medications, such as antiarrhythmics, antihistamines, digitalis, tricyclic antidepressants, diuretics, and antipsychotics were all excluded from this study.

QT was calculated from the beginning of the QRS to the end of T wave, which comes back to the isoelectric line. For each QT, two consecutive calculations were done, and the average value of two readings was recorded. Bazett's formula: $QTc=QT/\sqrt{R-R}$ interval was performed to calculate QTc. RR interval was measured as the average of three complexes. Tpe-interval was determined as the interval from the peak of T wave to the end of T wave, where the wave reached the isoelectric line. Tpe-interval was measured mostly in V5. However, if V5 is not suitable for calculation, V4 or V6 leads were used (8, 12, 13).

The calculation was done only in leads with T wave amplitude >1.5 mm. The Tpe/QTc ratio was calculated from these measurements.

Statistical Analysis

Continuous parameters with normal distribution were presented as means and standard deviation, and non-normally distributed parameters were presented as median and interquartile range. Normal distribution was evaluated by Kolmogorov–Smirnov test.

Continuous parameters with normal distribution were compared with Student's t-test and non-normally distributed parameters were compared with Mann–Whitney U test between groups.

Categorical parameters were presented as percentages and compared between groups with the Chi-square test or Fisher's exact test.

Paired Student's t-test was used to compare repeated measurements (at baseline and 6 months) for ECG and laboratory parameters. The correlation coefficients and significance between LDL-C and ECG parameters were calculated by Pearson's or Spearman's test. Changes in LDL-C and ECG parameters before and after statin therapy were represented as Δ, and correlation between ΔLDL-C and ECG parameters were analyzed.

A p-value<0.05 was statistically significant. Statistical analyses were conducted using the Statistical Package for the Social Sciences 115 (SPSS 20.0) for Windows (IBM SPSS Corp.; Armonk, NY, USA).

RESULTS

Our study enrolled 46 consecutive patients with newly diagnosed FH (21 females; mean age 41±12 years) and 46 healthy controls (19 females; mean age 40±8 years). Baseline characteristics include gender, age, body mass index, smoking, hypertension, and diabetes mellitus. Table 1 shows similarity between groups with respect to these baseline characteristics. Left ventricular ejection fraction and mean systolic and diastolic blood pressure were also found similar between the two groups.

Total cholesterol, LDL-C, and triglyceride levels were found significantly higher in the FH patients than in the control subjects (Table 1). Levels of both total cholesterol (343±49 mg/dL vs. 206±45 mg/dL; p<0.001) and LDL-C (260±42 mg/dL vs. 138±40 mg/dL; p<0.001) were shown to decrease significantly at the sixth month of the intensive statin treatment. Although, effective reduction was detected in total cholesterol (39.4%±13.1%) and LDL-C (49.2%±16.2%) levels with statin therapy, posttreatment levels of both remained significantly higher than control subjects (for both; p<0.001). ECG evaluation revealed normal sinus rhythm for both FH and control groups. The mean baseline Tpe-interval was found significantly prolonged in the FH patients compared to the control group (90.7±9.3 ms vs. 77.6±7.3 ms; p<0.001) (Table 2). Tpe/QTc ratio was shown to be significantly higher in the FH patients than control subjects (0.219±0.02 vs. 0.193±0.01; p<0.001) (Figure 1). Although QT (399±10.5 ms vs. 389±8.5 ms; p=0.608) and QTc (414±19.5 ms vs. 402±16.9 ms; p=0.094) intervals were prolonged in FH group, it was not statistically significant (Table 2). In FH patients, the Tpe-interval (90.7±9.3 ms vs. 81.3±8.3 ms; p<0.001) and Tpe/QTc ratio (0.219±0.02 vs. 0.201±0.02; p<0.001) were significantly reduced after intensive statin therapy compared to baseline. The QT and QTc intervals did not show any significant change after the statin therapy (for both; p>0.05) (Table 3). Compared to the control group, the Tpe-interval (81.3±8.3 ms vs. 77.6±7.3 ms; p=0.027) and Tpe/QTc ratio (0.201±0.02 vs. 0.193±0.01; p=0.021) were still significantly higher in the FH patients after the statin therapy.

Table 1. Baseline characteristic and laboratory parameters of FH patients and control group

Parameter	FH (n=46)	Control (n=46)	p
Age, years	41±12	40±8	0.379
Female, n (%)	21 (45.7)	19 (41.3)	0.674
Smoking, n (%)	10 (21.7)	9 (19.6)	0.797
Hypertension, n (%)	8 (17.4)	6 (13.0)	0.562
Diabetes mellitus, n (%)	3 (6.5)	2 (4.3)	1.000
Body mass index (kg/m^2)	27.3±4.2	24.9±3.6	0.324
Systolic BP (mmHg)	128±11	125±8	0.191
Diastolic BP (mmHg)	74±10	71±9	0.171
LVEF (%)	65.3±2.5	66.2±3.1	0.878
Total cholesterol, (mg/dL) Median (IQR)	338 (60)	157 (46)	<0.001
LDL–C (mg/dL) Median (IQR)	253 (16)	91 (11)	<0.001
HDL–C (mg/dL)	42 (17)	47 (6)	0.228
Triglyceride (mg/dL)	139±31	83±22	<0.001
eGFR	94±22	89±13	0.652

BP: blood pressure; eGFR: estimated glomerular filtration rate; HDL–C: high–density lipoprotein–cholesterol; FH: familial hypercholesterolemia; LDL–C: low–density lipoprotein–cholesterol; LVEF: left ventricular ejection fraction

Table 2. Comparison of electrocardiographic findings of the patients with FH and control subjects

Variables	Pretreatment (n=46)	Control (n=46)	p
HR, beats/min	65±6.3	65±4.5	0.374
PR, ms	137±21	141±19	0.477
QRS, ms	89±8	91±6	0.731
QT, ms	399±10.5	389±8.5	0.211
QTc, ms	414±19.5	402±16.9	0.087
Tpe–interval, ms	90.7±9.3	77.6±7.3	<0.001
Tpe/QT	0.227±0.02	0.199±0.01	<0.001
Tpe/QTc	0.219±0.02	0.193±0.01	<0.001

The number of patients with 50%≥LDL-C reduction after intensive statin therapy was 20 (43.5%). No significant difference was found between patients with 50%≥LDL-C reduction and without after statin therapy in terms of Tpe-interval (82.3±8.4 vs. 80.6±8.3; p=0.493) and Tpe/QTc ratio (0.204±0.01 vs. 0.200±0.02; p=0.462). A strong and positive correlation was found between basal LDL-C

Figure 1. Comparison of Tpe-interval and Tpe/QTc ratio between FH (pre and post statin therapy) and control group

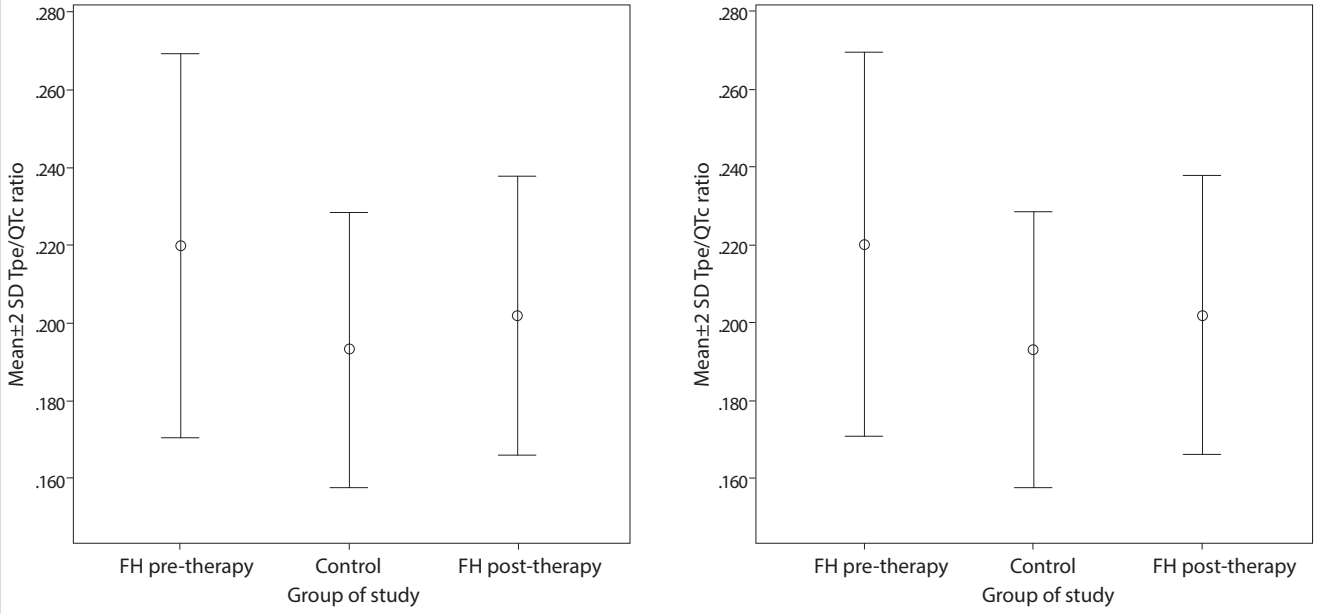
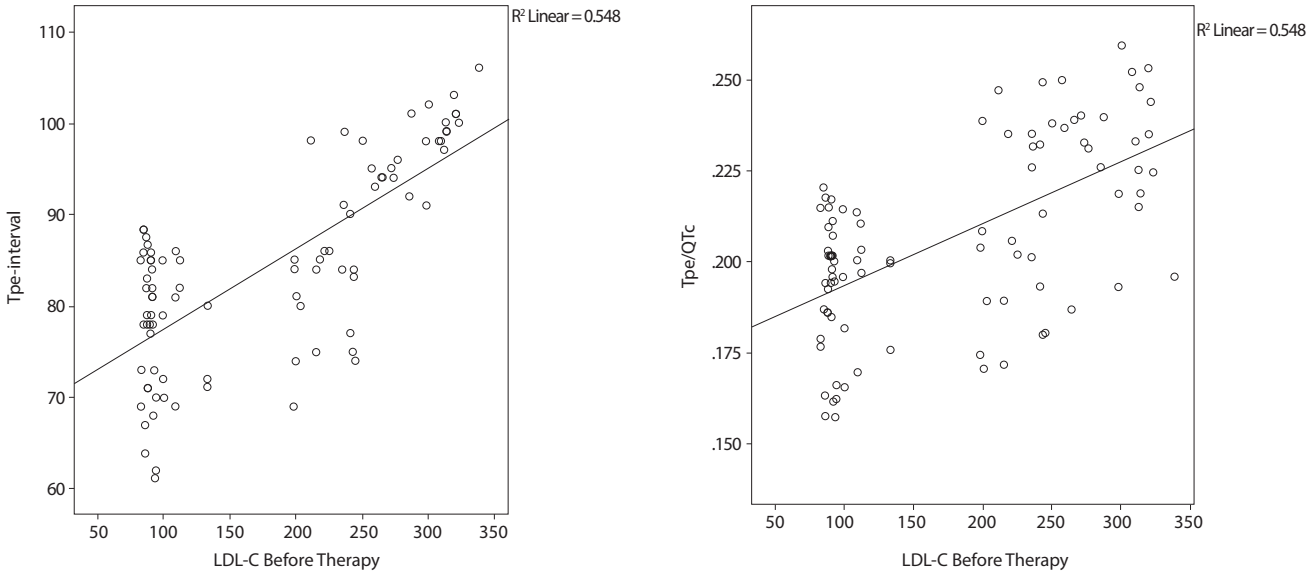


Figure 2. Correlation between TPe-inteval and Tpe/QTc ratio and LDL-C level before therapy



and Tpe-interval (r=0.740, 95% CI: 0.639–0.820; p<0.001) and Tpe/QTc ratio (r=0.597, 95% CI: 0.549–0.706; p<0.001) (Figure 2). However, a significant but weak association was found between LDL-C and Tpe-interval (r=0.349, 95% CI: 0.170–0.512; p<0.001) and Tpe/QTc ratio (r=0.217, 95% CI: 0.001–0.424; p=0.038) after therapy. No significant correlation was found between ΔLDL-C and ΔTpe-interval (r=0.215; 95% CI: −0.356 to −0.561 p=0.172) and ΔTpe/QTc (r=248; 95% CI: −0.50 to −0.566; p=0.113) after statin therapy.

DISCUSSION

In the present study, we showed that Tpe-interval and Tpe/QTc ratio were prolonged in treatment-naive patients with FH as compared to control subjects. Tpe-interval and Tpe/QTc ratio were also shown to be decreased significantly after the statin therapy in patients with FH. To our best knowledge, this is the first report that demonstrated prolonged Tpe-interval and increased Tpe/QTc ratio in FH patients and their improvement after intense LDL-C lowering with statins. Moreover, our results

Table 3. Comparison of electrocardiographic findings of the patients with FH (pre– and posttreatment)

Variables	Pretreatment (n=46)	Posttreatment (n=46)	p
HR, beats/min	65±6.3	63±4.5	0.091
QT, ms	399±10.5	393±9.2	0.608
QTc, ms	414±19.5	404±17.7	0.094
Tpe–interval, ms	90.7±9.3	81.3±8.3	<0.001
Tpe/QT	0.227±0.02	0.206±0.02	<0.001
Tpe/QTc	0.219±0.02	0.201±0.02	<0.001

showed that improvement of ECG parameters might explain effects of intensive statin therapy.

Previous studies have reported that lipid-lowering therapy (LLT) reduces coronary artery events and mortality (14, 15). The beneficial effects of LLT could be attributed to the reduction of ventricular arrhythmias and sudden death (14-16). This hypothesis was first evaluated by De Sutter et al. (17) who reported that in patients with CAD, receiving an implantable cardioverter-defibrillator (ICD), LLT reduction was associated with occurrence of inappropriate shocks. Similarly, Mitchell et al. (18) have shown that LLT was associated with a 40% reduction in relative hazard of recurrence of ventricular tachycardia/fibrillation in patients with an ICD for secondary prevention. In addition, the results of Multicenter Automatic Defibrillator Implantation Trail II (MADIT-II) have shown that use of statin decreases the risk of ventricular fibrillation and sudden cardiac death (19). Vrtovec et al. (20) demonstrated that atorvastatin treatment had shortened the QTc interval and thus might have reduced the risk of arrhythmias in patients with advanced heart failure. These clinical trials supported the evidence of favorable effects of statin on ventricular arrhythmias. Previous experimental studies had also demonstrated that hypercholesterolemia produced significant cardiac proarrhythmic neural and electrical remodeling (1, 21, 22). These neural and electrical remodeling processes may contribute to of the presence of ventricular arrhythmia (1, 21, 23). In their study, Liu et al. (21) showed that hypercholesterolemia-induced significant nerve sprouting and sympathetic hyperintervention, prolonged action potential duration and QTc interval, and increased the repolarization dispersion in a rabbit model. In this study, the researchers had also shown that the neural and electrophysiological remodeling induced by hypercholesterolemia was associated with increased ventricular vulnerability to fibrillation. Simvastatin had shown to significantly reduce the vulnerability of ventricular fibrillation via mechanism of reduction of hypercholesterolemia-induced neural and electrophysiological remodeling in another study conducted by the same researchers (1). Experimental studies provide strong evidence for the hypercholesterolemia-induced life-threatening ventricular arrhythmias. Both experimental and clinical trials support the beneficial effects of statins on life-threatening ventricular arrhythmias.

The T wave is indicative of ventricular repolarization in ECGs. The Tpe-interval is the marker of the total DVR. An increased Tpe-interval is associated with malignant ventricular arrhythmias (24-26). Yayla et al. (27, 28) in their studies, have shown that both Tpe-interval and Tpe/QTc ratio are increased in patients with systemic sclerosis and aortic stenosis that might explain increased frequency of ventricular arrhythmias in these cases.

Tpe/QTc ratio was suggested as a more accurate measure of DVR because Tpe-interval is affected by the variations in body weight and heart rate (8).

Our study showed that both Tpe-interval and Tpe/QTc ratio are markers of ventricular transmural dispersion of repolarization and are significantly longer in FH patients than the control subjects. Our results are in line with previous reports of association between hypercholesterolemia and repolarization dispersion. Our study showed significant decrease in Tpe-interval and Tpe/QTc ratio after LLT with statins. This result is important to provide further evidence for both experimental and clinical studies that evaluated the effects of the statin therapy in ventricular repolarization and life-threatening ventricular arrhythmias.

Our results might be explained by either long-term exposure to high-level LDL-C or beneficial effects of statin therapy. Long-term exposure to elevated LDL-C levels in asymptomatic FH patients might have led to prolonged Tpe-interval and Tpe/QTc ratio because of subclinical atherosclerosis, microvascular dysfunction, and endothelial dysfunction (29-31). Impaired subclinical left ventricular systolic functions had also been shown previously in asymptomatic FH patients compared to control subjects by 2D strain echocardiography (32). Although after intensive statin therapy, Tpe-interval and Tpe/QTc ratio were significantly decreased compared to baseline, but it remained prolonged than control subjects. Additionally, there were no significant differences seen between patients with ≥50% LDL-C reduction attained and without. This result might support the effect of long-term exposure to high levels of LDL-C as a leading cause of prolonged Tpe-interval and Tpe/QTc ratio.

The decrease of Tpe-interval and Tpe/QTc ratio might be explained by possible pleiotropic effects of statin therapy (33). Many studies have shown the effect of statin therapy on regression of atherosclerosis, both in symptomatic and asymptomatic patients (34-36). Kayikcioglu et al. (33) have shown improvement of endothelial functions and normalization of ischemic findings even in normolipidemic patients with cardiac syndrome-x after only 3 months of statin therapy. Therefore, the favorable effect that we observed in the Tpe-interval and Tpe/QTc ratio after such a short statin therapy might be the result of improvement in endothelial functions. Thus, we suggest that with a longer statin therapy it might be possible to normalize the Tpe-interval and Tpe/QTc ratio in FH patients.

Our study has several limitations. It is a single-center study with low number of patients and a short follow-up duration of only 6 months. Therefore, the clinical outcomes such as ventricular

arrhythmic episodes could not be evaluated. Our study population consisted of newly diagnosed treatment-naive FH patients. Moreover, the diagnosis of FH was confirmed genetically in all subjects. Therefore, this population represents a more homogeneous FH population. Another important limitation is that patients received atorvastatin or rosuvastatin, which had different effect on ECG parameters.

CONCLUSION

This prospective observational study presented an evidence that suggested Tpe-interval and Tpe/QTc ratios were increased in treatment-naive FH patients. These parameters were significantly decreased after an intensive statin therapy. These results support the possible effects of hypercholesterolemia and statin therapy on Tpe-interval and Tpe/QTc ratio. All these findings should be confirmed in prospective, multicenter studies. These markers might be better predictor of sudden death and ventricular arrhythmias in FH patients. Therapies such as PCSK9 inhibitors or combination of LLT should be evaluated for their effects on arrhythmogenic substrate in patients with FH.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University School of Medicine (2016/104).

Informed Consent: All participants signed informed consent forms before study inclusion.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.K., S.K.; Design - M.K., S.K., E.S.; Supervision E.V., M.K.; Resources - Y.Ç., E.S., E.V.; Materials - M.K., Y.Ç.; Data Collection and/or Processing - M.K., S.K., E.S., Y.Ç.; Analysis and/or Interpretation - E.V., M.K.; Literature Search - M.K., S.K.; Writing Manuscript - S.K, M.K, E.S.; Critical Review - E.V., M.K.; Other - Y.Ç, M.K.

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REFERENCES

1. Liu Y-B, Lee Y-T, Pak H-N, Lin S-F, Fishbein MC, Chen LS, et al. Effects of simvastatin on cardiac neural and electrophysiologic remodeling in rabbits with hypercholesterolemia. Heart Rhythm 2009; 6: 69-75. [Crossref]
2. Tamargo J, Caballero R, Gómez R, Núñez L, Vaquero M, Delpón E. Lipid-lowering therapy with statins, a new approach to antiarrhythmic therapy. Pharmacol Ther 2007; 114: 107-26. [Crossref]
3. Buber J, Goldenberg I, Moss AJ, Wang PJ, McNitt S, Hall WJ, et al. Reduction in life-threatening ventricular tachyarrhythmias in statin-treated patients with nonischemic cardiomyopathy enrolled in the MADIT-CRT (Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy). J Am Coll Cardiol 2012; 60: 749-55. [Crossref]
4. Xia Y, Liang Y, Kongstad O, Liao Q, Holm M, Olsson B, et al. In vivo validation of the coincidence of the peak and end of the T wave with full repolarization of the epicardium and endocardium in swine. Heart Rhythm 2005; 2: 162-9. [Crossref]
5. Kongstad O, Xia Y, Liang Y, Hertervig E, Ljungström E, Olsson B, et al. Epicardial and endocardial dispersion of ventricular repolarization. A study of monophasic action potential mapping in healthy pigs. Scand Cardiovasc J 2005; 39: 342-7. [Crossref]
6. Porthan K, Viitasalo M, Toivonen L, Havulinna AS, Jula A, Tikkanen JT, et al. Predictive value of electrocardiographic T-wave morphology parameters and T-wave peak to T-wave end interval for sudden cardiac death in the general population. Circ Arrhythm Electrophysiol 2013; 6: 690-6. [Crossref]
7. Laurita KR, Girouard SD, Akar FG, Rosenbaum DS. Modulated dispersion explains changes in arrhythmia vulnerability during premature stimulation of the heart. Circulation 1998; 98: 2774-80. [Crossref]
8. Antzelevitch C, Viskin S, Shimizu W, Yan G-X, Kowey P, Zhang L, et al. Does Tpeak-Tend provide an index of transmural dispersion of repolarization? Heart Rhythm 2007; 4: 1114-6. [Crossref]
9. Hovingh GK, Davidson MH, Kastelein JJ, O'connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 2013; 34: 962-71. [Crossref]
10. Olshausen KV, Witt T, Pop T, Treese N, Bethge K-P, Meyer J. Sudden cardiac death while wearing a Holter monitor. Am J Cardiol 1991; 67: 381-6. [Crossref]
11. Kuo C-S, Munakata K, Reddy CP, Surawicz B. Characteristics and possible mechanism of ventricular arrhythmia dependent on the dispersion of action potential durations. Circulation 1983; 67: 1356-67. [Crossref]
12. Chua KC, Rusinaru C, Reinier K, Uy-Evanado A, Chugh H, Gunson K, et al. Tpeak-to-Tend interval corrected for heart rate: A more precise measure of increased sudden death risk? Heart Rhythm 2016; 13: 2181-5. [Crossref]
13. Panikkath R, Reinier K, Uy-Evanado A, Teodorescu C, Hattenhauer J, Mariani R, et al. Prolonged Tpeak to Tend interval on the resting electrocardiogram is associated with increased risk of sudden cardiac death. Circ Arrhythm Electrophysiol 2011; 4: 441-7. [Crossref]
14. LaRosa JC, He J, Vupputuri S. Effect of statins on risk of coronary disease: a meta-analysis of randomized controlled trials. Jama 1999; 282: 2340-6. [Crossref]
15. Pyörälä K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease: a subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). Diabetes care 1997; 20: 614-20. [Crossref]
16. Tekin A, Tekin G, Sezgin AT, Müderrisoğlu H. Short-and long-term effect of simvastatin therapy on the heterogeneity of cardiac repolarization in diabetic patients. Pharmacol Res 2008; 57: 393-7. [Crossref]
17. De Sutter J, Tavernier R, De Buyzere M, Jordaens L, De Backer G. Lipid lowering drugs and recurrences of life-threatening ventricular arrhythmias in high-risk patients. J Am Coll Cardiol 2000; 36: 766-72. [Crossref]
18. Mitchell LB, Powell JL, Gillis AM, Kehl V, Hallstrom AP, Investigators A. Are lipid-lowering drugs also antiarrhythmic drugs?: an analysis of the antiarrhythmics versus implantable defibrillators (AVID) trial. J Am Coll Cardiol 2003; 42: 81-7. [Crossref]
19. Vyas AK, Guo H, Moss AJ, Olshansky B, McNitt SA, Hall WJ, et al. Reduction in ventricular tachyarrhythmias with statins in the Multicenter Automatic Defibrillator Implantation Trial (MADIT)-II. J Am Coll Cardiol 2006; 47: 769-73. [Crossref]
20. Vrtovec B, Okrajsek R, Golicnik A, Ferjan M, Starc V, Radovancevic B. Atorvastatin therapy increases heart rate variability, decreases QT variability, and shortens QTc interval duration in patients with advanced chronic heart failure. J Card Fail 2005; 11: 684-90. [Crossref]
21. Liu Y-B, Wu C-C, Lu L-S, Su M-J, Lin C-W, Lin S-F, et al. Sympathetic nerve sprouting, electrical remodeling, and increased vulnerability to ventricular fibrillation in hypercholesterolemic rabbits. Circ Res 2003; 92: 1145-52. [Crossref]

22. Luo T-Y, Wu C-C, Liu Y-B, Fu Y-K, Su M-J. Dietary cholesterol affects sympathetic nerve function in rabbit hearts. J Biomed Sci 2004; 11: 339-45. [Crossref]
23. Pinto JM, Boyden PA. Electrical remodeling in ischemia and infarction. Cardiovasc Res 1999; 42: 284-97. [Crossref]
24. Hevia JC, Antzelevitch C, Bárzaga FT, Sánchez MD, Balea FD, Molina RZ, et al. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. J Am Coll Cardiol 2006; 47: 1828-34. [Crossref]
25. Zareba W, Moss AJ, le Cessie S. Dispersion of ventricular repolarization and arrhythmic cardiac death in coronary artery disease. J Am Coll Cardiol 1994; 74: 550-3. [Crossref]
26. Antzelevitch C. Tpeak-Tend interval as an index of transmural dispersion of repolarization. Eur J Clin Invest 2001; 31: 555-7. [Crossref]
27. Yayla Ç, Yayla ME, Yayla KG, Ilgen U, Akboğa MK, Düzgün N. The assessment of Tp-e interval and Tp-e/QT ratio in patients with systemic sclerosis. Arch Rheumatol 2016; 31: 139-44. [Crossref]
28. Yayla Ç, Bilgin M, Akboğa MK, Gayretli Yayla K, Canpolat U, Dinç Asarcikli L, et al. Evaluation of Tp-E interval and Tp-E/QT ratio in patients with aortic stenosis. Ann Noninvasive Electrocardiol 2016; 21: 287-93. [Crossref]
29. Caballero P, Alonso R, Rosado P, Mata N, Fernández-Friera L, Jiménez-Borreguero LJ, et al. Detection of subclinical atherosclerosis in familial hypercholesterolemia using non-invasive imaging modalities. Atherosclerosis 2012; 222: 468-72. [Crossref]
30. Neefjes LA, Gert-Jan R, Alexia R, Nieman K, Galema-Boers AJ, Langendonk JG, et al. Accelerated subclinical coronary atherosclerosis in patients with familial hypercholesterolemia. Atherosclerosis 2011; 219: 721-7. [Crossref]
31. Miname MH, Ribeiro MS, Parga Filho J, Avila LF, Bortolotto LA, Martinez LR, et al. Evaluation of subclinical atherosclerosis by computed tomography coronary angiography and its association with risk factors in familial hypercholesterolemia. Atherosclerosis 2010; 213: 486-91. [Crossref]
32. Saracoglu E, Kılıç S, Vuruşkan E, Düzen I, Çekici Y, Kuzu Z, et al. Prediction of subtle left ventricular systolic dysfunction in homozygous and heterozygous familial hypercholesterolemia: Genetic analyses and speckle tracking echocardiography study. Echocardiography 2018; 35: 1289-99. [Crossref]
33. Kayikcioglu M, Payzin S, Yavuzgil O, Kultursay H, Can LH, Soydan I. Benefits of statin treatment in cardiac syndrome-X. Eur Heart J 2003; 24: 1999-2005. [Crossref]
34. Crouse JR, Raichlen JS, Riley WA, Evans GW, Palmer MK, O'Leary DH, et al. Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR Trial. Jama 2007; 297: 1344-53. [Crossref]
35. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. Jama 2006; 295: 1556-65. [Crossref]
36. Rodriguez-Granillo GA, Agostoni P, Garcia-Garcia HM, Biondi-Zoccai GG, Mc Fadden E, Amoroso G, et al. Meta-analysis of the studies assessing temporal changes in coronary plaque volume using intravascular ultrasound. Am J Cardiol 2007; 99: 5-10. [Crossref]

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Original Research

Is it Possible to Regain Lost Minerals to Initial Enamel Lesions Using Enamel Matrix Derivatives Combined with Casein Phosphopeptide Amorphous Calcium Phosphate: An *in Vitro* Study

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ABSTRACT

Objective: This study aimed to assess the efficacy of enamel matrix derivatives (EMD) used in combination with casein phosphopeptide amorphous calcium phosphate (CPP-ACP) to recover minerals to the enamel after artificial caries formation.

Methods: Fifty enamel samples were prepared from the buccal surfaces of extracted human third molars. The samples were divided into five groups: (G1) Untreated enamel samples (control), (G2) Application of 500 ppm sodium fluoride (NaF), (G3) CPP-ACP, (G4) EMD, and (G5) CPP-ACP + EMD. All of the samples were placed in an acidic buffer solution for 96 hours to simulate a carious lesion. Ca/P ratios were calculated using energy dispersive X-ray spectroscopy (EDX). After seven days of the remineralization procedure, the mineral contents of the samples were re-measured. All the data were analyzed statistically.

Results: There were no statistically significant differences in the mineral contents of the samples between the groups after demineralization ($p>0.05$). The Ca/P ratios of G2, G3, G4, and G5 increased significantly ($p<0.05$) after remineralization. The highest levels of the Ca/P ratio were obtained in G5.

Conclusion: Despite the limitations of this in vitro study, the combined use of CPP-ACP and EMD may increase the remineralization potential. Furthermore, this procedures may be an alternative for providing enamel remineralization in future clinical trials.

Keywords: Casein phosphopeptide amorphous calcium phosphate, enamel matrix derivatives, enamel remineralization, fluoride, SEM-EDX

INTRODUCTION

Dental caries is an infectious disease of the teeth that results in the dissolution and destruction of hard tissues such as the enamel and dentin (1). The first sign of dental caries is the initial caries lesion defined as "subsurface enamel porosity from carious demineralization" (2), and it can be reversed by the recovery of minerals to the lost structure (3). Some methods or materials like the application of topical fluoride or CPP-ACP provided aided remineralization (4). Fluoride is a classic anti-caries agent, although it is more effective for sound enamel than caries lesions (5) since its effectiveness is limited by the availability of calcium and phosphate ions (6). CPP, which is the protective factor in milk, was obtained by the digestion of casein with trypsin enzyme, using the selective precipitation method (7). CPP can stabilize calcium phosphate as a CPP-ACP complex (8), and these complexes promote remineralization by increasing calcium phosphate in the dental plaque (9).

More recently, biomimetic remineralization approaches are receiving increasing attention as a promising anti-caries therapy (10). The biomimetic synthesis of materials such as enamel can provide a noninvasive alternative treatment for early carious lesions. Amelogenin is the most common enamel matrix protein that plays a role in the formation and growth of the enamel crystal structure (11). Regarding the interaction of amelogenin and calcium phosphate, it is believed that amelogenin modulates the calcium phosphate nanocrystalline structure and plays an important role in enamel biomineralization (11). Amelogenin, calcium, and phosphate ions are important substances for the formation of organized hydroxyapatite crystals *in vitro* (12). Enamel matrix derivatives (EMD) are commercially available derivatives of enamel matrix proteins (EMP). EMD includes 90% amelogenin and 10% pig enamel matrix protein derivatives, which have been shown to promote periodontal ligament cell proliferation and collagen production, and also to enhance *in vitro* mineralization (13).

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In light of these knowledge,the null hypothesis of the present *in vitro* study was that CPP-ACP and EMD would have a positive synergistic effect on the recovery of the lost structure of the enamel surface. This *in vitro* study aimed to evaluate the remineralization potential of CPP-ACP and EMD in initial caries lesions by EDX and make comparisons between the groups.

METHODS

Preparation of the Enamel Samples

Fifty impacted human third molar teeth were collected for this study, and ethics committee approval was obtained from the İnönü University/Turkey Clinical Research Ethics Committee (2013/146). The teeth were stored in 4°C deionized water containing 0.2% thymol for at most 30 days. The samples were evaluated using a stereomicroscope (Nikon, Tokyo, Japan) at 2× magnification to choose thesound enamel surfaces. The teeth were then cut using the IsoMet ™ Low Speed Precision Cutter (Buehler, Germany). The enamel surface of the blocks was polished on a polishing machine (Ecomet 3, Bueller, IL, USA) and water-cooled to obtain a smooth enamel surface. Then, they were covered with two coats of acid-resistant nail varnish, except for a 3×3 mm^2 enamel window.

Caries Lesion Formation

Early artificial caries lesions were produced in 50 enamel samples. The samples were placed in a demineralization solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 50 mM acetic acid, and 1 M KOH. The pH of the solution was adjusted to 5.0, andthe demineralization was performed at 37°C for 96 hours (14).

pH-Cycling Model

The samples were divided into five groups: (G1) Untreated enamel samples (control), (G2) 500 ppm sodium fluoride (NaF, Oral-B Stages, Oral-B Laboratories, Netherlands), (G3) CPP-ACP (Tooth Mousse, containing 10% CPP-ACP; GC Int., Tokyo, Japan), (G4) EMD gel (Emdogain, lyophilized protein fractions dissolved in acetic acid, Straumann, Biora, Sweden), and (G5) CPP-ACP + EMD. pH-cycling was carried out using a remineralization solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 150 mM KCl, 1 M KOH, and pH 7.0) and demineralization solution (2.2 mM CaCl2, 2.2 mM NaH_2PO_4, 50 mM acetic acid, and 1 M KOH, pH 5.0) (15). The experimental design included 2 demineralization/remineralization cycles a day for 10 days: immersion of the enamel samples in 10 mL of demineralizing solution, twice daily (at 9 AM and 9 PM) for 30 minutes; application of the solutions (described above) for 4 minutes(in group 5;CPP-ACP was applied for 4 minutes firstly, washing in distilled water for 10 seconds, then EMD gel was applied for 4 minutes); washing in distilled water for 10 seconds; immersion in 100 mL of the remineralizing solution (16).

Main Points:

- The combined use of CPP-ACP and EMD increased Ca/P% ratio at the enamel surface.
- CPP-ACP showed close efficacy to the fluoride in enamel remineralization.
- The use of EMD alone was not sufficient to reverse the mineral loss from the enamel.

SEM-EDX Analysis

SEM-EDX analysis was performed to determine the mineral content of the enamel samples after caries lesion formation and pH-cycling. Elemental analysis was performed on both halves of the exposed enamel surfaces in 20 Kv and fluoride (F) %, calcium (Ca) %, and phosphate (P) % values were recorded atomically. The Ca/P% ratio was then calculated. Image analysis was also performed using an SEM to determine the structure of the initial carious lesions and the variations after remineralization.

Statistical Analysis

After demineralization and application of the remineralization agents to the demineralized samples, a paired t-test was performed for each group to evaluate the mineral changes in the samples. The one-way ANOVA test was conducted to compare mineralization differences between the groups after demineralization and remineralization. In addition, the post-hoc Tukey test was used to compare the differences between the groups after remineralization.

RESULTS

The mean atomic values of the F%, Ca%, and P% values were recorded atomically using SEM-EDX, and the Ca/P% ratios of all the study groups were statistically compared (Table 1).

The SEM-EDX analysis method was used after applying the demineralization procedure, and it revealed that there was no statistically significant difference between the groups in terms of the mean atomic Ca/P% ($p<0.05$). After the remineralization, the mean atomic Ca/P% ratio in all groups, except for the control group, exhibited a statistically significant increase ($p<0.05$). According to the paired t-test, the highest Ca/P% ratio after treatment was obtained in group 5, whereas the lowest Ca/P% ratio was obtained in group 1 (Table 2).

Due to the intergroup comparison of the mean Ca/P% ratios after remineralization using the post-hoc Tukey test, the mean Ca/P% ratio of group 5 was found to be higher than that of group 2. However, the difference between these values was not found to be statistically significant ($p>0.05$). The mean Ca/P% ratio obtained from groups 2 and 5 was higher than the mean Ca/P% ratio obtained from group 3, and the mean Ca/P% ratio obtained from group 3 was higher than that obtained from groups 1 and 4 ($p<0.05$). The mean Ca/P% ratio obtained from group 4 was higher than that obtained from group 1. However, the difference between these values was not found to be statistically significant ($p>0.05$; Table 3).

By evaluating the SEM images according to the treatment groups, we observed the enamel surfaces of the groups (Figure 1). The small particles and cracks found in the control groups (a)could be due to the dissolved Ca/P ions from the enamel subsurface. Prism structure could be seen in NaF (b) and CPP-ACP+EMD (e) with smaller pores and showed a dense packed precipitation layer. CaF_2 deposition was also observed

Table 1. Comparison of the mean atomic values of the F%, Ca%, P%, and Ca/P% ratiosin the demineralization and remineralization periods

		Groups					
Elements %		**G1**	**G2**	**G3**	**G4**	**G5**	**OWA P**
F	Demineralization	13,44±1,29	13.44±0.84	13.57±1.05	13.45±1.06	13.59±0.84	0.054 0.99
	Remineralization	13,61±1,32	17.10±0.48	12.60±1.19	15.25±0.78	15.21±1.10	**37.862***
Ca	Demineralization	15,53±1,79	15.03±1.73	14.37±0.94	14.23±0.89	14.06±1.46	1.887 0.12
	Remineralization	15,43±0,83	17.81±0.29	20.81±1.37	15.59±1.11	18.29±1.26	**44.22***
P	Demineralization	8,74±0,81	8.56±0.85	8.34±0.30	8.38±0.73	8.09±0.56	1.305 0.28
	Remineralization	8,63±0,61	8.15±0.23	10.03±0.94	8.40±0.72	8.29±0.77	**11.923***
Ca/P	Demineralization	1,77±0,10	1.75±0.09	1.72±0.08	1.70±0.10	1.73±0.09	0.90 0.46
	Remineralization	1,79±0,08	2.18±0.06	2.07±0.12	1.86±0.09	2.21±0.11	**37.86***

Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD.
OWA: One-way Anova,
*: statistically significant difference (p<0.05)

Table 2. Comparison of the mean atomic values of the Ca/P% ratiosin the demineralization and remineralization periods

	GROUPS					
	G1 n=10	**G2 n=10**	**G3 n=10**	**G4 n=10**	**G5 n=10**	**OWA P**
Demineralization	1.77±0.10	1.75±0.09	1.72±0.08	1.70±0.10	1.73±0.09	0.90 0.46
Remineralization	1.79±0.08	2.18±0.06	2.07±0.12	1,86±0,09	2.21±0.11	**37.86***
Difference	+0.01±0.14	+0.42±0.12	+0.35±0.16	+0,15±0,14	+0.47±0.14	
PTT (P)	0.801	**0.0001***	**0.0001***	**0.007***	**0.0001***	

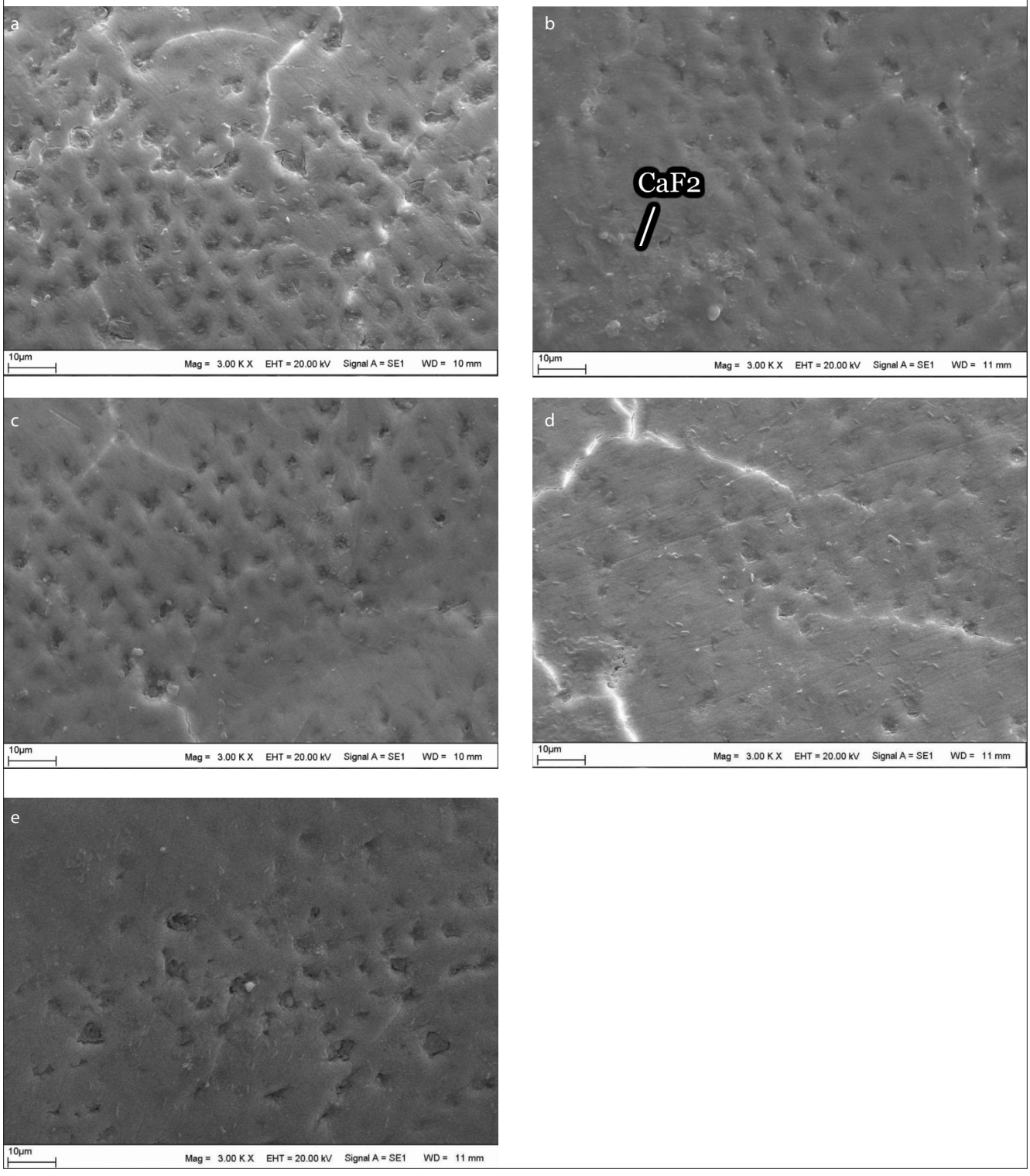
Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD.
OWA: One-way Anova, PTT: Paired T-Test,
*: statistically significant difference (p<0.05)

Table 3. Comparison of the mean atomic values of the Ca/P% ratios between the groups after remineralization

Group	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**
G1	G3	**0.0001***	G2	G3	**0.014***	G3	G4	**0.0001***
	G4	0.118		G4	0.0001*		G5	**0.003***
	G5	**0.0001***		G5	0.556		G2	**0.014***
	G2	**0.0001***		G1	0,0001*		G1	**0.0001***
Group	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**			
G4	G3	**0.0001***	G5	G3	**0.003***			
	G5	**0.0001***		G4	**0.0001***			
	G2	**0.0001***		G2	0.556			
	G1	0.118		G1	**0.0001***			

Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD
PHT: Post Hoc Tukey,
*: statistically significant difference (p<0.05)

Figure 1. a-e. Scanning Electron Microscope (SEM) images (× 3.00 k). a) G1: Control b) G2: NaF c) G3: CPP-ACP d) G4: EMD, and e) G5: CPP-ACP+EMD



in the NaF (b) group, but CPP-ACP+EMD (e) seemed to have a less porous structure than the NaF (b) group. In the CPP-ACP groups (c) and EMD (d), patch-like structures were clearly seen while some enlarged pores were also observed (Figure 1a-e).

DISCUSSION

This study evaluated the effectiveness of using EMD combined with CPP-ACP on the remineralization of initial caries lesions, and it was found that using the two materials in combination may

have a synergistic effect on biomimetic remineralization. These materials may be proposed as a new treatment alternative forinitial caries lesions. However, there is need for further research to clarify this.

The progression of caries or enabling of remineralization depends on the balance between pathological (demineralization) and protective factors (improving remineralization and decreasing bacteria formation) (17). Among the protective factors, fluoride is the most commonly known agent that supports remineralization. Sodium fluoride (NaF), which has a very high water solubility, is the simplest fluoride compound that can be used (18). When toothpaste containing fluoride with an ionic bond (NaF) is used, a CaF_2 layer accumulates on the dental hard tissue while teeth are being brushed. This reserve is used over time, and fluoride concentrations rise in the enamel and saliva (19). A toothpaste containing 500 ppm NaF was used with the positive control group in this study. Controlling the decay process with the fluoride treatment may not be sufficient in individuals who are at a higher risk of caries. Therefore, new anti-caries agents and delivery systems have recently been developed.

EMD is a purified acidic extract of EMP that is secreted from Hertwing's epithelial root sheath in the development of the swine teeth at the embryonic stage (20). EMP that mainly consists of amelogenin, has a hydrophobic structure (21). EMD consist of 90% amelogenin and 10% proline-rich non-amelogenin, tuftelin, tuft protein, serum, ameloblastin, amelin, and saliva proteins (22). EMD regulates enamel biomineralization by inducing and guiding crystal formation, supporting crystal growth, protecting the mineral phase, combining mineral ions, and regulating the growth rate (23).

Wang et al. (24) obtained many new hydroxyapatite crystals using EMP and showed that it plays an important role in hydroxyapatite crystals formation and growth. It was therefore demonstrated that EMP induces enamel remineralization. Chen et al. (25) used EMP to obtain hydroxyapatite nanorods, and thus simulated the enamel mineralization process. These rods were similar to mature enamel not only in terms of their chemical composition, but also in terms of their size. Xiang et al. (26) investigated the remineralization effect of EMDs on initial enamel carious lesions. According to the results of this study, it was found that EMD plays an essential role in promoting the remineralization of initial carious lesions. However, the complete remineralization of such lesions could not be achieved. Considering the findings in these studies, our study aimed to enhance the understanding of the remineralization activity of EMD. Therefore, we used casein phosphopeptide amorphous calcium phosphate, which is a remineralization agent containing Ca and P.

We also used an SEM in this study to determine the structure of the initial carious lesion on the enamel and the changes that occurred after the treatment. Moreover, the changes created by the administered treatment agents at the mineral level in the enamel samples were evaluated using a microanalytical technique with EDX. SEM is a well-defined technique being used in almost every field of modern medicine, and it is generally used as a supportive technique in various studies. Energy dispersive X-ray spectroscopy (EDS, EDX, or XEDS) is an analytical technique that can be used in combination with SEM for the chemical characterization or elemental analysis of samples (27).

It has been reported in the literature that CPP-ACP remineralization pastes increase the Ca/P% ratio on demineralized enamel surfaces, and thus have the potential ofrecovering minerals to dental hard tissues (28, 29). Recently, biomineralization of the enamel with a regrown enamel-like mineral layer has been considered in the treatment of initial caries lesions. Self-assembling peptide (30), amelogenin (31), or EMD (26, 32) can be used for this purpose. In our study, SEM-EDX measurements showed that all the groups except the control group exhibited remineralization. It was found that EMD could play an essential role in promoting the remineralization of the initial carious lesions, andprevious studies also support this finding (23, 26, 32). However, when used alone, it could not provide complete remineralization of such lesions. CPP-ACP administration provided an effective remineralization of the enamel and therefore, CPP-ACP agent can be an alternative to fluoride in enamel remineralization. The fact that the Ca/P% ratio was higher in the group that received the combination of Emdogain and CPP-ACP compared to the groups that received these materials separately supports the opinion that EMD is an important promoter of enamel bio-remineralization and a modulator of calcium phosphate nanocrystal structures (11). Furthermore, the highest Ca/P% ratio was obtained from the group that received the combination of Emdogain and CPP-ACP. SEM images also supported the data obtained in our study using the EDX microanalytical method.

CONCLUSION

In this study, we found that using a combination of CPP-ACP and EMD could enhance the remineralization activity of each other. However, further studies are required to evaluate the profit-loss relationship and the remineralization activity in terms of the cost, in order to create a standard procedure with regard to the frequency of application, and to evaluate the possible effects on oral-dental and general health in the case of combined CPP-ACP and EMD use.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of İnönü University (2013/146).

Informed Consent: Written informed consent was obtained from participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - G.K.; Design - G.K., Ç.G., S.Y.; Supervision - G.K.; Resources - G.K., Ç.G.; Materials - G.K.; Data Collection and/or Processing - G.K.; Analysis and/or Interpretation - G.K., Ç.G., S.Y; Literature Search - G.K.; Writing Manuscript - G.K.; Critical Review - G.K., Ç.G., S.Y.; Other - G.K., Ç.G., S.Y.

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REFERENCES

1. Somasundaram P, Vimala N, Mandke LG. Protective potential of casein phosphopeptide amorphous calcium phosphate containing paste on enamel surfaces. J Conserv Dent 2013; 16: 152-6. [Crossref]
2. Gocmen GB, Yanikoglu F, Tagtekin D, Stookey GK, Schemehorn BR, Hayran O. Effectiveness of some herbals on initial enamel caries lesion. Asian Pac J Trop Biomed 2016; 6: 846-50. [Crossref]
3. Wang X, Wang Y, Wang K, Ren Q, Li H, Zheng S, et al. Bifunctional anticaries peptides with antibacterial and remineralizing effects. Oral Dis 2019; 25: 488-96. [Crossref]
4. Nakata T, Kitasako Y, Sadr A, Nakashima S, Tagami J. Effect of a calcium phosphate and fluoride paste on prevention of enamel demineralization. Dent Mater J 2018; 30; 37: 65-70. [Crossref]
5. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling Peptide P11-4 and Fluoride for Regenerating Enamel. J Dent Res 2018; 97: 148-54. [Crossref]
6. Pinto de Souza SCT, Araújo KC, Barbosa JR, Cancio V, Rocha AA, Tostes MA. Effect of dentifrice containing fTCP, CPP-ACP and fluoride in the prevention of enamel demineralization. Acta Odontol Scand 2018; 76: 188-94. [Crossref]
7. Buckshey S, Anthonappa RP, King NM, Itthagarun A. Remineralizing Potential of Clinpro® and Tooth Mousse Plus® on Artificial Carious Lesions. J Clin Pediatr Dent 2019; 43: 103-8. [Crossref]
8. Attiguppe P, Malik N, Ballal S, Naik SV. CPP-ACP and Fluoride: A Synergism to Combat Caries. Int J Clin Pediatr Dent 2019; 12: 120-5. [Crossref]
9. Fatemeh M, Marjan S, Homa N, Mahsa S. CPP-ACP: Effect on Dental Plaque Acidity after Water Rinsing Following Topical Fluoride Therapy. J Clin Pediatr Dent 2017; 41: 22-6. [Crossref]
10. Lv X, Yang Y, Han S, Li D, Tu H, Li W, et al. Potential of an amelogenin based peptide in promoting reminerlization of initial enamel caries. Arch Oral Biol 2015; 60: 1482-7. [Crossref]
11. Fan Y, Wen ZT, Liao S, Lallier T, Hagan JL, Twomley JT, et al. Novel amelogenin-releasing hydrogel for remineralization of enamel artificial caries. J Bioact Compat Polym 2012; 27: 585-603. [Crossref]
12. Tian K, Peng M, Ren X, Liao C, Fei W. Regeneration of tooth-like hydroxyapatite depended on amelogenin functional section monolayer: a new approach for tooth repair. Med Hypotheses 2012; 79: 143-6. [Crossref]
13. Kubasad G, Ghivari S, Garg K. Replantation of an avulsed tooth with an extended extra oral period. Indian J Dent Res 2012; 23: 822-5. [Crossref]
14. Mohd Said SN, Ekambaram M, Yiu CK. Effect of different fluoride varnishes on remineralization of artificial enamel carious lesions. Int J Paediatr Dent 2017; 27: 163-73. [Crossref]
15. Oliveira GM, Ritter AV, Heymann HO, Swift E Jr, Donovan T, Brock G, et al. Remineralization effect of CPP-ACP and fluoride for white spot lesions in vitro. J Dent 2014; 42: 1592-602. [Crossref]
16. Peric TO, Markovic DL, Radojevic VJ, Heinemann RM, Petrovic BB, Lamovec JS. Influence of pastes containing casein phosphopeptide-amorphous calcium phosphate on surface of demineralized enamel. J Appl Biomater Funct Mater 2014; 12: 234-9. [Crossref]
17. Featherstone JD, Domejean S. The role of remineralizing and anticaries agents in caries management. Adv Dent Res 2012; 24: 28-31. [Crossref]
18. Rugg-Gunn A, Banoczy J. Fluoride toothpastes and fluoride mouthrinses for home use. Acta Med Acad 2013; 42: 168-78. [Crossref]
19. Altenburger MJ, Bernhard J, Schicha TD, Wrbas KT, Hellwig E. Comparison of in vitro fluoride uptake from whitening toothpastes and a conventional toothpaste in demineralised enamel. Schweiz Monatsschr Zahnmed 2010; 120: 104-13.
20. Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol1997; 24: 658-68. [Crossref]
21. Kobayashi E, Fujioka-Kobayashi M, Saulacic N, Schaller B, Sculean A, Miron RJ. Effect of enamel matrix derivative liquid in combination with a natural bone mineral on new bone formation in a rabbit GBR model. Clin Oral Implants Res 2019; 30: 542-9. [Crossref]
22. Youssef AR, Emara R, Taher MM, Al-Allaf FA, Almalki M, Almasri MA, Siddiqui SS. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. BMC Oral Health 2019; 19: 133. [Crossref]
23. Ieong CC, Zhou XD, Li JY, Li W, Zhang LL. Possibilities and potential roles of the functional peptides based on enamel matrix proteins in promoting the remineralization of initial enamel caries. Med Hypotheses 2011; 76: 391-4. [Crossref]
24. Wang ZW, Zhao YP, Zhou CR, Li H. The study on the enamel remineralization by enamel matrix proteins' inducing. Sichuan Da Xue Xue Bao Yi Xue Ban 2008; 39: 579-82.
25. Chen H, Clarkson BH, Sun K, Mansfield JF. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J Colloid Interface Sci 2005; 288: 97-103. [Crossref]
26. Xiang C, Ran J, Yang Q, Li W, Zhou X, Zhang L. Effects of enamel matrix derivative on remineralisation of initial enamel carious lesions in vitro. Arch Oral Biol 2013; 58: 362-9. [Crossref]
27. Ellingham STD, Thompson TJU, Islam M. Scanning Electron Microscopy-Energy-Dispersive X-Ray (SEM/EDX): A Rapid Diagnostic Tool to Aid the Identification of Burnt Bone and Contested Cremains. J Forensic Sci 2018;63: 504-10. [Crossref]
28. Zhou C, Zhang D, Bai Y, Li S. Casein phosphopeptide-amorphous calcium phosphate remineralization of primary teeth early enamel lesions. J Dent 2014; 42: 21-9. [Crossref]
29. Hegde MN, Moany A. Remineralization of enamel subsurface lesions with casein phosphopeptide-amorphous calcium phosphate: A quantitative energy dispersive X-ray analysis using scanning electron microscopy: An in vitro study. J Conserv Dent 2012; 15: 61-7. [Crossref]
30. Alkilzy M, Santamaria RM, Schmoeckel J, Splieth CH. Treatment of Carious Lesions Using Self-Assembling Peptides. Adv Dent Res 2018; 29: 42-7. [Crossref]
31. Ruan Q, Zhang Y, Yang X, Nutt S, Moradian-Oldak J. An amelogenin-chitosan matrix promotes assembly of an enamel-like layer with a dense interface. Acta Biomater 2013; 9: 7289-97. [Crossref]
32. Schmidlin P, Zobrist K, Attin T, Wegehaupt F. In vitro re-hardening of artificial enamel caries lesions using enamel matrix proteins or self-assembling peptides. J Appl Oral Sci 2016; 24: 31-6. [Crossref]

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Original Research

Serum Adropin Level in Patients with Isolated Coronary Artery Ectasia

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ABSTRACT

Objective: Isolated coronary artery ectasia (iCAE) is characterized by localized or diffuse dilatation of the coronary arteries, and the exact mechanism underlying iCAE is unclear. However, endothelial dysfunction is related to iCAE. Adropin has a regulatory effect in the coronary artery endothelium. This study aimed to determine the relationship of adropin level to iCAE.

Methods: Fifty patients with iCAE and 32 age- and sex-matched control subjects with normal coronary angiography (CAG) findings were selected from 4546 patients who underwent CAG between July 2019 and October 2019. Blood sample for adropin analysis were collected just before CAG.

Results: No significant difference was found between groups in terms of baseline characteristics and laboratory parameters. The mean adropin level in patients with iCAE was significantly lower than that in control subjects (0.33±0.35 vs. 0.55±0.35 ng/mL, p<0.001). In receiver operating characteristic analysis, the cut-off value of adropin ≤0.341 had 76.0% sensitivity and 84.37% specificity for predicting iCAE (area under the curve: 0.839, p<0.001). Multivariate analysis included age, left ventricular ejection fraction, and adropin level, which showed that adropin (per 1 ng/dL decrease) (odds ratio: 0.973 [0.956–0.990]; p=0.002) was independently associated with iCAE.

Conclusion: The present study showed that adropin level is significantly lower in patients with iCAE patients and is independently associated with iCAE.

Keywords: Adropin, coronary ectasia, endothelial dysfunction

INTRODUCTION

Coronary artery ectasia (CAE) is characterized by localized or diffuse dilatation of the coronary arteries. CAE is determined as the ratio of dilated segment of the coronary artery to the adjacent normal segment of >1.5 (1). The prevalence of CAE was 1.2%– 4.9% in different studies (1-3). The exact mechanism underlying CAE is unclear. However, atherosclerosis, inflammation, and endothelial dysfunction have been suggested to be possible mechanisms (2,3). The possible mechanism in almost half of patients with CAE is atherosclerosis. However, in a small number of patients, CAE occurs without significant atherosclerosis, which is called isolated CAE (iCAE). The prevalence of iCAE is 0.1%–0.79% in studies (1-3). The endothelium plays an important role in the maintenance of vascular homeostasis, and dysfunction of the endothelium is related to CAE (4, 5).

Adropin is a protein that participates in the maintenance of energy homeostasis and insulin response. Moreover, adropin has vascular effect by increasing the eNOS protein levels and mRNA expression in the coronary artery endothelium (6). Hence, adropin has a regulating effect in the coronary artery endothelium. We aimed to determine the relationship of adropin level to CAE.

METHODS

Patients

Between July 2019 and October 2019, all consecutive patients (n=4546) underwent elective coronary artery angiography (CAG) for angina pectoris, or significant myocardial ischemia was evaluated using non-invasive stress test in our center. Of those, 132 (2.9%) patients were diagnosed with CAE. Patients with acute coronary syndrome, atherosclerotic stenosis in the coronary arteries, left ventricular ejection fraction (LVEF) <40%, acute heart failure, severe valvular disease or significant left ventricular hypertrophy (septal thickness> 15 mm) in echocardiography, previously known inflammatory/autoimmune disorders, malignancy, and chronic infection were excluded from the study. After exclusion criteria, 50 (37.8%) patients with iCAE were included. The control subjects were 32 age- and sex-matched healthy subjects with normal coronary arteries. Detailed medical characteristics of patients and control subjects were recorded after CAG. All participants signed informed consent forms before study inclusion. The local ethic committee of Adana Health Practice and Research Center approved this research (2019-518).

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Hypertension (HT) was defined as systolic blood pressure (BP) ≥140 mmHg (≥130 mmHg for diabetes mellitus [DM]) and/or diastolic BP ≥90 mmHg (≥80 mmHg for DM), and use of antihypertensive drugs.

Type 2 DM was defined as use of anti-diabetic medications or fasting blood glucose of ≥126 mg/dL. Hyperlipidemia was defined as total cholesterol level ≥200 mg/dL and/or use of lipid-lowering medications. Family history was defined as history of coronary artery disease or sudden cardiac death in any first-degree relative (male <55 years or female <65 years).

Angiographic Analysis

Routine coronary angiography was performed for all patients, and all images were recorded with coronary angiography digitized system (Siemens, Munchen, Germany). All CAG images were recorded and analyzed by two experienced interventional cardiologists blinded to the patient's clinical status. CAE was determined as exceeding coronary artery dilatation 1.5-fold than the normal segment of the coronary artery.

Blood Sample Collection and Analyses

Venous blood specimens were collected in citrate tubes for adropin analysis just before the CAG procedure. The specimens were centrifuged for 30 min at 4000 cycles. Plasma was placed in an Eppendorf tube and kept at –80°C until the assay was performed. Frozen serum was thawed slowly for 24 h, and the adropin levels were measured after the samples reached room temperature. Serum adropin levels were analyzed using a commercially available enzyme-linked immunosorbent assay kit (Cusabio Biotech Co., Wuhan, China).

Statistical Analyses

Continuous variables with normal distribution were described as mean (±SD). Variables with normal and not normal distribution were compared using Student t-test and Mann Whitney U-test, respectively. Categorical variables are presented as numbers and percentages and compared using the chi-squared or Fisher exact test. The receiver operating characteristics (ROC) was used to determine the sensitivity and specificity of adropin cut-off values for iCAE.

Binary logistics regression analysis was performed to determine the variable associated with iCAE, Statistical Package for the Social Sciences (SPSS 20.0) for Windows (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis, and $p<0.05$ was considered statistically significant.

RESULTS

The study included 50 patients (58.7±11.2 years, 32.0%, n=16 women) and 32 (55.6±8.2 years, 43.8%, n=14 women) control subjects.

Main Points:

- The exact mechanism underlying isolated coronary artery ectasia is unclear. In present study we showed that Adropin, which has a regulatory effect in the coronary artery endothelium, level is significantly lower in patients with isolated coronary artery ectasia.

Baseline medical and demographic characteristics of the patients and control subjects are summarized in Table 1. Demographic and medical characteristics of study groups were similar. The laboratory parameters of the groups are summarized in Table 2.

No significant difference was found between the groups in terms of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, hemoglobin, fasting blood glucose, C-reactive protein, and troponin level. Compared with the control subjects, the mean adropin level was significantly lower in iCAE (0.55±0.35 ng/mL vs. 0.33±0.35, $p<0.001$) (Figure 1). The correlation between adropin level and demographic and laboratory parameters are shown in Table 3. A statistically significant but weak correlation was found between adropin level and mean platelet volume and LVEF. In the ROC analysis, a cut-off value

Table 1. Baseline characteristics of the study population

Variables	iCAE (+) (n=50)	Control (n=32)	p
Age, years	58.7±11.2	55.6±8.2	0.183
BMI (kg/m^2)	24.8±5.7	25.8±6.6	0.515
Female, % (n)	32.0 (16)	43.8 (14)	0.281
Diabetes mellitus, % (n)	38.0 (19)	34.4 (11)	0.740
Hypertension, % (n)	62.0 (31)	59.4 (19)	0.812
Smoker, % (n)	46.0 (23)	43.6 (14)	0.842
Family history of coronary artery disease, % (n)	10.0 (5)	12.5 (4)	0.724
Systolic blood pressure (mmHg)	122±19	116±14	0.166
Diastolic blood pressure (mmHg)	76±10	73±13	0.259
LVEF, %	56.1±3.9	54.7±7.6	0.241

Figure 1. Comparison of adropin level of study groups

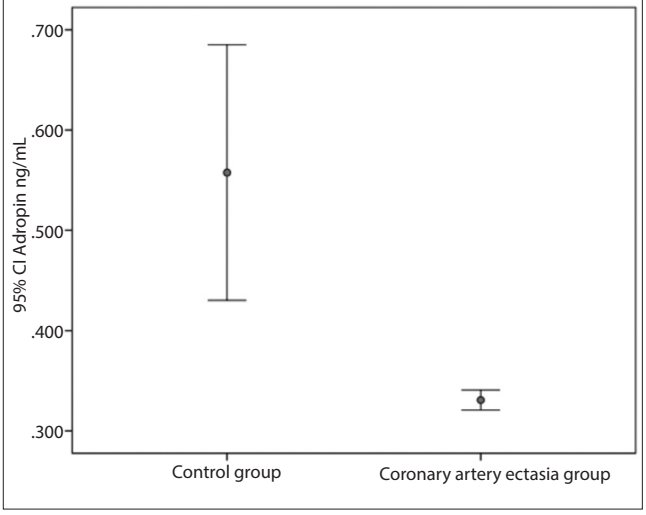

Table 2. Comparison of laboratory parameters of the study groups

Parameters	iCAE (+) (n=50)	Control (n=32)	p
Hemoglobin, g/dL	13.7±1.8	13.2±1.5	0.192
Hematocrit, %	40.2±5.1	39.4±4.1	0.406
White blood cell count, $\times 10^3$/mL	8.3±2.2	8.4±2.4	0.960
Platelet count, $\times 10^3$/mL	259±70	269±50	0.514
Lymphocyte count, $\times 10^3$/mL	2.7±1.2	2.6±0.9	0.752
Neutrophil count, $\times 10^3$/mL	4.6±1.5	4.7±1.5	0.667
Monocyte count, $\times 10^3$/mL	0.7±0.2	0.7±0.1	0.991
Mean platelet volume, fL	8.59±0.9	8.84±1.02	0.235
Neutrophil/lymphocyte ratio	1.95±1.01	1.87±0.5	0.725
Glucose (mg/dL)	121±40	134±46	0.184
Creatinine (mg/dL)	0.81±0.16	0.78±0.15	0.421
C-reactive protein (mg/L)	2.83±2.9	2.1±1.7	0.235
CK-MB (ng/mL)	1.44±1.1	1.36±0.98	0.727
Troponin (ng/mL)	7.1±3.3	5.7±2.2	0.378
Total cholesterol (mg/dL)	209±47.8	212±34	0.744
HDL-cholesterol (mg/dL)	43±9.7	45±8.3	0.396
LDL-cholesterol (mg/dL)	141±43	147±26	0.546
Triglyceride (mg/dL)	190±94	184±97	0.780
Adropin (ng/mL)	0.33±0.35	0.55±0.35	<0.001

Figure 2. Receiver operating characteristic analysis of adropin level

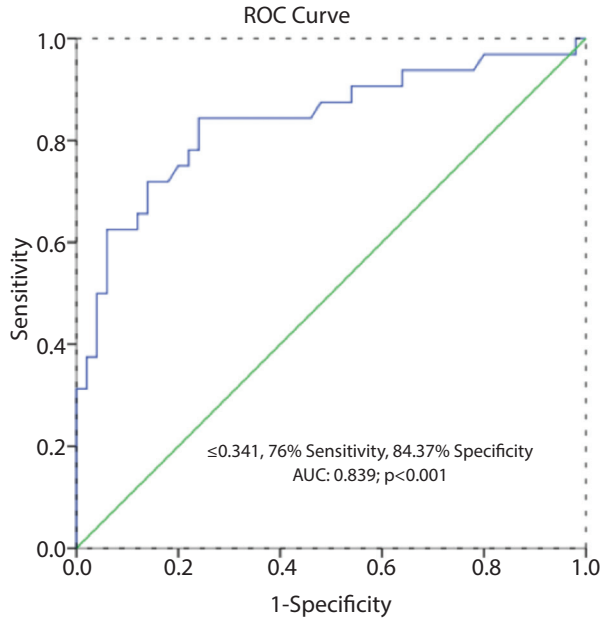

Table 3. Correlation between serum adropin level and baseline characteristics and laboratory parameters of patients

	Adropin level	
Variable	**r**	**p**
Age	−0.167	0.133
BMI	−0.122	0.901
Female	−0.10	0.376
Diabetes mellitus	0.041	0.713
Hypertension	−0.03	0.817
Smoker	−0.12	0.282
Family history of coronary artery disease	0.016	0.883
Systolic blood pressure (mmHg)	0.02	0.862
Diastolic blood pressure (mmHg)	0.104	0.354
LVEF	−0.343	0.002
Hemoglobin	−0.134	0.231
Platelet count	0.098	0.383
Lymphocyte count	−0.058	0.605
Neutrophil/lymphocyte ratio	−0.081	0.470
Mean platelet volume	0.219	0.048
Glucose	0.071	0.526
Creatinine	−0.118	0.292
C-reactive protein	−0.090	0.424
Troponin	0.090	0.422
Total cholesterol	−0.063	0.571
HDL-cholesterol	0.040	0.723
LDL-cholesterol	0.044	0.692
Triglyceride	−0.066	0.554

of adropin ≤0.341 had 76.0% sensitivity and 84.37% specificity for predicting iCAE (area under the curve, 0.839; 95% confidence interval [CI], 0.743–0.935; p<0.001] (Figure 2). Univariate and multivariate logistic regression analyses were performed to determine the risk factors of iCAE, and the results are summarized in Table 4. Univariate analysis showed that LVEF (OR, 1.192; 95% CI, 1.053–1.349; p=0.005) and adropin (OR, 0.976; 95% CI, 0.962–0.990; p<0.001) were predictors of iCAE. The multivariate analysis included age, LVEF, and adropin and showed that adropin (per 1 ng/mL decrease) (OR, 0.973 [0.956–0.990]; p=0.002) was independently associated with iCAE.

DISCUSSION

In our study, plasma adropin level was significantly decreased in patients with iCAE compared with the healthy control subjects. Decreased serum adropin level was also independently associated with iCAE.

Table 4. Logistic regression analysis of possible predictors for isolated coronary artery ectasia

Analysis		Univariate		Multivariate
Variables	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**
Age	0.183	1.031 (0.986–1.078)		
Female	0.283	1.653 (0.661–4.135)		
Body mass index (per 1 kg/m^2 decrease)	0.496	0.975 (0.905–1.050)		
LVEF (per 1% decrease)	0.005	1.192 (1.053–1.349)		
Mean platelet volume	0.234	0.747 (0.463–1.207)		
Glucose	0.186	0.993 (0.983–1.003)		
Systolic blood pressure	0.168	1.020 (0.992–1.048)		
Hemoglobin	0.193	1.193 (0.915–1.557)		
C–reactive protein	0.248	1.147 (0.909–1.446)		
Adropin (per 1–ng/dL decrease)	<0.001	0.976 (0.962–0.990)	0.002	0.973 (0.956–0.990)

CAE is characterized by localized or diffuse dilatation of the coronary arteries with 1.2%–4.9% prevalence in different CAG studies. Although the exact mechanism underlying of CAE remain unclear, different mechanisms have been suggested.

CEA is considered a variant of atherosclerosis, mainly due to an extensive exaggerated positive remodeling of the coronary artery (7). The most probable mechanism for this inappropriate remodeling is the thinning of the tunica media associated with enzymatic degradation of the extracellular matrix and chronic inflammation (8, 9).

Markis et al. (10) suggested that CAE is caused by tunica media damage. Another potential causative mechanism is chronic overstimulation of the endothelium by nitric oxide (NO). Although nearly half of patients with CAE have atherosclerosis as the underlying disease, in the other half, CAE develops without atherosclerosis and is defined as iCAE (11, 12). The most important underlying cause of patients with iCAE is chronic inflammation and endothelial dysfunction as mentioned above. Several studies have previously shown a relationship between inflammation markers, such as plasma interleukin-6, plasma soluble adhesion molecules (ICAM-I, VCAM-I, and E-selectin), and C-reactive protein, and iCAE development (12-15). In addition, Kocaman et al. (16) showed that monocyte, leukocyte, and neutrophil levels in patients with iCAE were significantly higher than those in normal controls. Doğduş et al. (11) have recently determined that the increase may have occurred in the early pathogenesis of iCEA. The vascular endothelium has many different mechanisms to maintain normal vascular physiology. NO is a potent endogenous vasodilator and has an important role in flow-mediated vasodilation (17-20). NO also suppresses vascular smooth muscle proliferation and inhibits platelet adhesion to the endothelium (18, 19). Inflammatory cell-mediated NO production results in high NO levels, and toxin production degrades elastin and disrupts the extracellular matrix. Decreased NO level in the vascular endothelium is related to vasoconstriction, ischemia, and atherosclerosis. In addition, Gurlek et al. (21) have shown that the NO level in patients with CAE patients was significantly lower than that in normal subjects.

Adropin is a recently identified protein encoded by energy homeostasis-associated gene (*Enho*) and mainly expressed in the heart, brain, liver, and coronary endothelial cells (22). Adropin is involved in regulating lipid metabolism, improving insulin resistance, protecting vascular endothelial cells, and has anti-inflammatory effects. The positive effects of adropin can be achieved by increasing endothelial NO synthase expression. Studies have shown that decreased serum adropin level is associated with endothelial dysfunction (20, 23). Moreover, Decreased adropin levels may play a role in the cause and progression of atherosclerosis by weakening the protective effect of the endothelium (6, 23-25). Yu et al. (26) showed that serum adropin levels in patients with acute coronary artery disease were significantly lower than those in healthy controls. Similarly, Demircelik et al. (27) showed that the adropin level in patients with occluded saphenous vein graft is lower than that in those with potent saphenous graft. In summary, adropin is associated with vascular endothelial function, inflammation, and atherosclerosis. Similarly, iCAE is associated with endothelial dysfunction and inflammation. Because adropin and iCAE share similar pathogenic mechanisms, we hypothesized that adropin level and iCAE may be associated. Our results supported our hypothesis and showed that adropin level in iCAE patients is lower than that in normal control subjects. Moreover, adropin is an independent predictor for iCAE.

Our study has many limitations. First, the study primarily has a cross-sectional design and relatively small population and is single center. Thus, the molecular mechanism underlying the relationship between adropin and iCAE could not be clarified. Second, the correlation between adropin and other inflammatory markers, such as P-selectin and interleukin-6, was not evaluated. Third, because of the lack of follow-up, the effect of adropin in the clinical outcomes was not evaluated in patients with iCAE.

CONCLUSION

Our study showed that adropin level is significantly lower in patients with iCAE, and adropin is independently associated with iCAE.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Adana Health Practice and Research Center approved this research. / (2019-518).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.K.; Design - M.K.; Supervision - M.K.; Resources - F.Y.; Materials - M.K., F.Y.; Data Collection and/or Processing - F.Y.; Analysis and/or Interpretation - M.K., F.Y., M.K.; Literature Search - M.K., F.Y., M.K.; Writing Manuscript - M.K.; Critical Review - M.K.

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REFERENCES

1. Çekici Y, Kılıç S, Saraçoğlu E, Çetin M, Veysel Düzen İ, Yılmaz M. The Relationship between Blood Viscosity and Isolated Coronary Artery Ectasia. Acta Cardiol Sin 2019; 35: 20-6. [Crossref]
2. Erdoğan T, Kocaman SA, Çetin M, Durakoğlugil ME, Kırbaş A, Canga A, et al. Increased YKL-40 levels in patients with isolated coronary artery ectasia: an observational study. Anadolu Kardiyol Derg 2013; 13: 465-70.
3. Kundi H, Gok M, Kiziltunc E, Cetin M, Cicekcioglu H, Guven Cetin Z, et al. "Relation between monocyte to high-density lipoprotein cholesterol ratio with presence and severity of isolated coronary artery ectasia". Am J Cardiol 2015; 116: 1685-9. [Crossref]
4. Balin M, Celik A, Kobat MA. The association between soluble lectin-like oxidized low-density lipoprotein receptor-1 levels and patients with isolated coronary artery ectasia. J Thromb Thrombolysis 2012; 33: 239-45. [Crossref]
5. Antonopoulos AS, Siasos G, Oikonomou E, Mourouzis K, Mavroudeas SE, Papageorgiou N, et al. Characterization of vascular phenotype in patients with coronary artery ectasia: the role of endothelial dysfunction. Int J Cardiol 2016; 215: 138-9. [Crossref]
6. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M, et al. Adropin is a novel regulator of endothelial function. Circulation 2010; 122: S185-92. [Crossref]
7. Devabhaktuni S, Mercedes A, Diep J, Ahsan C. Coronary artery ectasia-a review of current literature. Curr Cardiol Rev 2016; 12: 318-23. [Crossref]
8. Liu R, Chen L, Wu W, Chen H, Zhang S. "Extracellular matrix turnover in coronary artery ectasia patients". Heart Vessels 2016; 31: 351-9. [Crossref]
9. Antoniadis AP, Chatzizisis YS, Giannoglou GD. Pathogenetic mechanisms of coronary ectasia. Int J Cardiol 2008; 130: 335-43. [Crossref]
10. Markis JE, Joffe CD, Cohn PF, Feen DJ, Herman MV, Gorlin R. Clinical significance of coronary arterial ectasia. Am J Cardiol 1976; 37: 217-22. [Crossref]
11. Doğduş M, Güler TE. İzole koroner arter ektazili hastalarda aterojenik dislipideminin araştırılması. Kocaeli Tıp Dergisi 2019; 8: 173-81.
12. Bilik MZ, Kaplan İ, Yıldız A, Akıl MA, Acet H, Yüksel M, et al. Apelin levels In Isolated coronary artery ectasia. Korean Circ J 2015; 45: 386-90. [Crossref]
13. Triantafyllis AS, Kalogeropoulos AS, Rigopoulos AG, Sakadakis EA, Toumpoulis IK, Tsikrikas S, et al. Coronary artery ectasia and inflammatory cytokines: link with a predominant Th-2 immune response? Cytokine 2013; 64: 427-32. [Crossref]
14. Eid MD, Abdelaziz AA, Hassan NA. "Isolated coronary artery ectasia debate: Inflammation versus atherosclerosis." The Egyptian Heart Journal 2012; 64: 185-90. [Crossref]
15. Li JJ. "Inflammation in coronary artery diseases." Chin Med J 2011; 124: 3568-75.
16. Kocaman SA, Taçoy G, Sahinarslan A, Cengel A. Relationship between total and differential leukocyte counts and isolated coronary artery ectasia. Coron Artery Dis 2008; 19: 307-10. [Crossref]
17. Levine AB, Punihaole D, Levine TB. "Characterization of the role of nitric oxide and its clinical applications." Cardiology 2012; 122: 55-68. [Crossref]
18. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 2012; 10: 4-18. [Crossref]
19. Giles TD, Sander GE, Nossaman BD, Kadowitz PJ. Impaired vasodilation in the pathogenesis of hypertension: focus on nitric oxide, endothelial-derived hyperpolarizing factors, and prostaglandins. J Clin Hypertens 2012; 14: 198-205. [Crossref]
20. Park, KH, Park WJ. Endothelial dysfunction: clinical implications in cardiovascular disease and therapeutic approaches. J Korean Med Sci 2015; 30: 1213-25. [Crossref]
21. Gürlek A, Esenboğa K, Özcan ÖU, Çiçek ÖF, Arıbal Ayral P, Özelçi Kavas G, et al. Serum nitric oxide levels in patients with coronary artery ectasia. Anatol J Cardiol 2016; 16: 947-52. [Crossref]
22. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 2011; 8: 785-6. [Crossref]
23. Zhao ZW, Ren YG, Liu J. Low serum adropin levels are associated with coronary slow flow phenomenon. Acta Cardiol Sin 2018; 34: 307-12.
24. Oruc CU, Akpinar YE, Dervisoglu E, Amikishiyev S, Salmaslıoglu A, Gurdol F, et al. Low concentrations of adropin are associated with endothelial dysfunction as assessed by flow-mediated dilatation in patients with metabolic syndrome. Clin Chem Lab Med 2017; 55: 139-44. [Crossref]
25. Wu L, Fang J, Chen L, Zhao Z, Luo Y, Lin C et al. Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. Clin Chem Lab Med 2014; 52: 751-58. [Crossref]
26. Yu HY, Zhao P, Wu MC, Liu J, Yin W. Serum adropin levels are decreased in patients with acute myocardial infarction. Regul Pept 2014; 190: 46-9. [Crossref]
27. Demircelik B, Cakmak M, Nazli Y, Gurel OM, Akkaya N, Cetin M, et al. Adropin: a new marker for predicting late saphenous vein graft disease after coronary artery bypass grafting. Clin Invest Med 2014: E338-44. [Crossref]

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Original Research

The Effect of Epinephrine Administration on Return of Spontaneous Circulation and One-Month Mortality with Cardiopulmonary Arrest Patients

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ABSTRACT

Objective: The objective of this study is to determine the effect of epinephrine administration on the return of spontaneous circulation (ROSC) and one-month mortality in patients with cardiopulmonary arrest.

Methods: We conducted this study between August 1, 2016 and May 31, 2017. Importantly, we included the witnessed cases (≥18 years) of in-emergency department cardiopulmonary arrest (IEDCA) and out-of-hospital cardiopulmonary arrest (OHCA) in the study. We divided the patients into two groups: the adrenaline group (Group 1) and the non-adrenaline group (Group 2). Thereafter, we investigated ROSC and one-month mortality in them.

Results: We included 183 patients (50.3% of males and 49.7% of females with a mean age of 64.2±16.8 years) in the study. The percentages of IEDCA and OHCA cases were 25.1% and 74.9%, respectively. Epinephrine was administered to 100 (54.6%) patients (Group 1). Among these patients, 15.9% (n=29) of the patients had shockable rhythms (ventricular fibrillation, pulseless ventricular tachycardia) and 84.1% (n=154) of them had non-shockable rhythms (asystole, pulseless electrical activity) as the initial rhythm. ROSC and one-month mortality rate of these patients were 24% (n=44) and 72.8% (n=36), respectively. The one-month mortality rates of Group 1 (30% of patients had IEDCA and 70% of patients had OHCA) and Group 2 were 43.8% and 56.2%, respectively (p=0.0231). The ROSC and one-month mortality rates of Group 1 and Group 2 cases, whose initial rhythm was a shockable rhythm, were 26.6% and 50% vs. 42.8% and 66.6%, respectively.

Conclusion: In this study, we found no significant difference in terms of obtaining ROSC between the shockable rhythm and ROSC in the IEDCA and OHCA cases (p=0.963 and p=0.141, respectively). The effect of epinephrine administration on patients with IEDCA and OHCA whose ROSC was obtained on one-month mortality was not statistically significant (p>0.05).

Keywords: Cardiac arrest, cardiopulmonary resuscitation, epinephrine, in-emergency department, out-of-hospital

INTRODUCTION

In 2017, the USA's Cardiac Arrest Register to Enhance Survival reported 76,215 out-of-hospital cardiac arrest (OHCA) cases. Most of these cases were either unwitnessed cases (51.1%) or cases whose rhythms were non-shockable rhythm (81.6%) (1). The mortality rate for OHCA cases is approximately 90%, and they mostly occur at home (68.5%), public areas (21%), and care homes (10.5%) (2). Although the incidence and survival rates for

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in-emergency department cardiopulmonary arrest (IEDCA) cases are not clear, like OHCA, it occurs in every 1–6 of 1,000 hospitalized patients whose non-shockable rhythm ratio is high (72.3%), and the discharge rate stands at approximately 15% (3, 4).

The literature shows that the incidence and mortality rate for non-shockable rhythms are higher. In many studies, aside from cardiopulmonary resuscitation (CPR), drugs such as bicarbonate, atropine, calcium chloride, vasopressin, and epinephrine have been used to contribute toward survival. However, no promising results have been achieved, with most of these drugs remaining at the level of 2B recommendation (5, 6). Numerous studies have shown that epinephrine increases the likelihood of coronary artery perfusion and the return of spontaneous circulation (ROSC) in patients with cardiopulmonary arrest (CPA). Additionally, epinephrine reduces hospital discharge and neurological survival rates. Its effects on brain perfusion are still debatable (5-7).

The effect of epinephrine on ROSC is undeniable; however, the risk/benefit profile of this drug is still a controversial issue due to the fact that most of the cases that are followed up after having an arrest in the intensive care units are linked with a high or mortality rate or are discharged with sequela. Considering the long-term hospitalization of patients in intensive care after ROSC, the expectations of their relatives, the costs, the need to investigate whether epinephrine actually provides benefits or causes harm are increased. To clarify the uncertainty associated with the use of epinephrine during CPR, this study intends to investigate the effects of this drug on ROSC and one-month mortality rates by comparing epinephrine with placebo in the witnessed OHCA and IEDCA cases.

METHODS

We prospectively conducted this study between August 1, 2016 and May 31, 2017. Importantly, we obtained the Ethics committee approval from Gaziantep University (Date: July 25, 2016 and Decision no: 2016–217). The informed consent was postponed until the resolution of patients' emergency condition, after which consent was obtained from the patients' legal representatives. We included a total of 183 cases (137 OHCA and 46 IEDCA cases) occurring during the shifts of 12 emergency medicine specialists from four hospitals (three from each hospital) and four medical emergency teams in the study. The first CPR on OHCA cases was performed by at least two paramedics working for Turkish emergency medical health services. Ongoing CPR for patients upon arrival and CPR for all the cases experiencing a cardiac arrest in the emergency departments were managed by emergency medical specialists.

Main Points:

- Administration of epinephrine to patients with shockable rhythm decreased the ROSC ratio.
- Administration of epinephrine to patients with non-shockable rhythm increased the chances of ROSC.
- One-month mortality was higher in patients with non-shockable rhythm who were administered with epinephrine.
- Epinephrine administration had no significant effect on either ROSC or one-month mortality in the OHCA and IEDCA.

Inclusion Criteria:

- Patients who were witnessed cases of OHCA and IEDCA and underwent CPR in the emergency departments of the abovementioned four hospitals.
- Only patients with CPA in the emergency departments were included in the study.

Exclusion Criteria:

- ≥18 years old
- Cardiac arrest during pregnancy
- Arrest due to anaphylaxis
- Asthma-induced arrest
- Patients ≥85 years in the terminal period who had an arrest
- For OHCA, cases wherein ≥30 minutes had elapsed between the occurrence of the incident and arrival of the paramedics at the scene were excluded from the study, in addition to unwitnessed cases.

Structure:

The patients were divided into two groups: patients administered with epinephrine (Group 1) and patients without epinephrine administration (Group 2). Each group was then consequently divided into subgroups, namely patients who were in the emergency department and patients who were out of the hospital at the time of arrest.

Medicine Preparation and Administration Techniques:

We informed the individuals who were performing CPR about the study beforehand. We asked them to administer medication every three minutes for each case on an average and continue CPR for a minimum of 45 minutes for patients for whom no ROSC could be achieved. No limitation was imposed on the dose of epinephrine to be administered. The persons who had not previously performed CPR in the hospital or emergency ambulance (nurses or paramedics) were given separate training in advance and were described as third parties. The study coordinators put in black, non-transparent plastic bags with epinephrine (1 mg of epinephrine and 9 cc of normal saline) and without epinephrine (10 cc of saline only) into the boxes along with an equal number of normal saline and delivered them to the third parties. When CPR performers asked for epinephrine, the third parties took the bags out of the boxes at random and administered it to the patients through intravenous push over 5 seconds. Those who performed CPR were unaware as to whether epinephrine was present in the syringes prepared for the patients. The third parties recorded the contents of the bags taken out of the boxes in the previously prepared forms and delivered them to the study coordinators on a weekly basis.

Parameters considered for the study:

- ROSC
- One-month mortality rate
- Where the arrest occurred, that is OHCA or IEDCA?
- Start time of epinephrine and administered doses
- The first detected rhythm (whether shockable or not)

- Start time of chest compression
- The presence of chronic diseases and risk factors
- Lactate, pH, and pCO2 concurrently checked with CPR
- Activation time
- Arrival time of emergency medical intervention teams
- Whether endotracheal intubation (ETI) was performed for brought-in patients

Statistical Analysis

We tested the normality of the distribution of numerical data using the Shapiro–Wilk test. We employed the Student T-test for the comparison of variants that complied with normal distribution. We used the Mann–Whitney U test to compare the variables that did not have a normal distribution in the two groups. We used the Chi-Square test to determine the relationship between categorical variables and used SPSS 22.0 package software for the analyses (IBM SPSS Corp.; Armonk, NY, USA). A p value <0.05 was accepted as statistically significant for this study.

RESULTS

We prospectively conducted this study between August 1, 2016 and May 31, 2017. We included 183 patients with CPA (92 males [50.3%] and 91 females [49.7%]) in the study. The mean age of female patients having arrests (66.69±17.61 years) was higher than male patients (59.58±16.58 years; p=0.005). The mean age of all the patients was 64.2 years (±16.8) (age range: 19–99 years). In total, 46 (25.1%) of the cases were IEDCA and 137 (74.9%) were OHCA. The initial CPR of the three out-of-hospital arrest cases was performed by the relatives of the patients within the specified period of time, whereas the initial CPR of the 134 arrest cases was performed by at least two paramedics working for the emergency ambulance services.

Chronic diseases identified in the medical history of patients with CPA were as follows: diabetes mellitus (DM) (24%, n=44), coronary artery disease (CAD) (21.2%, n=39), hypertension (HT) (16.9%, n=31), chronic obstructive pulmonary disease (6.6%, n=12), obstructive sleep apnea syndrome (3.3%, n=6), cerebrovascular disease (6.6%, n=12), chronic heart failure (9.8%, n=18), and chronic renal failure (3.3%, n=6).

The mean values of arterial blood gas results taken within five minutes of the arrival of patients at the hospital were as follows: pH: 7.03 (±0.17), lactate: 11.2 (±4.7) mg/dL, and PCO_2: 57.6 (±17.65) mm/Hg. ETI was performed on all the IEHCA cases (n=46) during resuscitation. When admitted to the emergency department, 91 of the OHCA cases (66.4%) received ETI, 38 (27.7%) received a bag valve mask, and 6 (4.4%) received a laryngeal mask airway as respiratory support. Meanwhile, two patients (1.7%) received esophageal intubation.

According to the first rhythms detected inside and outside of the hospital, we divided the cases of arrest into two groups: shockable (ventricular fibrillation, pulseless ventricular tachycardia) and non-shockable (pulseless electrical activity [NEA], asystole) rhythms. In both groups, the rate of non-shockable rhythm was higher (76% vs. 24% and 87% vs. 13%; respectively).

In total, 54.6% (n=100) of patients with CPA were randomly administered epinephrine (Group 1), whereas 45.4% (n=83) of the patients were not administered with epinephrine (Group 2). In the IEDCA cases, chest compression was started in the first minute of arrest. The mean initial chest compression and breathing times for OHCA cases were 8.9±4.4 and 9.2±4.4 minutes, respectively. The first ROSC time was 31.8±18.1 minutes for all the patients on an average. ROSC was obtained in 44 patients (24%) during CPR. Of the patients for whom ROSC was obtained, 24 (54.5%) were in Group 1 and 20 (45.5%) were in Group 2. The one-month mortality rate was 72.8% (n=36) in the patients for whom ROSC was obtained. Although the ROSC ratio was higher in male patients (28.3%) than female patients (19.8%), this difference was not statistically significant (p=0.180). One-month mortality was higher among the female patients, but it was not statistically significant (p=0.054).

The mean time interval between reporting of an arrest case in Group 1 and epinephrine administration at the scene was 16.33±9.87 minutes, and the mean administered dose of epinephrine was 6.2±5.7 mg. The mean dose of epinephrine (n=4 mg) for patients for whom ROSC was obtained was less than the average dose of epinephrine (n=7 mg) administered to the patients for whom no ROSC could be obtained. However, the difference was not statistically significant (p=0.140).

The start times of chest compressions in Groups 1 and 2 were 7±3.6 and 6.8±6.4 minutes, respectively. Although the initial administration of epinephrine delayed the start of chest compression, it was not statistically significant (p=0.828).

ROSC was obtained for 44 (24%) patients in both the groups, and the initial rhythm of 10 (34.4%) of these patients was found to be shockable. We found that although the administration of epinephrine in the patients with shockable rhythm decreased the ROSC ratio (26.6% vs. 42.8%), the administration of epinephrine in patients with non-shockable rhythm increased the chance of ROSC (23.5% vs. 20.2%), but that this ratio was not statistically significant (Table 1).

There was no statistically significant relationship between the groups in terms of whether the initial rhythm was shockable or not as well as one-month mortality (p=0.079 and p=0.344, respectively) (Table 2). However, one-month mortality was higher in Group 2 cases whose first rhythm was shockable and Group 1 cases whose initial rhythm was non-shockable according to the table (66.6% vs. 50% and 90% vs. 85.8%, respectively).

When we looked at the effect of administering epinephrine to the patients for whom ROSC was obtained on one-month mortality, epinephrine was found to increase the one-month mortality rate, but this ratio was not statistically significant (Table 3).

The mean age of the patients for whom ROSC was obtained was smaller (p=0.003). While prolonged chest compression did not increase the likelihood of ROSC (p=0.001), early respiratory support (p=0.004) and chest compression (p=0.007) increased

Table 1. Relationship between ROSC in Group 1 and 2 according to the Initial Rhythm

	Group 1		Group 2	
	ROSC		ROSC	
	Yes	None	Yes	None
Shockable Rhythm (N: 15/14)	4 (26.6%)	11 (73.4%)	6 (42.8%)	8 (57.2%)
Non-Shockable Rhythm (N: 85/69)	20 (23.5%)	65 (76.5%)	14 (20.2%)	55 (79.8%)
p	0.795		0.087	

Table 2. The Effect of Epinephrine on One-Month Mortality according to the Initial Rhythm

	Group 1		Group 2	
	One-Month Mortality		One-Month Mortality	
	Survived	Did not survive	Survived	Did not survive
Shockable Rhythm (N: 4/6)	2 (50%)	2 (50%)	2 (33.3%)	4 (66.6%)
Non-Shockable Rhythm (N: 20/14)	2 (10%)	18 (90%)	2 (14.2%)	12 (85.8%)
p	0.079		0.344	

Table 3. Relationship between Group 1 and 2 and ROSC and One-Month Mortality

Patients for whom ROSC was obtained	Group 1	Group 2	Total	p
	24 (54.5%)	20 (45.5%)	44	0.988
One-Month Mortality				
Survived	4 (16.7%)	4 (20%)	8 (18%)	
Did not survive	20 (83.3%)	16 (80%)	36 (82%)	0.776

Table 4. Comparison between ROSC and Variables

Variables	ROSC (n:44)	Ex (n:139)	p
Age	56.27±17.8	65.28±16.79	0.003
Minute When Chest Compression Was Started	5.16±3.89	7.52±5.34	0.007
Duration of Chest Compression (Minutes)	33±19.92	58.29±17.17	0.001
Minute When Respiratory Support Was First Started	5.41±4	7.92±5.32	0.004
pH	7.05±0.16	7.03±0.18	0.733
Lactate (mg/dL)	10.96±4.74	11.34±4.7	0.640
pCO_2 (mm/Hg)	55.87±18.13	58.2±17.53	0.447

the likelihood of ROSC. There was no statistically significant relationship among pH, lactate, and pCO_2 values in the patients for whom ROSC was obtained (Table 4).

There was no statistically significant difference in the IEDCA and OHCA cases between the shockable rhythms and obtaining ROSC (p=0.963 and p=0.141, respectively).

Table 5. The Effect of Epinephrine Administration on One-Month Mortality in IEDCA and OHCA Patients for Whom ROSC Was Obtained

Patients for Whom ROSC Was Obtained	One-Month Mortality				
	Survived		Did not survive		Total
	Group 1 N (%)	Group 2 N (%)	Group 1 N (%)	Group 2 N (%)	ROSC
IEDCA	1 (25%)	3 (75%)	6 (30%)	7 (43.8%)	17
OHCA	3 (75%)	1 (25%)	14 (70%)	9 (56.2%)	27
p	0.158		0.231		

Table 6. Comparison of Group 1 and 2 and Categorical Variables in Terms of One-Month Mortality

Categorical Variables	One-Month Mortality		
	Survived	Did not survive	p
Age			
Group 1	4 (48.5±19.5)	20 (61.3±15.7)	0.388
Group 2	4 (54.5±12.6)	16 (52.3±20.4)	0.963
Minute When Chest Compression Was Started			
Group 1	4 (5.2±2.9)	20 (5.9±3.9)	0.627
Group 2	4 (2±2)	16 (4.9±4.1)	0.249
Minute When Chest Compression Ended (Minutes)			
Group 1	4 (33±16)	20 (35.5±19.2)	1.000
Group 2	4 (20.2±12.2)	16 (33±23.1)	0.249
Minute When Respiratory Support Was Started			
Group 1	4 (5.2±2.9)	20 (6.4±4.1)	0.477
Group 2	4 (2.5±3)	16 (4.9±4.1)	0.335
pH			
Group 1	4 (7.11±0.08)	20 (7.04±0.15)	0.431
Group 2	4 (7.15±0.14)	16 (7.0±0.18)	0.122
Lactate			
Group 1	4 (8±4.7)	20 (11.8±3.5)	0.210
Group 2	4 (7.9±4.3)	16 (11.3±5.8)	0.290
pCO_2			
Group 1	4 (67.8±10.4)	20 (57.6±17.59)	0.210
Group 2	4 (40±1.7)	16 (54.6±20.4)	0.099

The mean chest compression time of eight patients who survived for a month after ROSC was 26.6±14.8 minutes, whereas it was 34.1±20.7 minutes in 36 patients who died within a month. It was established that prolonging chest compressions did not contribute to one-month mortality (p=0.360).

The effect of epinephrine administration on one-month mortality in patients with IEDCA and OHCA for whom ROSC was obtained was not statistically significant (p≥0.05) (Table 5).

No statistically significant result was detected in the comparison of age, duration of chest compression and respiratory support, pH, lactate, pCO_2, and one-month mortality between Groups 1 and 2 (p≥0.05) (Table 6).

DISCUSSION

The Turkish Statistical Institute stated that circulatory system diseases were the most common cause of death in 2017, contributing to 39.7% of deaths. This rate was 36% in Gaziantep, where this study was conducted, and most of the patients were in the

age group of 75–84 years (50.3% of patients were females, 49.7% of patients were males) (8). The average life expectancy according to the Turkish Statistical Institute's data for 2016 was 78 years (75.3 vs. 80.7 years; male vs. female) (9). In a randomized study where epinephrine was administered, Perkins et al. (10) found that the mean age of male and female patients in the OHCA cases was similar (69.7 and 69.8 years, respectively), but the rate of arrest was approximately twice as high in males (65% and 35%, respectively). In a study on patients with arrest, Koscik et al. (11) found that the mean age of OHCA cases was 68.8±17 years (62% of male patients). In another study on patients who had suffered from OHCA, the mean age of the cases was found to be 65 years, and most (73%) of them were male patients (12). Meanwhile, in Turkey, according to a study conducted by Oğuztürk et al. (13) that examined IEDCA cases, the mean age was 63.4 years (58.6% of them were males. The results of our study show that most of the patients were male, and the mean age was consistent with the literature; however, it was below the average life expectancy found by the Turkish Statistical Institute.

In their study on comorbidity and survival in the OHCA cases, Hirlekar et al. found that heart failure (29%), myocardial infarction (24%), and diabetes (23%) were the most common chronic diseases. In the same study, patients with cardiac and respiratory failure and renal dysfunction who had an in-hospital arrest were found to have the lowest rate of survival (14, 15). A separate study examining the characteristics of patients in whom CPA occurred during the interventional radiological procedures found that most patients had diabetes, HT, and renal failure (16). Meanwhile, in our study, the most common chronic diseases in CPA cases were DM, CAD, and HT. According to these results, CPA is more likely to occur in the patients with comorbidities.

A study evaluating blood gas analysis in OHCA cases reported that patients for whom ROSC was obtained had high pH and low lactate and pCO_2 levels, and that patients with a pCO_2 level less than 75 mm Hg were 3.3 times more likely to have successful ROSC (17). Another study found a strong correlation between lactate along with pH parameters and patient mortality within the first five days (18). Dadeh et al. (19) found no significant relationship between the initial lactate levels and ROSC with neurological survival in the non-traumatic OHCA cases. In our study, we found no statistically significant relationship among pH, lactate, and pCO2 values in the patients for whom ROSC was obtained. We think that this result is due to the extended resuscitation attempts in the cases for whom ROSC was obtained in accordance with the literature guidelines. Therefore, we cannot regard it as a predictive value in terms of ROSC and one-month mortality.

In a study of patients with OHCA, 74.1% were administered with ETI, 24.2% were administered with supraglottic airway (SGA) devices, and the rest of them received both airway techniques. Additionally, the chest compression fraction with supraglottic devices was found to be higher as compared to the other methods (20). Another study conducted in Finland showed that the rate of ETI use was 67.3% in patients with OHCA, whereas the rate of supraglottic device use was 30.2% (21). A different study examining OHCA and airway methods found that 52.3% of the patients received pre-hospital ETI and 15% of them were administered with a pre-hospital SGA device. According to the results of this study, the comparison of ETI and SGA revealed that patients receiving ETI had higher rates of discharge with ROSC, survival, and good neurological outcomes (22). In our study, ETI was applied to all the IEDCA and most of the OHCA cases (66.4%). The comparison of the type of respiratory device used and ROSC found no statistically significant difference between them (p=0.419). Therefore, especially in the cases where pre-hospital airway interventions may interrupt chest compressions, priority should be given to effective chest compressions, and ventilation options that can be performed without interrupting chest compressions should be chosen.

A retrospective study examined the effect of epinephrine on neurological survival in about 200,000 witnessed OHCA cases whose initial rhythm was non-shockable rhythm. It was shown that epinephrine may increase the neurological survival in the cases where the epinephrine administration time is ≤19 minutes for patients brought to the hospital in ≥11 minutes (23). In a similar study, Hansen et al. (24) found that in the cases with an epinephrine administration time of less than 10 minutes, each minute's delay compromised survival and a positive neurological outcome. Many other similar studies have emphasized the importance of early epinephrine administration (25-27). In this study, the epinephrine administration time in the OHCA cases was longer than the time recommended in the literature (16.33±9.87 minutes). As the time for reaching the patient was within the acceptable limits, we attributed this delay to the difficulty of accessing the collapsed vascular structures outside of the hospital. We believe that such situations can be overcome by providing training and opportunities to the paramedics that promote alternative methods, such as intraosseous access. In epinephrine-administered OHCA cases, the low ROSC and one-month survival rates may have been due to the inability to administer epinephrine at an early stage in this study.

Fisk et al. (28) noted in their study that increasing the dose of epinephrine in OHCA cases did not had a significant impact on patient discharge and neurological outcomes. In a systematic review study, Lin et al. (29) mentioned that the advantage of epinephrine administered in a standard dose over placebo and high doses of epinephrine in patients with OHCA was still not clear, be it in terms of hospital discharge or good neurological survival, and that more studies were needed to determine the optimal dose. There are also studies suggesting that high-dose epinephrine is harmful and should not be administered (30). In this study, we found that increasing the dose of epinephrine did not contribute to the success of ROSC. Despite the large number of clinical studies, it is not clear within which regimen it should be administered to generate a positive impact on survival and good neurological outcomes.

A review study of the effects of epinephrine on cardiac arrest cases found that epinephrine contributed to ROSC but did not help with survival or good neurological outcomes (30). According to many other studies, epinephrine contributes to ROSC but delivers no benefits to survival and neurological re-

covery (31-34). On the contrary, some studies have shown that epinephrine decreases mortality, especially in patients with non-shockable rhythm (35, 36). The results of the PARAMEDIC 2 study showed that the ROSC and one-month survival rates of the patients in the epinephrine group were higher as compared to the placebo group. However, it was reported that this result may lead to more negative results (10). In this study, not administering epinephrine to patients with shockable rhythm and administering epinephrine to patients with non-shockable rhythm further increased the one-month mortality. While this situation can be explained by the small number of cases and the fact that most of the cases had non-shockable first rhythm, it may also have been caused by the delay in the administration of epinephrine.

An analysis of OHCA cases in Japan between 2007 and 2010 showed that the rate of ROSC was higher when epinephrine was administered to those whose first rhythm was non-shockable (18.5% vs. 5.7%) as well as in those with shockable first rhythm who were not administered epinephrine (28.1% vs. 21.6%, respectively). According to the same study, the one-month survival rate was found to be higher in the cases with shockable and non-shockable first rhythm who were not administered epinephrine (28.8% vs. 16.5% and 4.2% vs. 3.9%, respectively) (37). Another study conducted in Japan reported that the survival rates were higher in patients with shockable and non-shockable rhythms who were administered adrenaline (5.4% vs. 4.7%) (34). A study that analyzed OHCA cases showed that the ROSC and one-month survival rates of patients with shockable first rhythm who were not administered epinephrine were higher as compared to the patients administered with epinephrine (27.7% vs. 22.8% and 27% vs. 15.4%, respectively) (38). In this study, the results indicate that administering epinephrine to patients with shockable rhythm decreased the ROSC ratio, whereas withholding it increased the one-month mortality rates. On the contrary, administering epinephrine to patients with non-shockable rhythm increased the chances of ROSC, whereas the one-month mortality rate was higher in the patients with shockable rhythm who were administered with epinephrine.

CONCLUSION

According to the results of our study, ROSC was obtained for 44 (24%) patients, whereas a one-month survival was achieved for eight patients (1.4%). Although the administration of epinephrine to patients with shockable rhythm decreased the ROSC ratio, the administration of epinephrine to patients with non-shockable rhythm increased the chances of ROSC. There was no statistically significant relationship between the groups whether the first rhythm was shockable or not as well as one-month mortality, but one-month mortality was higher in patients with non-shockable rhythm who were administered with epinephrine. In conclusion, epinephrine administration had no significant effect on either ROSC or one-month mortality in the OHCA and IEDCA cases. A large number of placebo-controlled, prospective, randomized, and double-blind studies should be performed to demonstrate the efficacy of epinephrine, especially due to the small number of in-hospital arrest cases.

Study Limitations

The fact that staff providing emergency health care for pre-hospital OHCA cases administered adrenaline later than the time recommended in the guidelines may have limited the effects of the adrenaline. One of the most important reasons for this action is not having alternative vascular access (such as intraosseous insertion). The inequality in the distribution of in-hospital and out-of-hospital arrest cases was another factor served as the limitation of this study because of the insufficient number of cases.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: 25/07/2016; No:2016-217).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Cardiac Arrest Registry to Enhance Survival. "2017 Annual Report". Available at https://mycares.net/sitepages/uploads/2018/2017flipbook/index.html?page=16.Accessed December 18, 2018.
2. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. Circulation 2018; 137: e67-e492. [Crossref]
3. Nolan JP, Soar J, Smith GB, Gwinnutt C, Parrott F, Power S, et al. Incidence and outcome of in-hospital cardiac arrest in the United Kingdom National Cardiac Arrest Audit. Resuscitation 2014; 85: 987-92. [Crossref]
4. Schluep M, Gravesteijn BY, Stolker RJ, Endeman H, Hoeks SE, et at. One-year survival after in-hospital cardiac arrest: A systematic review and meta-analysis. Resuscitation 2018; 132: 90-100. [Crossref]
5. American Heart Association " 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care". Available at https://eccguidelines.heart.org/wp-content/uploads/2015/10/2015-AHA,Guidelines Highlights-English.pdf. Accessed December 18, 2018.
6. Soar J, Nolan JP, Böttiger BW, Perkins GD, Lott C, Carli P, et al. European resuscitation council guidelines for resuscitation 2015: section 3. Adult advanced life support. Resuscitation 2015; 95: 100-47. [Crossref]

7. Ristagno G, Tang W, Huang L, Fymat A, Chang YT, Sun S, et al. Epinephrine reduces cerebral perfusion during cardiopulmonary resuscitation. Crit Care Med 2009; 37: 1408-15. [Crossref]
8. Turkish Statistical Institute. "Life Table 2014-16". Available at http://www.tuik.gov.tr/PreHaberBultenleri.do?id=24640. Accessed October 23, 2018.
9. Turkish Statistical Institute. "Death Statistics, 2017 ". Available at http://www.tuik.gov.tr/PreHaberBultenleri.do?id=27620. Accessed October 23, 2018.
10. Perkins GD, Ji C, Deakin CD, Quinn T, Nolan JP, Scomparin C, et al. A randomized trial of epinephrine in out-of-hospital cardiac arrest. N Engl J Med 2018; 379: 711-21. [Crossref]
11. Koscik C, Pinawin A, McGovern H, Allen D, Media DE, Ferguson T, et al. Rapid epinephrine administration improves early outcomes in out-of-hospital cardiac arrest. Resuscitation 2013; 84: 915-20. [Crossref]
12. Boyce LW, Vlieland TV, Bosch J, Wolterbeek R, Volker G, Exel HJV, et al. High survival rate of 43% in out-of-hospital cardiac arrest patients in an optimised chain of survival. Neth Heart J 2015; 23: 20-5. [Crossref]
13. Oğuztürk H, Turtay MG, Tekin YK, et al. Cardiac Arrests in the Emergency Ward and Our Experiences on the Cardiopulmonary Resuscitation. Kafkas J Med Sci 2011; 1: 114-7. [Crossref]
14. Hirlekar G, Jonsson M, Karlsson T, Hollenberg J, Albertsson P, Herlitz J. Comorbidity and survival in out-of-hospital cardiac arrest. Resuscitation 2018; 133: 118-23. [Crossref]
15. Hirlekar G, Karlsson T, Aune S, Ravn-Fischer A, Albertsson P, Herlitz J, et al. Survival and neurological outcome in the elderly after in-hospital cardiac arrest. Resuscitation 2017; 118: 101-6. [Crossref]
16. Rueb GR, Brady WJ, Gilliland CA, Patrie JT, Saad WE, Sabri SS, et al. Characterizing cardiopulmonary arrest during interventional radiology procedures. J Vasc Interv Radiol 2013; 24: 1774-8. [Crossref]
17. Kim YJ, Lee YJ, Ryoo SK, Sohn CH, Ahn S, Seo DW, et al. Role of blood gas analysis during cardiopulmonary resuscitation in out-of-hospital cardiac arrest patients. Medicine 2016; 95: e3960. [Crossref]
18. Von Auenmueller KI, Christ M, Sasko BM, Trappe HJ. The value of arterial blood gas parameters for prediction of mortality in survivors of out-of-hospital cardiac arrest. J Emerg Trauma Shock 2017; 10: 134-9. [Crossref]
19. Dadeh AA, Nuanjaroan B. Using initial serum lactate level in the emergency department to predict the sustained return of spontaneous circulation in nontraumatic out-of-hospital cardiac arrest patients. Open Access Emerg Med 2018; 10: 105. [Crossref]
20. Kurz MC, Prince DK, Christenson J, Carlson J, Stub D, Cheskes S, et al. Association of advanced airway device with chest compression fraction during out-of-hospital cardiopulmonary arrest. Resuscitation 2016; 98: 35-40. [Crossref]
21. Hiltunen P, Jäntti H, Silfvast T, Kuisma M, Kurola J, FINNRESUSCI Prehospital study group. Airway management in out-of-hospital cardiac arrest in Finland: current practices and outcomes. Scand J Trauma Resusc Emerg Med 2016; 24: 49. [Crossref]
22. McMullan J, Gerecht R, Bonomo J, Robb R, McNally B, Donnely J, et al. Airway management and out-of-hospital cardiac arrest outcome in the CARES registry. Resuscitation 2014; 85: 617-22. [Crossref]
23. Funada A, Goto Y, Tada H, Shimojima M, Hayashi K, Kawashiri MA, et al. Effects of Prehospital Epinephrine Administration on Neurologically Intact Survival in Bystander-Witnessed Out-of-Hospital Cardiac Arrest Patients With Initial Non-Shockable Rhythm: Based on Emergency Medical Service Response Time. Heart Vessels 2017; 33: 1525-33. [Crossref]
24. Hansen M, Schmicker RH, Newgard CD, Gruanu B, Scheuermeyer F, Cheskes S, et al. Time to Epinephrine Administration and Survival From Nonshockable Out-of-Hospital Cardiac Arrest Among Children and Adults. Circulation 2018; 137: 2032-40. [Crossref]
25. Ueta H. Is early epinephrine administration still effective when the response time takes longer? Resuscitation 2017; 118: e44. [Crossref]
26. Hayashi Y, Iwami T, Kitamura T, Nishiuchi T, Kajino K, Sakai T, et al. Impact of early intravenous epinephrine administration on outcomes following out-of-hospital cardiac arrest. Circ J 2012; 76: 1639-45. [Crossref]
27. Khera R, Chan PS, Donnino M, Girotra S, American Heart Association's Get With The Guidelines-Resuscitation Investigators. Hospital Variation in Time to Epinephrine for Nonshockable In-Hospital Cardiac ArrestClinical Perspective. Circulation 2016; 134: 2105-14. [Crossref]
28. Fisk CA, Olsufka M, Yin L, McCoy AM, Latimer AJ, Maynard C, et al. Lower-dose epinephrine administration and out-of-hospital cardiac arrest outcomes. Resuscitation 2018; 124: 43-8. [Crossref]
29. Lin S, Callaway CW, Shah PS, Wagner JD, Beyene J, Ziegler CP, et al. Adrenaline for out-of-hospital cardiac arrest resuscitation: a systematic review and meta-analysis of randomized controlled trials. Resuscitation 2014; 85: 732-40. [Crossref]
30. Long B, Koyfman A. Emergency medicine myths: Epinephrine in cardiac arrest. The J Emerg Med 2017; 52: 809-14. [Crossref]
31. Shao H, Li CS. Epinephrine in Out-of-hospital Cardiac Arrest: Helpful or Harmful? Chin Med J (Engl) 2017; 130: 2112. [Crossref]
32. Jacobs IG, Finn JC, Jelinek GA, Oxer HF, Thompson PT. Effect of adrenaline on survival in out-of-hospital cardiac arrest: A randomised double-blind placebo-controlled trial. Resuscitation 2011; 82: 1138-43. [Crossref]
33. Olasveengen TM, Sunde K, Brunborg C, Thowsen J, Steen PA, Wik L. Intravenous drug administration during out-of-hospital cardiac arrest: a randomized trial. Jama 2009; 302: 2222-9. [Crossref]
34. Hagihara A, Hasegawa M, Abe T, Nagata T, Wakata Y, Miyazaki S. Prehospital epinephrine use and survival among patients with out-of-hospital cardiac arrest. Jama 2012; 307: 1161-8. [Crossref]
35. Nakahara S, Tomio J, Nishida M, Morimura N, Ichikawa M, Sakamoto T. Association between timing of epinephrine administration and intact neurologic survival following out-of-hospital cardiac arrest in Japan: a population-based prospective observational study. Acad Emerg Med 2012; 19: 782-92. [Crossref]

36. Tomio J, Nakahara S, Takahashi H, Ichikawa M, Nishida M, Morimura N, et al. Effectiveness of prehospital epinephrine administration in improving long-term outcomes of witnessed out-of-hospital cardiac arrest patients with initial non-shockable rhythms. Prehosp Emerg Care 2017; 21: 432-41. [Crossref]
37. Nakahara S, Tomio J, Takahashi H, Ichikawa M, Nishida M, Morimura N, et al. Evaluation of pre-hospital administration of adrenaline (epinephrine) by emergency medical services for patients with out of hospital cardiac arrest. BMJ 2013; 347: f6829. [Crossref]
38. Goto Y, Maeda T, Goto YN. Effects of prehospital epinephrine during out-of-hospital cardiac arrest with initial non-shockable rhythm: an observational cohort study. Crit Care 2013; 17: R188. [Crossref]

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Original Research

Herbal Drug BNO 1016 Versus Fluticasone Propionate Nasal Spray in the Treatment of Chronic Rhinosinusitis without Nasal Polyps: A Preliminary Report

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ABSTRACT

Objective: Current evidence supports the use of herbal drugs in the reduction of symptoms of acute (ARS) and chronic rhinosinusitis (CRS). Intranasal corticosteroids are the first line treatment option for treating CRS with or without nasal polyps. This study was designed to compare the safety and efficacy of plant medication BNO 1016 and fluticasone propionate nasal spray (FPNS) for treating CRS without nasal polyps (CRSsNP).

Methods: Forty subjects with CRSsNP were randomly divided into two treatment groups that comprised 20 patients each. The patients from Group 1 were treated with herbal drug BNO 1016, tablets of 160 mg, 3×1/d per os, for 28 d. The patients from Group 2 used FPNS 200 µg once daily, 2 puffs in each nostril in the morning for 28 d. We evaluated the nasal total symptom score (TSS), individual scores for each symptom (nasal congestion/obstruction, rhinorrhea/postnasal discharge, facial pain with the sense of pressure, headache, loss of the sense of smell), total endoscopic score (TES), and individual endoscopic signs (edema of the nasal mucosa, nasal secretion, and nasal crusting), before and after the therapy.

Results: TSS was lower on Day 7 ($p=0.008$), Day 14 ($p=0.004$), Day 21 ($p<0.001$), and Day 28 ($p=0.002$) in patients treated with BNO 1016. Moreover, the TES was lower on Day 21 ($p=0.001$) and Day 28 ($p=0.002$) in subjects who were on therapy with BNO 1016. No adverse events were noted in Group 1; however, in patients treated with intranasal glucocorticoids, 2 patients reported mild nasal bleeding, and 1 reported a sense of dryness in the nose.

Conclusion: BNO 1016 could be a good alternative to intranasal corticosteroids in the treatment of CRSsNP without adverse events.

Keywords: Glucocorticoids, ınflammation, plants, medicinal, rhinitis, sinusitis

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of the sinonasal mucosa, with complaints (nasal congestion/obstruction, rhinorrhea/postnasal discharge, facial pain with the sense of pressure, headache and loss of the sense of smell) that persist for >12 wk. In addition to the symptoms, diagnosis is also based on signs of mucosal edema with or without nasal polyps during a nasal endoscopy. Furthermore, computed tomography (CT) scan of the paranasal sinuses might show changes within the ostiomeatal complex or the sinuses (1, 2).

The etiology of CRS remains unknown. The mechanisms involved in the translation from acute rhinosinusitis (ARS) to CRS are debatable. Repeated viral, bacterial, and fungal infections; cigarette smoking; air pollution; allergic reactions of the nasal mucosa; neurogenic inflammation; as well as innate and adaptive immune dysfunction are the focus of discussion (3, 4).

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The prevalence of CRS in Europe is estimated to be 7%–27% (around 11%). This disease considerably affects the quality of life. The first option in the pharmacological treatment of CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) is the use of intranasal corticosteroids; other medications, such as macrolide antibiotics, nasal irrigation with sea water isotonic and hypertonic solutions, systemic glucocorticoids, antihistamines, and biological drugs are also used (1, 2, 5).

The efficacy of the herbal medicinal product BNO 1016 that is available as tablet, syrup, and drops, has been assessed in several studies for the treatment of acute upper-airway infections (6-9). However, two controlled studies have evaluated the efficacy of BNO 1016 in the treatment of CRS (10, 11). The main constituents are the extracts of the following five medicinal plants: gentian (*Gentiana lutea*, root); primrose (*Primula veris*, flower); common sorrel (*Rumex acetosa*, herb); elder (*Sambucus nigra*, flower); and European vervain (*Verbena officinalis*, herb). Previous reports have shown clear mucolytic, secretomotoric, anti-inflammatory, virostatic, and bacteriostatic effects of these extracts (6-12). We aimed to investigate the safety and efficacy of BNO 1016 in comparison to that of corticosteroid fluticasone propionate nasal spray (FPNS) in the treatment of CRSsNP. To the best of our knowledge, this is the first study to compare the effects of BNO 1016 and intranasal glucocorticoid in CRS patients without nasal polyps.

METHODS

Participants

Adult patients diagnosed with CRSsNP were eligible for this randomized, non-inferiority, open-label, parallel arm, prospective study. The study period was May 2019 to October 2019 as per the principles of the Helsinki Declaration. The Ethics Committee of the Military Medical Academy, Belgrade, Serbia approved the protocol for investigation (Approval No. 05/2019). Written informed consent was obtained from all the subjects who participated in the study.

Main Points:

- This is the first study designed to compare the effects of herbal drug BNO 1016 and intranasal corticosteroid in the therapy of patients with CRSsNP.
- Our results demonstrated better efficacy of BNO 1016 on nasal congestion/obstruction, facial pain with the sense of pressure, headache, and loss of the sense of smell in comparison to fluticasone propionate nasal spray (FPNS).
- The endoscopic findings in CRSsNP patients were superior after the therapy with BNO 1016.
- No adverse events were noted in patients treated by BNO 1016, however, in patients treated with intranasal corticosteroids, 2 patients reported mild nasal bleeding, and 1 reported a sense of dryness in the nasal cavity.
- BNO 1016 could be a good alternative to intranasal corticosteroids in the treatment of CRSsNP without adverse events.

CRS was diagnosed as per the criteria of the International Consensus Statement on Allergy & Rhinology: Rhinosinusitis (ICAR 2016) (1) and the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2012) (2). Only patients with nasal symptoms that had lasted for >12 wk, with specific endoscopic findings without features of nasal polyps and with findings on paranasal sinuses CT scans were enrolled. CT scan was performed for all the participants before the start of therapy as per the Lund-Mackay scoring system (13). We did not perform post-treatment CT scans owing to the limitations noted in the local Ethics Committee Approval. Patients with CRSwNP, bronchial asthma, and non-steroid anti-inflammatory drug-exacerbated respiratory disease (N-ERD) were excluded from the study by the experienced rhinologist, allergist, and pulmonologist as per clinical findings, prior history of reaction to non-steroid anti-inflammatory drugs, and pulmonary function results.

Other exclusion criteria were as follows: age <18 years, age > 65 years; ARS; presence of choanal polyps and hamartomatous lesions in the nasal cavities; deformations of the nasal septum and hypertrophy of the inferior and/or middle turbinate that significantly impaired the nasal airflow and the application of nasal sprays; systemic diseases that affected nasal function, such as Churg-Strauss syndrome, primary ciliary dyskinesia, and granulomatosis with polyangiitis; allergies to medications used in the study; use of antibiotics, antihistamines, and glucocorticoids in the form of drops, sprays, or oral tablets within 4 wk before study initiation; pregnancy or lactation; previous paranasal sinus surgery; and cigarette smoking.

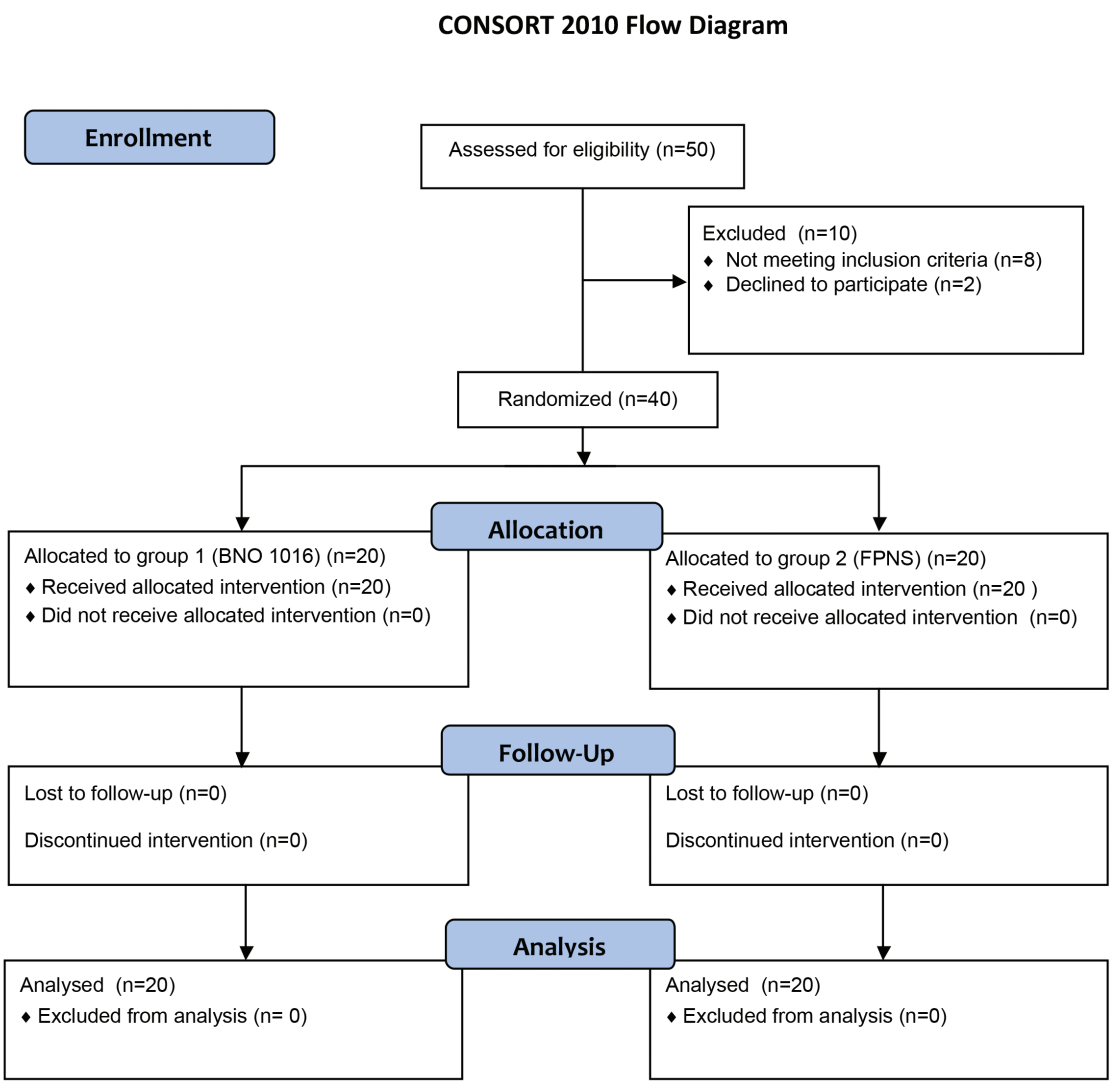
Treatment

We randomly divided CRSsNP patients into the following two study groups. Group 1 patients (n=20) were treated for 28 days with a herbal medicinal product BNO 1016 (Sinupret® forte, Bionorica, Neumarkt, Germany), oral tablets of 160 mg (Flixonase®), 3×1/day. The subjects from Group 2 (n=20) used FPNS (Glaxo Smith Kline Pharmaceuticals S.A., Burgos, Spain) 200 µg/day in the morning, two sprays in each nostril for 28 days, and these patients were informed about the correct application of INCS. Both, the investigators and the patients were aware of the drug being given. The patients did not use other medications simultaneously with herbal drug or intranasal corticosteroid.

Clinical Evaluation (Primary Outcomes)

The intensity of five nasal symptoms (nasal congestion/obstruction, rhinorrhea/postnasal discharge, facial pain with a sense of pressure, headache, and loss of the sense of smell) was evaluated by the patients on Day 0 and during Days 7, 14, 21, and 28 following treatment initiation. They used a visual analog scale (VAS) (0–10 cm; from 0=the absence of symptoms to 10=symptoms of maximal level). A 10-cm VAS was applied and explained for use in patients by the nurse following randomization. During the investigation, patients recorded their symptom scores and noted the use of medications on diary cards after taking the medications, and the investigator recorded the scores at the visits. The investigator evaluated treatment compliance based on the information in the diary cards.

Figure 1. Flow diagram, presented as per the CONSORT statement. Fifty patients (n=50) were involved in this study. Two (n=2) did not consent to participate, and eight (n=8) did not meet the inclusion criteria. Finally, 40 patients were recruited and assigned to Group 1 (n=20) and Group 2 (n=20)



An experienced rhinologist performed the nasal endoscopic examination at each visit using a 4 mm 0° and 30° endoscope (Karl Storz SE & Co., Tuttlingen, Germany) to assess the value of edema of the nasal mucosa, nasal secretion, and nasal crusting. Thereafter, the patients' local findings were scored as per the Likert endoscopic scoring system (14) as follows: 0, none; 1, mild; 2, moderate; 3, moderately severe; and 4, severe.

The parameters of clinical efficacy (main endpoints) were as follows: total symptom score (TSS; sum of the scores for nasal symptoms), individual symptom score (score for individual nasal symptom), total endoscopic score (TES; sum of the scores for all endoscopic signs – edema of the nasal mucosa, nasal secretion, nasal crusting), and individual endoscopic score for each endoscopic sign during Days 0, 7, 14, 21, and 28 following treatment initiation.

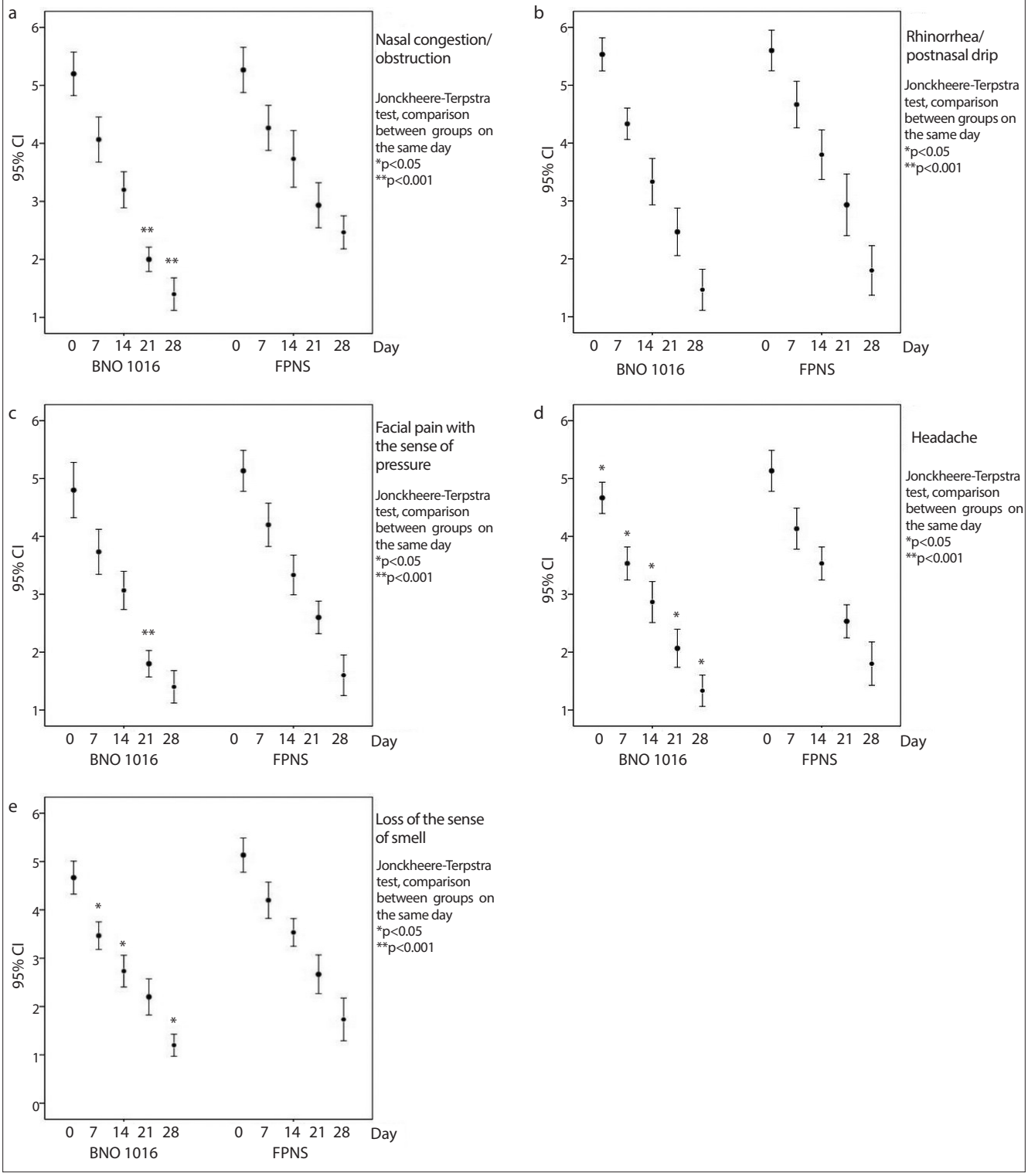
Safety

The reported adverse events were recorded throughout the study period, with severity grades classified as mild, moderate, and severe. At visits, nasal examination, laboratory tests, and vital signs assessment were performed. All the patients were aware of the potential adverse effects of both the medications.

Randomization

Randomization was performed as per the CONSORT statement. Fifty patients (n=50) with CRSsNP who were examined and treated at two hospitals were selected for the study. Two patients refused study participation, and eight did not meet the inclusion criteria. Thus, finally, 40 patients were included and divided into Group 1 (n=20) and Group 2 (n=20). The simple computer-generated procedure for participant randomization was used to allocate the patients into the study groups. The patients' eligibility was decided by

Figure 2. a-e. Comparison of the individual scores for nasal symptoms during treatment with two different medications: (a) Nasal congestion/obstruction; (b) Rhinorrhea/postnasal drip; (c) Facial pain with the sense of pressure; (d) Headache; and (e) Loss of the sense of smell. The levels of statistical significance: *p<0.05, **p<0.001

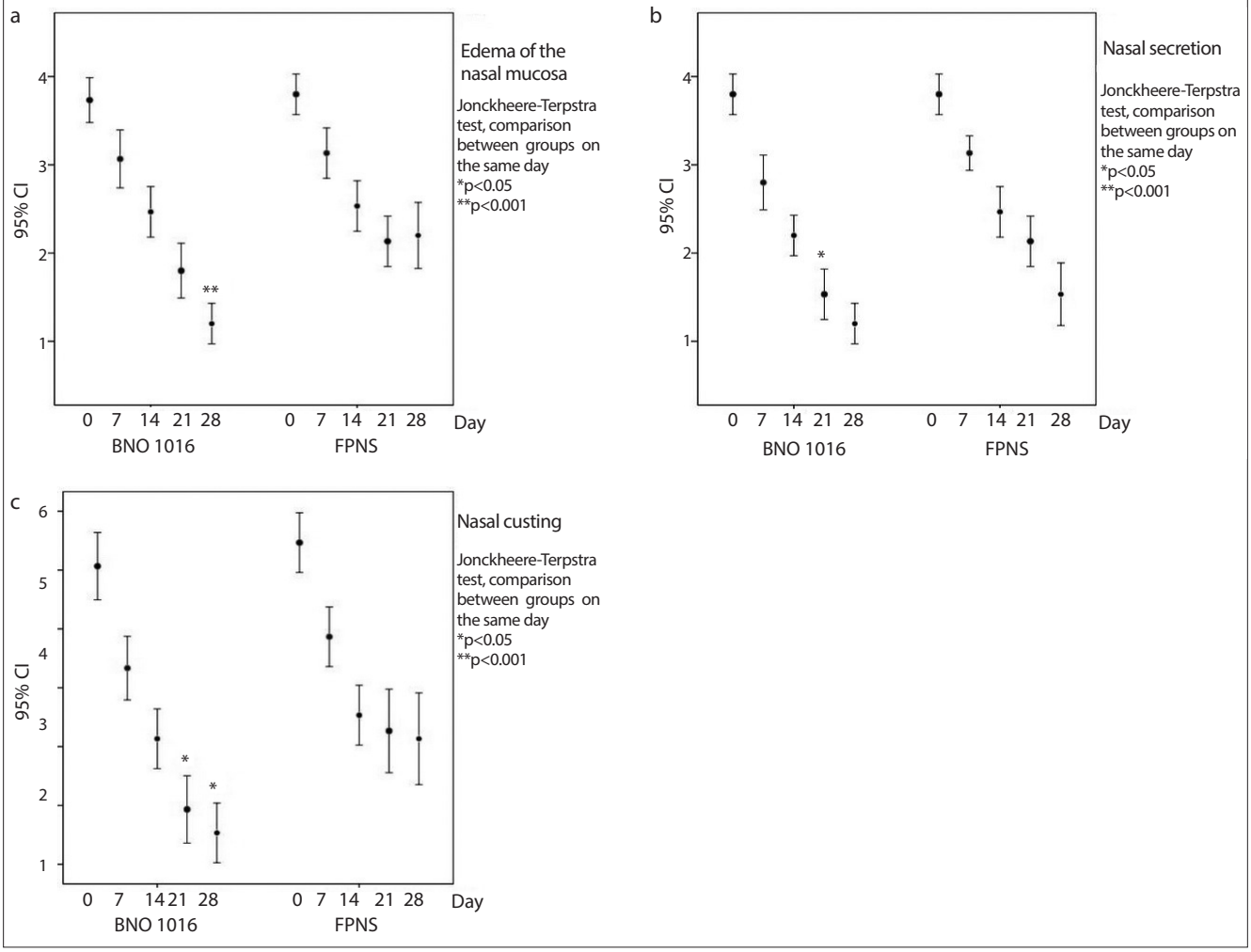


the investigator who then informed the nurse about the eligibility; thereafter, the nurse assigned the participants to either of the two study groups using computer-generated random allocation. The study profile has been presented in Figure 1.

Power of the Study and Statistical Analyses

A study power analysis indicated that 20 subjects would be required in each study group to reach a power level of at least 80% and a significance level of 5%. In our literature review,

Figure 3. a-c. Comparison of the individual scores for nasal endoscopic findings during treatment with two different medications: (a) Edema of the nasal mucosa; (b) Nasal secretion; and (c) Nasal crusting. The levels of statistical significance were as follows: *p<0.05, **p<0.001



we found no studies that have investigated the minimally import and difference in the symptom scores, assessed using the VAS for CRS patients; however, Devillier et al. (15) found that this value in patients with perennial allergic rhinitis could be rounded up to –1 unit for convenience. Therefore, the sample size was calculated as per the clinically relevant change for symptom scores and endoscopic scores of 1 point (IQR 1). The study parameters were expressed as median with interquartile range (IQR) values because the main variables were not distributed normally. We had several independent samples and assumed that they were arranged orderly; we used the nonparametric Jonckheere-Terpstra test for between-group comparisons of clinical median parameters on Days 0, 7, 14, 21, and 28 after treatment initiation. For paired comparisons within a group between the parameters of two successive visits (e.g. Day 0 *vs*. Day 7), for before-after effect and matched paired samples, we used the nonparametric Marginal Homogeneity test. We considered p-values <0.05 as statistically significant. For the statistical analysis, we used SPSS software (Statistical Package for the Social Sciences, version 15.0, (SPSS Inc.; Chicago, IL, USA).

RESULTS

Twenty patients who provided informed consent were enrolled in each group. There were no dropouts during the study period. Total 40 adult patients (23 men and 17 women, aged 25–61 y) who were diagnosed with CRSsNP were enrolled. We found no significant differences in the age and the pre-treatment Lund-Mackay CT score (p=0.587; p=0.482, respectively) (Table 1).

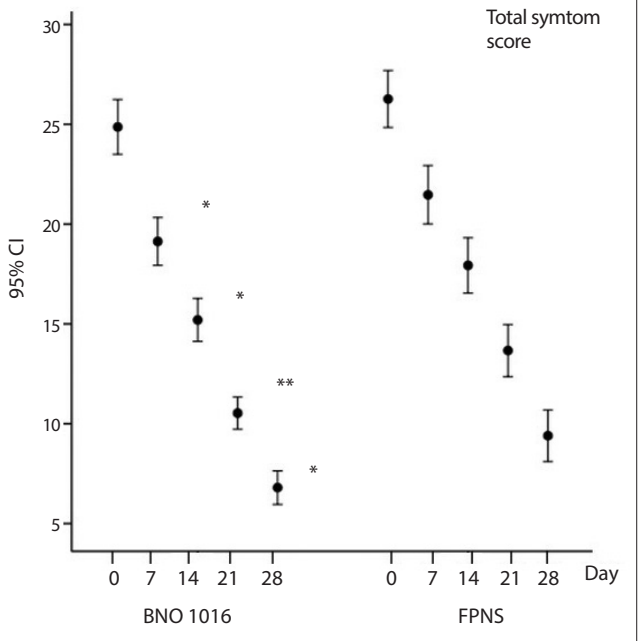
When we compared the individual symptom scores of the two groups, we found that Group 1 had significantly lower scores for nasal congestion/obstruction on Day 21 (p<0.001) and on Day 28 (p<0.001), significantly lower score for facial pain with the sense of pressure on Day 21 (p<0.001), lower scores for headache on Days 0, 7, 14, 21, and 28 (p=0.041; p=0.014; p=0.008; p=0.038; p=0.046, respectively), and lower scores for loss of the sense of smell during Day 7 (p=0.006), Day 14 (p=0.002), and Day 28 (p=0.039). The scores for rhinorrhea/postnasal discharge were not significantly different between the study groups from the start to the end of the investigation (Table 2, Figure 2 a-e).

Table 1. Baseline demographic characteristics and pre–treatment Lund–Mackay CT score of the study subjects

Characteristics	BNO 1016	FPNS	p
Patients	20		20
Age, years	44 (23)	46 (16)	0.587
Females	9 (45%)	8 (40%)	
Males	11 (55%)	12 (60%)	
Lund– Mackay CT score	11 (4)	9 (4)	0.482

Age: median (interquartile range – IQR)
Abbreviation: FPNS – fluticasone propionate nasal spray; CT – computed tomography

Figure 4. Comparison of total symptom score (TSS) during treatment with two different medications. Note the significantly lower TSS in Group 1 on Day 7 (p=0.009), Day 14 (p=0.003), Day 21 (p<0.001), and Day 28 (p<0.001)



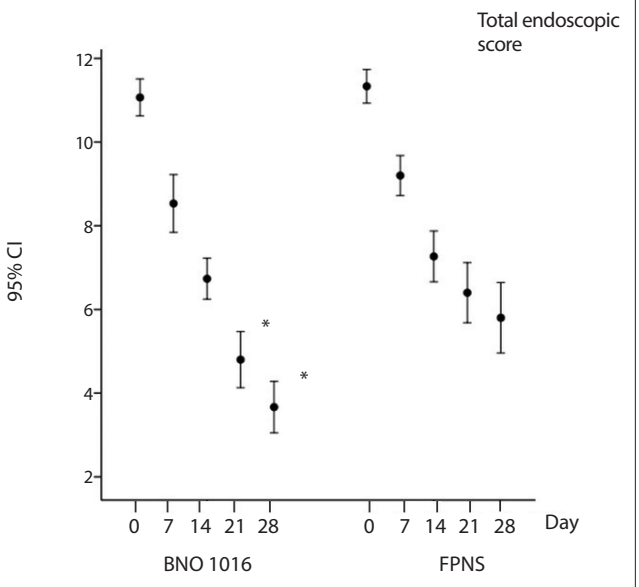
Jonckheere-Terpstra test, comparison between groups on the same day
*p<0.05 **p<0.001

Comparing the individual endoscopic scores, we found that Group 1 had a significantly lower score for edema of the nasal mucosa on Day 28 (p<0.001), lower score for nasal secretion on Day 21 (p=0.007), and lower scores for nasal crusting on Day 21 (p=0.008) and Day 28 (p=0.003) (Table 2, Figure 3a-c).

Finally, we found significantly lower TSS in Group 1 during Day 7 (p=0.008), Day 14 (p=0.004), Day 21 (p<0.001), and Day 28 (p=0.002) (Table 2, Figure 4). We found significantly lower TES in Group 1 on Day 21 (p=0.001) and Day 28 (p=0.002) (Table 2, Figure 5).

With respect to paired comparisons within a group between the parameters of two successive visits, we found significantly

Figure 5. Comparison of the total endoscopic score (TES) during treatment with two different medications. Note the significantly lower TES in Group 1 on Day 21 (p=0.002) and Day 28 (p=0.001)



Jonckheere-Terpstra test, comparison between groups on the same day
*p<0.05

decreased levels from Day 0 to Day 28 for individual symptom scores, individual endoscopic scores, TSS, and TES, except for nasal secretion and nasal crusting in Group 1 between the visits on Day 21 and Day 28 (p=0.061; p=0.087, respectively). Further, we found no significant differences in Group 2 for mucosal edema between the visits on Day 21 and Day 28 (p=0.618), for nasal crusting in the same group between the visits on the Day 14 and Day 21 (p=0.329), and between the visits on the Day 21 and Day 28 (p=0.583). All these results are presented in Table 3.

Group 1 patients exhibited no adverse events; however, Group 2 patients reported mild epistaxis, and one patient in Group 2 reported dryness in the nose.

DISCUSSION

To the best of our knowledge, only one study by Passali et al. has compared the efficacy and safety of BNO 1016 with intranasal corticosteroid in ARS treatment (16). The authors demonstrated better efficacy and safety of BNO 1016 in terms of the nasal symptoms and quality of life in comparison to that with fluticasone furoate nasal spray (16). Regarding the CRSsNP, only two randomized studies with BNO 1016 have been performed. The first randomized, placebo-controlled trial investigated 31 patients who had CRSsNP and were treated with BNO 1016 for 28 d (10). Of the 16 patients in the BNO 1016 group, 15 showed improvement in the radiological findings. However, of the 15 patients in the placebo group, only 6 showed improvement on paranasal sinus radiographies (10). In a recent, double blind, placebo-controlled study, Palm et al. (11) demonstrated that BNO 1016 can be recommended for the treatment of CRS patients for 3 mon with 3 times higher concentrations of drug constituents.

Table 2. Clinical parameters of the patients enrolled in this study. For between-group comparisons, we used a Jonckheere-Terpstra test

Parameter	BNO 1016 (N=20) Nr (%)/Median (IQR)	FPNS (N=20) Nr (%)/Median (IQR)	p
Nasal congestion/obstruction (Day 0)	5 (1)	5 (1)	0.782
Nasal congestion/obstruction (Day 7)	4 (1)	4 (1)	0.436
Nasal congestion/obstruction (Day 14)	3 (1)	4 (1)	0.072
Nasal congestion/obstruction (Day 21)	2 (0)	3 (1)	<0.001
Nasal congestion/obstruction (Day 28)	1 (1)	2 (1)	<0.001
Rhinorrhea/postnasal drip (Day 0)	6 (1)	6 (1)	0.564
Rhinorrhea/postnasal drip (Day 7)	4 (1)	5 (1)	0.113
Rhinorrhea/postnasal drip (Day 14)	3 (1)	4 (1)	0.057
Rhinorrhea/postnasal drip (Day 21)	2 (1)	3 (2)	0.151
Rhinorrhea/postnasal drip (Day 28)	1 (1)	2 (1)	0.226
Facial pain/sense of pressure (Day 0)	5 (1)	5 (1)	0.81
Facial pain/sense of pressure (Day 7)	4 (1)	4 (1)	0.083
Facial pain/sense of pressure (Day 14)	3 (0)	3 (1)	0.232
Facial pain/sense of pressure (Day 21)	2 (0)	3 (1)	<0.001
Facial pain/sense of pressure (Day 28)	1 (1)	2 (1)	0.388
Headache (Day 0)	5 (1)	5 (1)	0.041
Headache (Day 7)	4 (1)	4 (1)	0.014
Headache (Day 14)	3 (1)	4 (1)	0.008
Headache (Day 21)	2 (0)	3 (1)	0.038
Headache (Day 28)	1 (1)	2 (1)	0.046
Loss of the sense of smell (Day 0)	5 (1)	5 (1)	0.063
Loss of the sense of smell (Day 7)	3 (1)	4 (1)	0.006
Loss of the sense of smell (Day 14)	3 (1)	4 (1)	0.002
Loss of the sense of smell (Day 21)	2 (1)	3 (1)	0.121
Loss of the sense of smell (Day 28)	1 (0)	2 (1)	0.039
Edema of the nasal mucosa (Day 0)	4 (1)	4 (0)	0.682
Edema of the nasal mucosa (Day 7)	3 (0)	3 (0)	0.761
Edema of the nasal mucosa (Day 14)	2 (1)	3 (1)	0.731
Edema of the nasal mucosa (Day 21)	2 (1)	2 (0)	0.107
Edema of the nasal mucosa (Day 28)	1 (0)	2 (1)	<0.001
Nasal secretion (Day 0)	4 (0)	4 (0)	1.000
Nasal secretion (Day 7)	3 (1)	3 (0)	0.072
Nasal secretion (Day 14)	2 (0)	2 (0)	0.133
Nasal secretion (Day 21)	2 (1)	2 (0)	0.007

Table 2. Clinical parameters of the patients enrolled in this study. For between–group comparisons, we used a Jonckheere–Terpstra test (Continued)

Parameter	BNO 1016 (N=20) Nr (%)/Median (IQR)	FPNS (N=20) Nr (%)/Median (IQR)	p
Nasal secretion (Day 28)	1 (0)	1 (1)	0.112
Nasal crusting (Day 0)	4 (1)	4 (1)	0.264
Nasal crusting (Day 7)	3 (1)	3 (0)	0.139
Nasal crusting (Day 14)	2 (0)	2 (1)	0.248
Nasal crusting (Day 21)	1 (1)	2 (1)	0.008
Nasal crusting (Day 28)	1 (1)	2 (1)	0.003
Total symptom score (Day 0)	24 (4)	27 (2)	0.118
Total symptom score (Day 7)	18 (3)	23 (2)	0.008
Total symptom score (Day 14)	16 (2)	18 (4)	0.004
Total symptom score (Day 21)	10 (2)	14 (4)	<0.001
Total symptom score (Day 28)	6 (2)	9 (4)	0.002
Total endoscopic score (Day 0)	11 (1)	11 (1)	0.348
Total endoscopic score (Day 7)	8 (1)	9 (1)	0.112
Total endoscopic score (Day 14)	7 (1)	7 (2)	0.221
Total endoscopic score (Day 21)	5 (2)	6 (2)	0.001
Total endoscopic score (Day 28)	3 (1)	6 (2)	0.002

Abbreviations: IQR – interquartile range; FPNS – fluticasone propionate nasal spray

Our study showed that 1-month therapy with FPNS reduces the symptoms and local clinical signs in CRSsNP patients. We also demonstrated that treatment with herbal drug BNO 1016 leads to slightly more reduction in almost all symptoms and improved endoscopic findings. Thus, TSS is significantly lower after BNO 1016 treatment than after FPNS monotherapy on Days 7, 14, 21, and 28.

BNO 1016 is prepared using a mixture of the following five herbal extracts: gentian, primrose, common sorrel, elder, and European vervain. BNO 1016 is a drug with strong anti-inflammatory effects. In an experiment, the pleuritis was artificially induced in rats. The rats that were administered BNO 1016 extracts showed less pleural effusion and impaired neutrophil infiltration of the pleural tissue owing to the effects of polysaccharides and tannins from sorel and iridoids from vervain (17). BNO 1016 is shown to exert a strong virostatic effect against rhinoviruses, adenoviruses, respiratory syncytial virus, coxackie virus, influenza, and parainfluenza virus due to the inhibition of the enzyme neuraminidase, resulting in the inhibition of viral replication (6, 12). Therefore, BNO 1016 exerts antibacterial effects against Gram positive and Gram-negative bacteria (7). These anti-inflammatory and antimicrobial effects of BNO 1016 cause greater reduction in nasal symptoms and more improvement in the endoscopic findings compared to FPNS.

However, although significant improvement was observed in the rhinorrhea/postnasal discharge score for subjects in both the study groups from the start to the completion of treatment, there was no significant between-group difference in terms of the symptoms at all 4 time-points during the treatment period. This finding is in contrast to the lower endoscopically evaluated nasal secretion score in the BNO 1016 group. This interesting phenomenon could be attributed to the strong secretolytic and secretomotoric activity of BNO 1016. Dysfunction of mucociliary clearance is caused by chronic inflammation. Transport of the mucus that covers the respiratory epithelium is influenced by the transepithelial secretion of ions, especially chloride ions (Cl-). Cl- ion channels are dysfunctional in the respiratory epithelium of patients with ARS, CRS, and cystic fibrosis, and this disturbance in Cl- ion transport leads to impaired mucociliary clearance of pathogenic microorganisms and inflammatory products (18). Bioflavonoids, the main pharmacological component in BNO 1016, strongly activate transepithelial Cl- ion secretion, enhance Na+ ion and water molecule secretion, and increase ciliary beat frequency, resulting in hydration of nasal secretion and reduction of the viscosity of nasal fluid (18, 19). In contrast, intranasal corticosteroid application leads to decreased secretion in the nasal mucosal glands due to an anti-inflammatory effect (20). Thus, in patients treated with BNO 1016, accelerated nasal fluid clearance and decreased nasal secretion viscosity decreased the

Table 3. P–values (differences) for the comparison of clinical parameters in the same group of patients on consecutive visits during treatment with BNO 1016 (a) and fluticasone propionate nasal spray (b). For paired comparison within the group, we used a Marginal Homogeneity test

(a) Group 1 (BNO 1016)

Parameters/Day	Day 0 vs. Day 7	Day 7 vs. Day 14	Day 14 vs. Day 21	Day 21 vs. Day 28
Nasal congestion/obstruction	0.000	0.003	0.001	0.007
Rhinorrhea/postnasal discharge	0.000	0.001	0.003	0.001
Facial pain with the sense of pressure	0.000	0.002	0.000	0.014
Headache	0.000	0.002	0.005	0.002
Loss of the sense of smell	0.000	0.001	0.011	0.001
Edema of the nasal mucosa	0.002	0.003	0.004	0.003
Nasal secretion	0.000	0.003	0.004	0.061
Nasal crusting	0.000	0.003	0.007	0.087
Total symptom score	0.000	0.000	0.000	0.000
Total endoscopic score	0.000	0.000	0.001	0.003

(b) Group 2 (Fluticasone propionate nasal spray)

Parameters/Day	Day 0 vs. Day 7	Day 7 vs. Day 14	Day 14 vs. Day 21	Day 21 vs. Day 28
Nasal congestion/obstruction	0.000	0.005	0.001	0.008
Rhinorrhea/postnasal discharge	0.000	0.000	0.002	0.001
Facial pain with the sense of pressure	0.000	0.000	0.001	0.001
Headache	0.000	0.003	0.001	0.001
Loss of the sense of smell	0.000	0.002	0.003	0.001
Edema of the nasal mucosa	0.002	0.003	0.034	0.618
Nasal secretion	0.002	0.002	0.025	0.007
Nasal crusting	0.001	0.004	0.329	0.583
Total symptom score	0.001	0.000	0.000	0.000
Total endoscopic score	0.000	0.001	0.007	0.029

sense of rhinorrhea/postnasal discharge that is of similar intensity to the sense of nasal discharge in patients treated with FPNS.

There were no adverse effects in patients from the BNO 1016 group as compared to that in two subjects of the FPNS group who reported mild epistaxis and sensation of dryness in the nose. The use of 200 μg of FPNS daily in the morning during the 1-month therapy may theoretically cause the formation of small areas of atrophy in the nasal epithelium and mild nasal bleeding. The results of an experimental animal study that was conducted by Cho et al. (21) in a rabbit model of CRS showed that dry extracts from BNO 1011 suppress the atrophic changes of the ciliated epithelium and improve the histological characteristics of the lamina propria. This could explain the protective role of BNO 1016 in the human nasal mucosa.

The present study has certain limitations, such as a relatively small sample size. Further, we did not include the peak nasal inspiratory flow or other objective measurements of nasal patency. These parameters can increase the quality of results because the symptom scores are dependent on the subjective sensation of the patients. Although our study employed a prospective and randomized design, it was an open-label study wherein both, the researchers and participants were aware of which treatment was being administered. Thus, there is a need to further align herbal medicine with the requirements of evidence-based medicine, especially by organizing double blind, placebo-controlled studies that could provide better evidence of the efficacy of BNO 1016.

CONCLUSION

Our results demonstrated better efficacy of BNO 1016 on nasal congestion/obstruction, facial pain with the sense of pressure, headache, and loss of the sense of smell. The endoscopic findings in CRSsNP patients were superior after BNO 1016 treatment than after FPNS treatment. The absence of adverse events sug-

gests better safety of BNO 1016 treatment as compared to that of nasal corticosteroid monotherapy in CRSsNP patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Military Medical Academy, Belgrade, Serbia (Approval No. 05/2019).

Informed Consent: Written informed consent was obtained from the patient.

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REFERENCES

1. Orlandi RR, Kingdom TT, Hwang PH, Smith TL, Alt JA, Baroody FM, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. Int Forum Allergy Rhinol 2016; 6: S1-213. [Crossref]
2. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. A summary for otorhinolaryngologists. Rhinology 2012; 50: 1-12. [Crossref]
3. Prokopakis EP, Vlastos IM, Ferguson BJ, Scadding G, Kawauchi H, Georgalas C, et al. SCUAD and chronic rhinosinusitis. Reinforcing hypothesis driven research in difficult cases. Rhinology 2014; 52: 3-8. [Crossref]
4. Smith KA, Orlandi RR, Rudmik L. Cost of adult chronic rhinosinusitis: A systematic review. Laryngoscope 2015; 125: 1547-56. [Crossref]
5. Günel C, Başak HS, Bleier BS. Oral steroids and intraoperative bleeding during endoscopic sinus surgery. B-ENT 2015; 11: 123-8.
6. Jund R, Mondigler M, Stammer H, Stierna P, Bachert C. Herbal drug BNO 1016 is safe and effective in the treatment of acute viral rhinosinusitis. Acta Otolaryngol 2015; 135: 42-50. [Crossref]
7. Passali D, Cambi J, Passali FM, Bellussi LM. Phytoneering: a new way of therapy for rhinosinusitis. Acta Otorhinolaryngol Ital 2015; 35: 1-8.
8. Yaremchuk S, Zabolotny D, Vareniuk I, Makarchuk N, Veselsky S. Sinupret® oral drops protect against respiratory epithelium atrophy in experimental acute rhinitis. Clinical Phytoscience 2015; 1: 8. [Crossref]
9. Popovich VI, Koshel IV. Sinupret® as add-on therapy to saline irrigation for children with acute post-viral rhinosinusitis. Clinical Phytoscience 2017; 3: 10. [Crossref]
10. Richstein A, Mann W. Treatment of chronic rhinosinusitis with Sinupret. Schweiz Zschr Ganzheits Medizin 1999; 11: 280-3.
11. Palm J, Steiner I, Abramov-Sommariva D, Ammendola A, Mitzenheim S, Steindl H, et al. Assessment of efficacy and safety of the herbal medicinal product BNO 1016 in chronic rhinosinusitis. Rhinology 2017; 55: 142-51. [Crossref]
12. Glatthaar-Saalmüller B, Rauchhaus U, Rode S, Haunschild J, Saalmüller A. Antiviral activity in vitro of two preperations of the herbal medicinal product Sinupret® against viruses causing respiratory infections. Phytomedicine 2011; 19: 1-7. [Crossref]
13. Lund VJ, Mackay IS. Staging in rhinosinusitis. Rhinology 1993; 31: 183-4.
14. Pfaar O, Mullol J, Anders C, Hörmann K, Klimek L. Cyclamen europaeum nasal spray, a novel phytotherapeutic product for the management of acute rhinosinusitis: a randomized double-blind, placebo-controlled trial. Rhinology 2012; 50: 37-44.
15. Devillier P, Brüning H, Bergmann K-Christian. Determination of the minimally important difference in a nasal symptom score in house dust mite allergy. Allergy 2019; 74: 2191-8. [Crossref]
16. Passali D, Loglisci M, Passali GC, Cassano P, Rodriguez HA, Bellussi LM. A prospective open-label study to assess the efficacy and safety of a herbal medicinal product (Sinupret) in patients with acute rhinosinusitis. ORL J Otorhinolaryngol Relat Spec 2015; 77: 27-32. [Crossref]
17. Rossi A, Dehm F, Kiesselbach C, Haunschild J, Sautebin L, Werz O. The novel Sinupret® dry extract exhibits anti-inflammatory effectiveness in vivo. Fitoterapia 2012; 83: 715-20. [Crossref]
18. Virgin F, Zhang S, Schuster D, Azbell C, Fortenberry J, Sorscher EJ, et al. The bioflavonoid compound, Sinupret, stimulates transepithelial chloride transport in vitro and in vivo. Laryngoscope 2010; 120: 1051-6. [Crossref]
19. Kreindler JL, Chen B, Kreitman Y, Kofonow J, Adams KM, Cohen NA. The novel drug extract BNO 1011 stimulates chloride transport and ciliary beat frequency in human respiratory epithelial cultures. Am J Rhinol Allergy 2012; 26: 439-43. [Crossref]
20. Akpinar ME, Yigit O, Akakin D, Sarioz O, Ozkan N, Yildiz SD, et al. Topical glucocorticoid reduces the topical decongestant-induced histologic changes in an animal model nasal mucosa. Laryngoscope 2012; 122: 741-6. [Crossref]
21. Cho DY, Skinner D, Mackey C, Lampkin HB, Elder JB, Lim DJ, et al. Herbal dry extract BNO 1011 improves clinical and mucociliary parameters in a rabbit model of chronic rhinosinusitis. Int Forum Allergy Rhinol 2019; 9: 629-37. [Crossref]

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Original Research

Radioprotective Effects of Propolis and Caffeic acid phenethyl ester on the Tongue-Tissues of Total-Head Irradiated Rats

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ABSTRACT

Objective: This study aimed to investigate the radioprotective effects of propolis and caffeic acid phenethyl ester (CAPE) against radiation-induced damage in the tongue-tissues of rats exposed to total cranial gamma irradiation.

Methods: Fifty-four Sprague-Dawley rats were randomly divided into six groups. An appropriate control group was also studied. The animals were euthanized on day 10, and tongue-tissues were collected for evaluating biochemical oxidative parameters.

Results: Lipid hydroperoxides (LOOH), total oxidant status (TOS), oxidative stress index (OSI) levels and xanthine oxidase (XO) activity, and markers of oxidative stress were significantly higher, while paraoxonase (PON) activity was significantly lower for irradiated rats (IR) group compared to the other groups. When LOOH, TOS, OSI values, and XO activity in the propolis+IR and CAPE+IR groups were evaluated, there was no statistically significant difference between those ones and all control groups. In terms of the total -SH levels, the propolis+IR group was found to be significantly higher than all other groups. There was no significant difference in arylesterase (ARE) activity, ceruloplasmin (Cp) levels.

Conclusion: Propolis and CAPE reduce oxidative stress and have antioxidant effects that may be useful agents of ionizing radiation-induced tissue damage

Keywords: Antioxidants, caffeic acid phenethyl ester, irradiation, oxidative stress, propolis

INTRODUCTION

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals, which induce oxidative/nitrosative stress, play a role in the pathogenesis of many diseases (1-4). Studies have shown that head and neck cancer (HNC) is the sixth most common malignancy in the world, with an annual worldwide incidence of over 600,000 cases and 350,000 deaths per year (5, 6). HNCs include cancers of the buccal cavity, head and neck subset, larynx, pharynx, thyroid, salivary glands, and nose/nasal passages (6).

Radiotherapy is an indispensable modality method in cancer therapy, and approximately 2/3 of the patients are on radiotherapy. When the total dose required for effective local control with radiotherapy is exceeded, damage occurs in normal tissues in the field of irradiation. The resulting damage is also closely associated with the sensitivity to radiation. It is known that ionizing radiation forms free radicals (1, 6).

Radiotherapy-induced free radicals cause DNA breaks, and hence cell death, as well as inviting many diseases. An approach to reduce radiation-induced side effects is the systematic use of protective substances that protect normal tissues against radiation, but do not adversely affect the tumor such as amifostine and N-acetyl cysteine. Thus, early and late complications that could occur when the tumor is given a higher dose of radiation may be prevented (1, 7).

Propolis and an active component of its extract, caffeic acid phenethyl ester (CAPE), have been shown to have immunomodulatory, anti-humoral, cytotoxic, anti-metastatic, anti-inflammatory, and antioxidant properties. They inhibit lipoxygenase activities and suppress lipid peroxidation (7, 8).

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Efforts to diminish the toxicity of irradiation to normal cells, tissues, and organs have caused investigations to identify cytoprotective agents. Unfortunately, most of these radioprotectors possess toxic side effects limiting their role in medical treatment. Therefore, investigations for effective and non-toxic compounds with radioprotective capabilities caused increasing interest in naturally-occurring antioxidants such as *Nigella sativa*, CAPE, and propolis.

In this study, we focus on the protective effects of propolis and CAPE on oxidative stress due to radiation in rat lingual tissue by examining total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

It also investigates the radical scavenger activities involved in the radioprotective activities of these naturally-occurring substances and their possible mechanisms for the effects of lipid hydroperoxides (LOOH), xanthine oxidase (XO) activity, oxidant enzyme, and antioxidant systems, which contain enzymes such as paraoxonase (PON) and arylesterase (ARE), including low molecular weight free radical scavengers such as total sulfhydryl (-SH) groups and proteins such as ceruloplasmin (Cp) having antioxidant properties.

METHODS

Study Protocol, Rats, and Experimental Groups
In this study, 54 male Sprague-Dawley rats weighing 200±20 grams were used. A total of 6 groups were formed in the study, and there were 8 rats in the control groups and 10 rats in the other groups.

Group information was as follows
Sham control group (n=8): no propolis, no CAPE but sham irradiation.

The control group of propolis+irradiation (IR) (n=8): Only 1 ml of saline was given by an orogastric tube.

The control group of CAPE+IR (n=8): The amount of dimethyl sulfoxide (DMSO) used to dissolve CAPE was given intraperitoneally (IP) to this group.

IR group (n=10): received only IR of 5 Gy.

Propolis+IR group (n=10): received IR (5 Gy) plus propolis (80 mg $kg^{-1}day^{-1}$, by an orogastric tube, one hour before IR).

CAPE+IR group (n=10): received IR (5 Gy) plus CAPE (10 µmol $kg^{-1}day^{-1}$, IP, 30 min before IR). CAPE was dissolved in DSMO just before giving it to the rats.

Main Points:

- Ionizing radiation causes oxidative stress.
- Oxidative stress plays a role in the pathogenesis of many diseases.
- In the tongue-tissue, Propolis and Caffeic acid phenethyl ester significantly prevented oxidative stress caused by ionizing radiation.

This study was conducted at the Department of Medical Biochemistry after obtaining ethical approval from the Animal Ethics Committee of Gaziantep University School of Medicine (ethical committee number: 2017/2).

Biochemical Analysis
Except for Wistar-Albino male rats in the control groups, those in the other group were anesthetized with 50 mg/kg/ip ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) and the rats were placed in the prone position on the radiotherapy device. Irradiation of these rats was performed with a single dose of 5 Gy to the head area with a Co 60 teletherapy device and an area of 25 cm^2 with an SSD of 80 cm. The rats in the control groups were given a certain volume of isotonic serum. At the end of the experiment, the rats were first anesthetized with ketamine 50 mg/kg/ip. The rats were killed by decapitation. The brain tissue was homogenized with a phosphate buffer, and the supernatant was collected in 5 ependorf tubes and stored at –80°C until biochemical tests were performed. Biochemical analyses were performed by spectrophotometric methods. Group information was as follows.

All rats that received 80 mg/kg ketamine HCl (Pfizer Pharmaceuticals, Istanbul, Turkey) were anesthetized and placed in a tray in the prone position. The rats in the IR, propolis plus IR, and the IR plus TQ groups underwent a single dose of 5Gy irradiation using a Cobalt-60 teletherapy unit (Picker, C9, Maryland, NY, USA) from a source-to-surface distance of 80 cm by 5×5 cm anterior fields covering the total, while the rats in the control and sham control groups received sham irradiation. The irradiation rate was 0.49 Gy/min. The central axis dose was calculated at a depth of 0.5 cm.

Biochemical Analysis
All rats were anesthetized with ketamine hydrochloride 50 mg/kg ip on day 11. Then, all animals were killed by decapitation, and their tongue-tissues were taken. The tongue-tissues were homogenized in physiological saline solution (IKA-NERKE, GmBH & CO. KB D-79219, Staufen, Germany). The supernatant obtained after homogenization was collected using eppendorf tubes and stored at –80°C until biochemical analysis.

TAS, TOS, OSI, Cp, thiol-disulfide, and total SH levels were measured as previously described (9, 10). PON and ARE activities were measured with commercially-available kits (Rel Assay Diagnostics, Gaziantep, Turkey). LOOH levels were measured with the ferrous ion oxidation-xylenol orange method, as previously described (10). XO activity was measured spectrophotometrically with uric acid formation from xanthine with an increase in absorbance at 293 nm as previously described (1), which expressed as U/mg protein. The protein content was measured as described (11). All parameters were performed using a spectrophotometer (Shimadu U 1601, Japan).

Statistical Analysis
Analysis of the data obtained from the study was performed with Statistical Package for the Social Sciences for Windows version 11.5 (SPSS Inc.; Chicago, IL, USA). The Kolmogorov Smirnov test was used to test whether or not the data were normally distributed. The significance of the differences between the groups

Table 1. Antioxidant parameters assigned in the tongue–tissues of the rats

	Sham control group	The control group of propolis+IR	The control group of CAPE+IR	IR group	Propolis+IR group	CAPE+IR group
TAS (mmol Trolox Eq./g protein)	0.23±0.027	0.21±0.028[a]	0.22±.012[a]	0.21±0.013[a]	0.25±0.03	0.22±0.018[a]
Total–SH (µmol/g protein)	0.436±0.002[a]	0.445±0.0047[a]	0.455±0.003[a]	0.488±0.006[a]	0.560±0.009	0.460±0.007[a]
ARE (U/g protein)	8.76 1.0	8.31±0.99	8.6±0.45	8.14±0.74	8.49±0.84	8.77±0.69
Cp (mg/g protein)	106.±9.84	96.6±8.45	94.4±9.75	92.94±30.94	96.51±10.1	100.2±7.52

[a]: p<0.01vs. Propolis+IR group,

Figure 1. Mean± Standard deviation (SD) of xanthine oxidase (XO) activity assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR

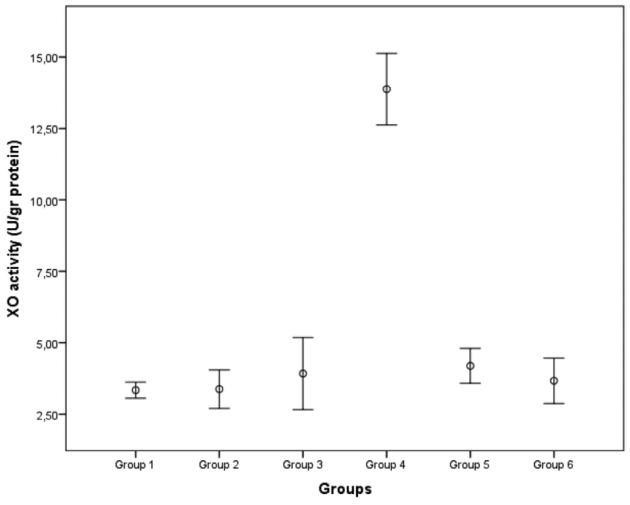
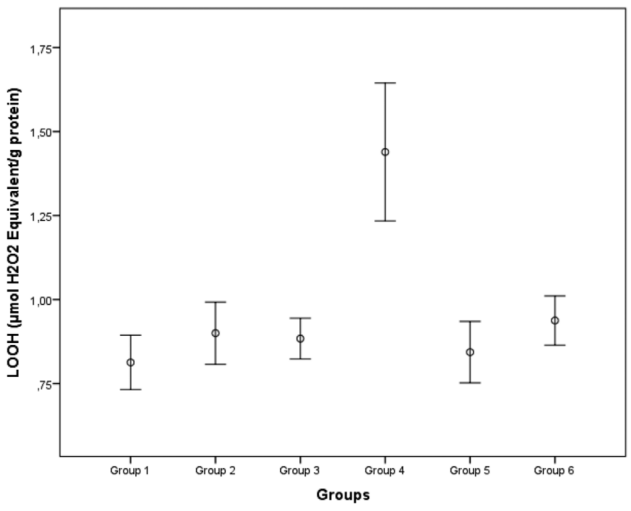


Figure 2. Mean±SD of lipid hydroperoxides (LOOH) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR



was analyzed using post-hoc, the lowest significant difference test procedure. Pearson correlation analysis was used to test the correlation between variables. Quantitative variables were expressed as mean±standard deviation, while qualitative variables were expressed as frequencies and percentages. P values <0.05 were considered statistically significant.

RESULTS

Antioxidant Parameters

As seen in Table 1, TAS values in the propolis+R group were significantly higher than those of all other groups, except for the sham control group. The total -SH levels of the propolis+IR group were found to be significantly higher than those of all other groups (p< 0.01). On the other hand, no statistically significant difference in Cp levels and ARE activity was found between the propolis+IR group and all groups.

Oxidant Parameters

As seen in Figures 1–4, there were statistically significant differences in certain parameters between the IR group and other groups. XO activity, LOOH, TOS, and OSI levels (p< 0.0001 for all parameters) were significantly higher, while PON activity was significantly lower for the IR group than in the other groups. When LOOH, TOS, OSI levels, and XO activity in the propolis+IR and CAPE+IR groups were evaluated, there were no statistically significant differences between those ones and the values for all control groups. This demonstrates that it reverses oxidative stress in irradiated rats given propolis and CAPE, and that these natural substances protect rats from ionizing radiation. In terms of XO activity, this protection appears to be even more evident.

DISCUSSION

Radiotherapy (RT) is a commonly-used therapeutic method in the precise and palliative treatment of cancer. The effects of RT are mediated by the production of free radicals, which react with the unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids. ROS, RNS, and other free radicals produced in biological systems are the major causes of oxidative/nitrosative stress (12, 13).

They can also mediate the activation of carcinogens to electrophilic, DNA-damaging components. For example, peroxyl

Figure 3. Mean±SD of total oxidant status (TOS) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR

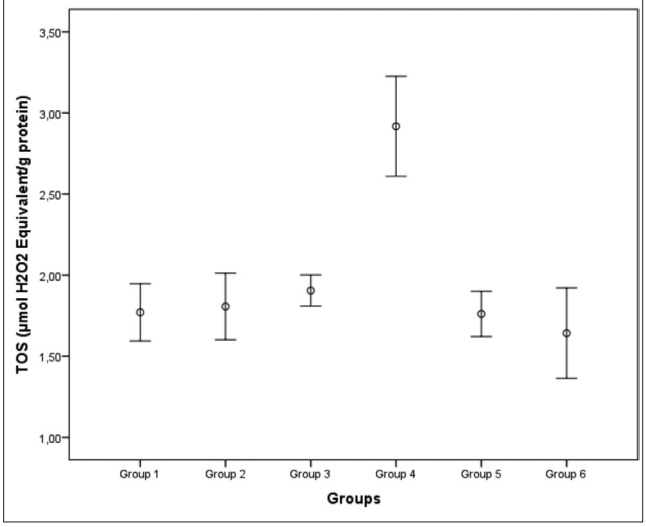
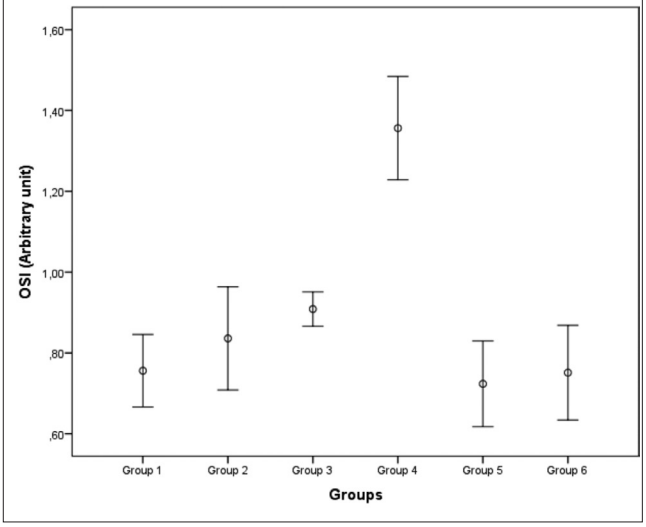


Figure 4. Mean±SD of oxidative stress index (OSI) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR



radicals may have an indirect role in carcinogens as mediators of oxidation related to hydroperoxide in carcinogens. Patients undergoing radiotherapy suffer serious side effects during and after treatment. When the total dose required for effective local control with radiotherapy is exceeded, the normal tissues in the irradiated area may be damaged. The damage that occurs in normal healthy tissues in this way varies with the sensitivity of the affected tissue to radiation. For this reason, it is essential to know the effects of radiation on many biological systems and organs. Investigation of the acute and long-term effects of ionizing radiation on tissues and cells are among the important topics in radiotherapy (7, 14). Many experimental studies have stated that the radicals formed caused by ionizing radiation prepare the ground for the formation of other ROS and RNS, which play a role in the pathophysiology of many diseases and cause cell death due to DNA breaks. Numerous studies have been carried out to prevent early and late complications, which may occur due to ionizing radiation. For this objective, the researchers have focused on bio-protectors that decrease or inhibit the level of the damage caused by ionizing radiation (1, 6, 7, 13).

In our previous study, we found a significant increase in XO activity in different tissues of irradiated rats when compared to other groups. In the current study, when the comparison is made in terms of oxidative stress markers, XO activity, LOOH, TOS, and OSI levels were significantly higher in the IR group compared to the other groups.

Lipid peroxidation is the primary cellular damage caused by free radical reactions. High lipid peroxidation is responsible for the formation of lipid hydroperoxides. Levels of LOOH, TOS, and OSI, markers of oxidative stress, were similar to those reported in previous studies (15, 16).

Interestingly, XO activity in these groups, supplemented with propolis and CAPE, was significantly lower than in these tissues of irradiated rats. Therefore, in rats exposed to irradiated rats only (IR group), there was a significant increase in XO activity, the oxidant enzyme. The findings of the present study are consistent with previous studies reporting the antioxidant effects of these natural substances. Also, the finding that these antioxidants provide protective effects against radiation-induced oxidative stress is consistent with previous reports of their radioprotective effects (7).

The paraoxonases (PON1, PON2, and PON3) are the protein products of a gene family that evolved via the duplication of a common precursor and have high structural homology with each other. PON1 and PON3 synthesized in the liver are both associated with high-density lipoprotein (HDL) particles and exhibit antioxidant and anti-inflammatory properties that hydrolyze lipid peroxides in low-density lipoproteins and HDL. PON2 and PON3 are intracellular enzymes modulating mitochondrial superoxide radical production and endoplasmic reticulum stress-induced apoptosis (17, 18). However, there has been an increasing interest in the biological function of PONs in human cancers. Changes of PON status encompassing genotype, activity and/or expression have been reported in patients affected by cancer, as well as in various cancer cells in vitro. Cells undergo neoplastic transformation through a number of different events in which the cell death program is regulated, and apoptosis resistance is impaired. There is evidence supporting the role of PON2 and PON3 enzymes in cancer cell survival, which can be ascribed to the antioxidant and anti-apoptotic activities of these cells. A role in cancer cell chemotherapeutic resistance and survival has also been ascribed to PON2 and PON3 (17). Previous reductions in PON activity have been reported in experimental and human studies (19, 20). The current study found that the PON activity in the tongue-tissue

of irradiated rats was statistically significantly lower than those when compared to all the other groups. Our results showed that PON activity was reduced only in irradiated rats and that propolis and CAPE administration reversed the decrease in PON activity in irradiated rats.

LOOH is a well-known marker of oxidative stress caused by glycolipid, cholesterol, and unsaturated phospholipids by peroxidative reactions under oxidative stress (21). The levels of LOOH have also been found to be increased in irradiated rats, and propolis and CAPE applications reversed an increase in LOOH levels in irradiated rats. Lipid hydroperoxides in studies done have been reported to inhibit PON activity, and researches with pomegranate juice have shown that it can maintain PON activity during lipoprotein oxidation. Also, for preservation, these studies have shown that pomegranate juice can increase PON activity (22). In this study, the reduction of PON activity and the consideration of high LOOH levels in irradiated rats can help to understand the underlying mechanisms of oxidative stress elevation caused by irradiation. The current study correlates prevention of the decrease in PON activity with protection of lipid peroxidation, in terms of reduced LOOH levels, by the administration of propolis and CAPE. The healing found in irradiated rats treated with these natural substances may provide an explanation of how these inhibitory effects occur.

One of the major limitations of this study is the lack of histological evaluation. Although biochemical analyzes suggest that propolis and CAPE exhibit radioprotective effects against oxidative damage in the tongue-tissue of irradiated rats, it may be reasonable to support these data with histological evaluations. Also, radio protectants are ideally expected to have selectivity for normal tissues, but not for tumor tissues from the effect of radiotherapy. However, this study does not provide any data for such a comparison with propolis and CAPE. This is another limitation of our work.

To our knowledge, this is the first study that simultaneously investigates the radioprotective effects of propolis and CAPE on the oxidative stress in the tongue-tissues of irradiated rats. The results obtained in the study suggest that propolis and CAPE exhibit radioprotective effects against oxidative stress in the tongue-tissues of irradiated rats. It is now apparent that the future approach to treating irradiation-associated complications can consider the use of propolis and CAPE having multi-pharmacological activities. ROS have been implicated in many disease processes, including aging and carcinogenesis, and have been associated with a variety of complications resulting from the treatment of cancer.

CONCLUSION

The treatment of free radical-induced diseases with antioxidants or free radical scavengers has become an important therapeutic method. Since free radicals are the main mediators of radiation-induced damage, a treatment that combines radiation with an antioxidant may provide a strategy to prevent radiation damage to normal tissues.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University School of Medicine (number: 2017/2).

Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

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REFERENCES

1. Akyuz M, Taysi S, Baysal E, Demir E, Alkis H, Akan M, et al. Radioprotective effect of thymoquinone on salivary gland of rats exposed to total cranial irradiation. Head Neck 2017; 39: 2027-35. [Crossref]
2. Taysi S, Tascan AS, Ugur MG, Demir M. Radicals, Oxidative/Nitrosative Stress and Preeclampsia. Mini-Rev Med Chem 2019; 19: 178-93. [Crossref]
3. Cikman O, Soylemez O, Ozkan OF, Kiraz HA, Sayar I, Ademoglu S, et al. Antioxidant Activity of Syringic Acid Prevents Oxidative Stress in l-arginine-Induced Acute Pancreatitis: An Experimental Study on Rats. Int Surg 2015; 100:891-6. [Crossref]
4. Baysal E, Gulsen S, Aytac I, Celenk F, Ensari N, Taysi S, et al. Oxidative stress in otosclerosis. Redox Rep 2017; 22: 235-9. [Crossref]
5. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917. [Crossref]
6. Singh AK, Pandey P, Tewari M, Pandey HP, Gambhir IS, Shukla HS. Free radicals hasten head and neck cancer risk: A study of total oxidant, total antioxidant, DNA damage, and histological grade. J Postgrad Med 2016; 62: 96-101. [Crossref]
7. Demir E, Taysi S, Al B, Demir T, Okumus S, Saygili O, et al. The effects of Nigella sativa oil, thymoquinone, propolis, and caffeic acid phenethyl ester on radiation-induced cataract. Wien Klin Wochenschr 2016; 128: 587-95. [Crossref]
8. Khayyo N, Taysi ME, Demir E, Ulusal H, Cinar K, Tarakcioglu M, et al. Radioprotective Effect of Caffeic Acid Phenethyl Ester on the Brain Tissue in Rats Who Underwent Total-Head Irradiation. Eur J Ther 2019; 25: 265-72. [Crossref]
9. Celik E, Taysi S, Sucu S, Ulusal H, Sevincler E, Celik A. Urotensin 2 and Oxidative Stress Levels in Maternal Serum in Pregnancies Complicated by Intrauterine Growth Restriction. Medicina (Kaunas) 2019; 55: 328. [Crossref]
10. Sezer U, Senyurt SZ, Gundogar H, Erciyas K, Ustun K, Kimyon G, et al. Effect of Chronic Periodontitis on Oxidative Status in Patients with Psoriasis and Psoriatic Arthritis. J Periodontol 2016; 87: 557-65. [Crossref]
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-54. [Crossref]
12. Deniz CD, Aktan M, Erel O, Gurbilek M, Koc M. Evaluation of the radioprotective effects of thymoquinone on dynamic thiol-disulphide homeostasis during total-body irradiation in rats. J Radiat Res 2019; 60: 23-8. [Crossref]

13. Ahlatci A, Kuzhan A, Taysi S, Demirtas OC, Alkis HE, Tarakcioglu M, et al. Radiation-modifying abilities of Nigella sativa and thymoquinone on radiation-induced nitrosative stress in the brain tissue. Phytomedicine 2014; 21: 740-4. [Crossref]
14. Hosseinimehr SJ. Trends in the development of radioprotective agents. Drug discovery today 2007; 12: 794-805. [Crossref]
15. Okumus S, Taysi S, Orkmez M, Saricicek E, Demir E, Adli M, et al. The effects of oral Ginkgo biloba supplementation on radiation-induced oxidative injury in the lens of rat. Pharmacogn Mag 2011; 7: 141-5. [Crossref]
16. Taysi S, Okumus S, Akyuz M, Uzun N, Aksor A, Demir E, et al. Zinc administration modulates radiation-induced oxidative injury in lens of rat. Pharmacogn Mag 2012; 8: 245-9. [Crossref]
17. Bacchetti T, Gianna F, Sahebkar A. The role of paraoxonase in cancer. Seminars in Cancer Biology. 2017.
18. Camps J, Iftimie S, Garcia-Heredia A, Castro A, Joven J. Paraoxonases and infectious diseases. Clin Biochem 2017; 50: 804-11. [Crossref]
19. Ustun K, Taysi S, Sezer U, Demir E, Baysal E, Demir T, et al. Radio-protective effects of Nigella sativa oil on oxidative stress in tongue-tissue of rats. Oral dis 2014; 20: 109-13. [Crossref]
20. Zhang C, Peng W, Wang M, Zhu J, Zang Y, Shi W, et al. Studies on protective effects of human paraoxonases 1 and 3 on atherosclerosis in apolipoprotein E knockout mice. Gene Ther 2010; 17: 626-33. [Crossref]
21. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res 1998; 39: 1529-42.
22. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr 2000; 71: 1062-76. [Crossref]

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Original Research

Investigation of Bacterial Presence in Cerebrospinal Fluid by Bioimpedance Technique

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ABSTRACT

Objective: This study aimed to determine the presence of bacteria and their colony numbers in cerebrospinal fluid (CSF) by bioimpedance measurements at 50 kHz phase angle (PA) and 5 kHz impedance values.

Methods: We evaluated the performance of the 18 Gauge probe for detecting several types of bacteria in CSF *ex-vivo*. We equally monitored the electrical differentiation between colony numbers. A quantity of 200 µl sterile CSF was used as the standard in each experiment and was inoculated with 1, 2, 5, and 10 colonies of Coagulase-negative staphylococci, *Streptococcus pneumoniae* and *Acinetobacter baumannii*, separately.

Results: PA and impedance values of CSF samples were compared with each other concerning different colony numbers. It was observed that, varying numbers of colony strains of *Acinetobacter baumannii*, Coagulase-negative staphylococci, and *Streptococcus pneumoniae* could be differentiated from sterile CSF using PA and impedance values. Only one colony of the *A. baumannii* strain could not be distinguished from sterile CSF due to its thin cell wall composition.

Conclusion: The bioimpedance probe was time-saving and could detect the presence of bacteria in CSF samples correctly. Moreover, the probe can be used in the rapid detection of bacteria in CSF during real-time examinations.

Keywords: Bacteria, bioimpedance probe, cerebrospinal fluid

INTRODUCTION

Cerebrospinal fluid (CSF) is a clear, plasma-like fluid that bathes the central nervous system (CNS). CNS infections are essential and challenging perspectives of clinical neurology. Immediate and accurate examination enables clinicians to introduce effective therapies; however, in conditions without the correct diagnosis, the patients may suffer from serious neurological deficits and sometimes even death. The CSF reflects the pathology of CNS and helps in early diagnosis and therapy (1). CSF culture is the gold standard method for the detection of bacteria. In CNS infections, the CSF culture results are positive in 70%–85% of the cases who have not received prior antimicrobial treatment. The identification of the causative agents may take up to 48 hours.

Impedance (Z) is an electrical quantity that represents the capacity of a material to resist alternating current flow. When an electrical potential is applied to a media, the current flows through the intracellular and extracellular spaces at high frequencies (β-dispersion; 10 kHz–10 MHz) and extracellular spaces at low frequencies (α-dispersion; 10 Hz–10 kHz). On the other hand, the cell membrane acts as an insulator in low frequencies and acts as a conductor in high frequencies. Resistance (R) and reactance (X_C) are the components of Z, which is calculated in each frequency with $Z^2=X_C^2+R^2$. X_C is related to the capacitance that causes the phase shift that is defined by the phase angle (PA) and calculated by arctan (X_C/R). The current applied to the cell, flows through the extracellular fluid because cell membranes act as capacitors at low frequencies. Membrane capacitance increases with an increase in cell membrane area increases. At higher frequencies, this capacitive effect is lost, and then the current passes through the extracellular and intracellular fluid, and the resistance decreases (2).

Bioimpedance methods have also been used for tissue differentiation, identifying intraneural needle placement, and tumor detection (3-6). Studies have shown that bioimpedance can be used in the clinical setting for the differentiation of tissues. The small size required is challenging to produce bioimpedance sensing hypodermic needle; Kari et al. (7) presented a thin bioimpedance probe needle of standard 22 G size. In our study, we practiced the

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same technology; however, we used the 18 G 1.2×89 mm spinal needle, which is connected to the bioimpedance analyzer.

Bioimpedance was simultaneously measured with the probe that was tested to determine the bacteria types and colony numbers in CSF at 50 kHz PA and 5 kHz Z values. Our primary aim was to assess the performance of the examination in detecting the presence of bacteria in CSF *ex-vivo*. Our secondary objective was to examine the electrical differentiation with different colony numbers. We hypothesized that if the probe could detect the presence of bacteria in CSF accurately, it could be used as an early predictive diagnostic tool in laboratories.

METHODS

Study Design

The study was conducted at SANKO University School of Medicine, with the approval of the SANKO University Ethics Committee (2018/10-07). At the Medical Microbiology laboratory, a quantity of 200 µl sterile CSF was used as the standard and was inoculated with 1, 2, 5, and 10 colonies of Coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae (S. pneumoniae)* and *Acinetobacter baumannii (A. baumannii)*, separately. A minimum of five bioimpedance measurements (50 kHz PA and 5 kHz Z values) were taken from each specimen within 1–2 min and their mean values were examined.

Bioimpedance Measurements

A bioimpedance analyzer (Quadscan 4000, Bodystat Inc.) was connected to an 18 G probe, and the experimental setup is shown in Figure 1. Using the probe, current was sent to the CSF samples in multiple (5, 50, 100, and 200 kHz) frequencies for bioimpedance measurements. We used only 5 kHz Z values since this frequency is suitable for the cellular sizes and the current information from the outside of the cells was sufficient to differentiate the CSF samples. Moreover, R, X_C, and PA values were recorded at a 50 kHz frequency. Many bioimpedance systems use 50 kHz as a frequency where the capacitor's X_C becomes relatively small so that the current is defined mostly by the R. The frequency at 50 kHz is one of the most essential and optimal frequency, thus we obtained the PA in this frequency. Moreover, most published studies have been carried out using devices with a frequency of 50 kHz to differentiate structures. Due to the logic of this reasoning, we chose to illustrate our PA results only for 50 kHz. All bioimpedance signals were examined by IGOR program (Wavemetrics, Lake Oswego, OR, USA).

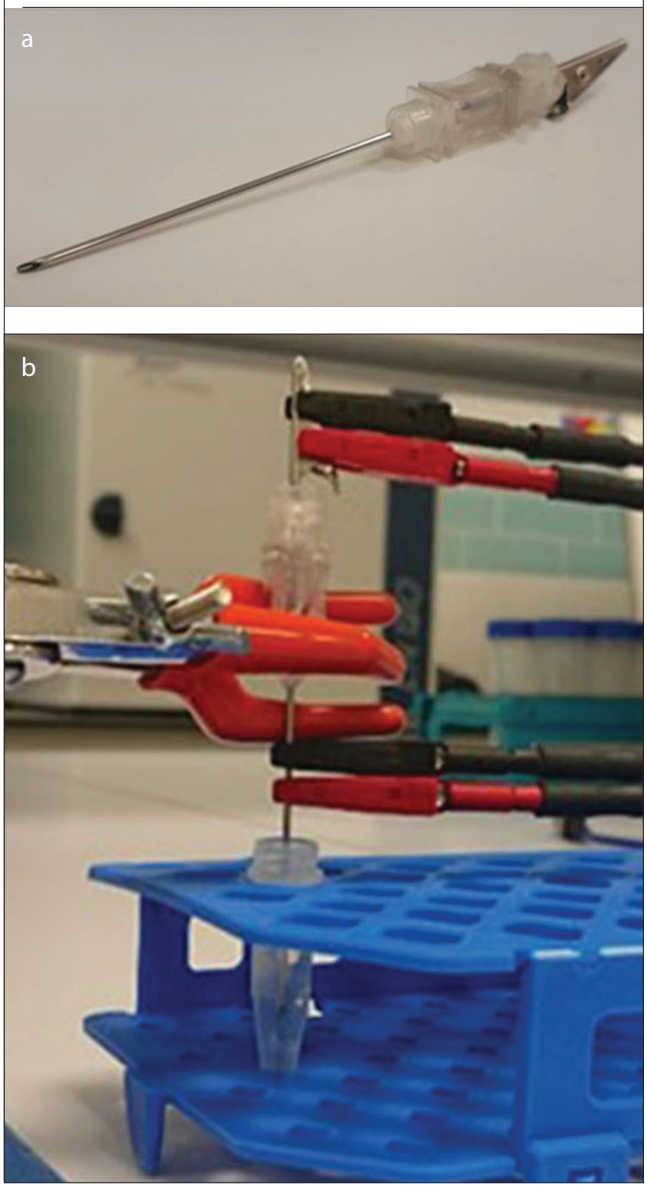
Suspension Preparation

Gram-negative *(A. baumannii)* and Gram-positive (CoNS and S. pneumoniae) bacterial strains which were isolated previously from various clinical samples were used. They were identified by the automated diagnostic system (Vitek2 Compact, Biomérieux, France) and were frozen at –80°C. CSF that was confirmed to be sterile after culture evaluation was stored at 2°C–8°C prior to use and was used as standard. Microorganisms were cultured onto 5% sheep blood agar (RTA Laboratories, Turkey) to prepare the suspension. The cultures were incubated at 37°C for 24 h in a CO_2 incubator, and then the microorganisms were transferred from plates to the sterile CSF. In our study, one colony contained 100 microorganisms (100 Colony-Forming Unit (CFU)/ml).

Main Points:

- Presence of bacteria and their colony numbers in CSF were investigated.
- Bioimpedance probe was time-saving and was able to detect the bacteria in CSF.
- 18 Gauge probe has the potential to be used during the real-time examination.

Figure 1. a, b. a) 18 G bioimpedance probe, b) Measurement set up



Statistical Analysis

IBM Statistical Package for the Social Sciences version 23.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analyses (8).

Figure 2. a-d. Impedance values of cerebrospinal fluid (CSF) samples at multiple frequencies with different colony numbers a) 1 colony, b) 2 colony, c) 5 colony and d) 10 colony (AB: *A. baumanni*; SP: *S. pneumoniae*)

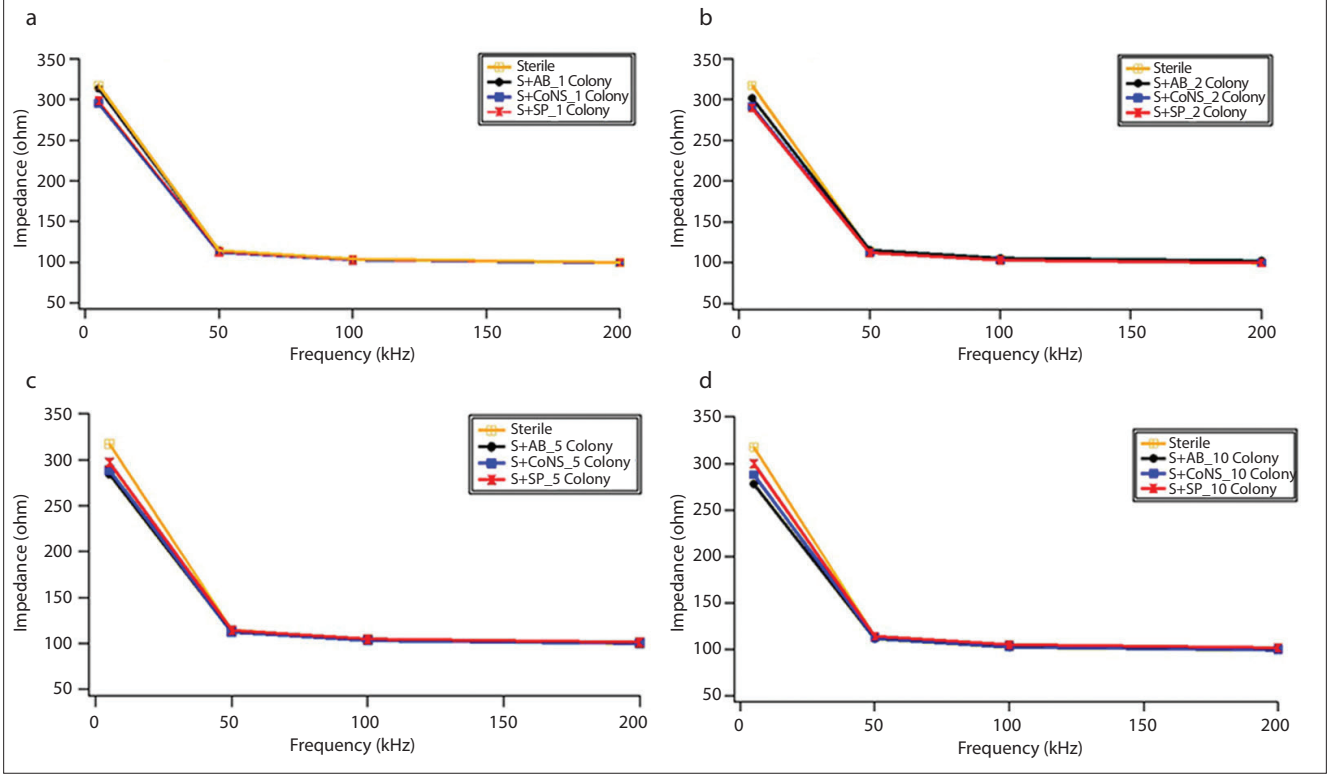


Table 1. Comparison of phase angle (PA) and impedance p–values for cerebrospinal fluid (CSF) samples at different colony numbers

p–values of PA and impedance at different colony numbers for *A. baumannii*, CoNS, S. pneumoniae and sterile CSF differentiation

Colony	PA	Impedance
1	0.001	0.001
2	0.001	0.0001
5	0.0001	0.0001
10	0.0001	0.0001

The Kruskal–Wallis test was used to compare the CSF samples according to PA at 50 kHz and impedance at 5 kHz. For pairwise comparisons, the Mann–Whitney U test was used with Bonferroni correction. A p- value <0.05 was considered to be statistically significant. The p-value was 0.013 for tests with Bonferroni correction.

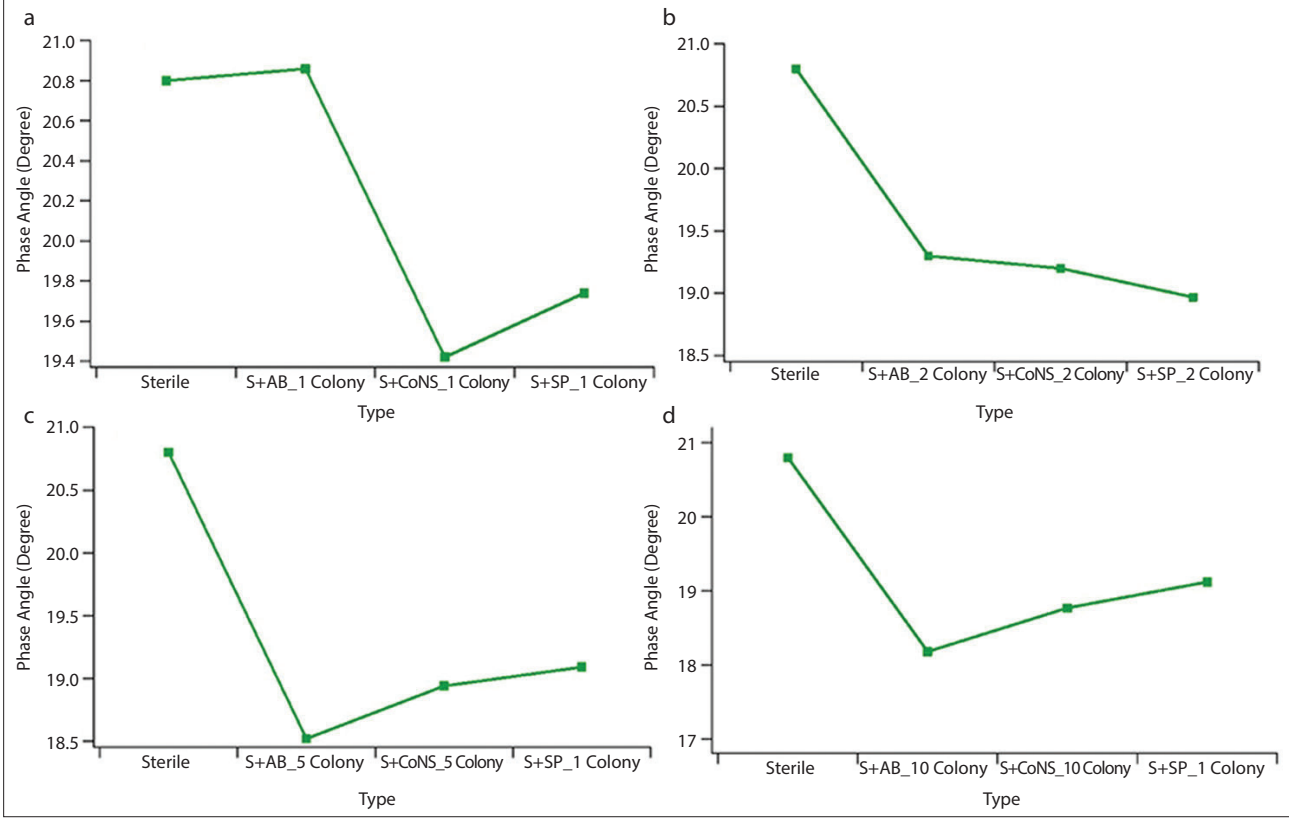
RESULTS

We found current at low frequency (5 kHz) exposed to a long resistive pathway around the one colony in CSF sample. However, the extracellular pathway decreased with the increase of colony number that caused a decrease in Z at low frequencies. In all the three cell types, Z was found high due to the strong dielectric characteristics of the cell membrane and the tissue interface acting as a capacitance in a low frequency (5 kHz). However, Z was low due to the loss of this capacitive effect of the membrane at high frequencies (50, 100, and 200 kHz) (Figure 2). The PA values of the sterile CSF were found higher than the non-sterile CSF with different colony numbers since the R values of non-sterile CSF samples were higher than sterile CSF (Figure 3). The values of PA at 50 kHz and the Z at 5 kHz obtained from different CSF samples were compared with each other (Table 1). The PA values of all CSF samples with different colony numbers were compared and it was observed that varying number of the colonies (1, 2, 5, 10) strains of *A. baumannii*, CoNS, and *S. pneumoniae* could be differentiated from sterile CSF by using their PA values. However, the sample with one colony of the *A. baumannii* strain could not be distinguished from sterile CSF by using PA value due to its thin cell wall composition (Table 2). Z values of all CSF samples were equally compared at different colony numbers. It was observed that varying numbers of the colonies (1, 2, 5, 10) strains of *A. baumannii*, CoNS, and S. pneumoniae could be differentiated from sterile CSF by using 5 kHz Z values (Table 2). However, the sample with one colony of the *A. baumannii* strain could not be distinguished from sterile CSF by using the 5 kHz Z value due to its thin cell wall composition.

DISCUSSION

According to the literature, the diameter of the *A. baumannii* cell was determined to be 0.9–1.6 µm (9), CoNS cell was defined

Figure 3. a-d. Phase angle (PA) values of different CSF samples at different colony numbers a) 1 colony, b) 2 colony, c) 5 colony and d) 10 colony (AB: *A. baumanni*; SP: *S. pneumoniae*)



as 0.5–1.5 µm in diameter (10), and *S. pneumoniae* cell wall was identified as 0.5–1.25 µm (11). Gram-positive (CoNS and *S. pneumoniae*) cell wall thickness is about 20–80 nm and more homogenous than that of the thin (2 nm) Gram-negative *(A. baumannii)* cell wall (12). The electrical characteristics of bacterial cells and their electrophysiology are fundamental for improving bioimpedance methods for the detection of bacterial cells. Like other biological cells, bacterial cells consist of structures that have various electrical features. The inner composition of a cell is sophisticated and contains vacuoles, mitochondria, nucleus, and many dissolved charged molecules. While the membrane of the cell is highly insulated, its interior is extremely conductive. The electrical conductivity of the cell membrane is about 10^{-7} S/m; however, the intracellular conductivity can be as high as 1 S/m. Bacterial suspension conductivity studies have investigated the electrical features of bacterial cell surface and related cell components (13).

Bioimpedance spectroscopy techniques have been used in some bacterial identification studies. In one study, Salmonella cell suspensions in deionized water solution were studied over a wide range of frequencies. It was reported that bacterial cell suspensions with different cell concentrations could result in various electrical Z spectral responses. It was stated that the Z of the cell suspension was related to the cell concentration which could present an alternative approach for quantifying bacterial cells in a label-free, cheap, and straightforward method (14). In another clinical study, it was demonstrated that the bioimpedance needle probe was a reliable tool for detecting spinal structures with a sensitivity of 100% and specificity 81% (15). In a certain study, 22 G and 24 G bioimpedance needle probes were employed for the detection of synovial fluid concentrations in the joints of 80 patients with active arthritis. It was found that the sensitivity and specificity of this probe for synovial fluid detection were 86% and 85% respectively (16). In another Z study, the authors demonstrated that the Z biosensor was capable of detecting Listeria as low as 1.6×10^2 CFU/mL in 1 h based on either the PA or the Z change analysis (17). In another study, *P. aeruginosa* was detected by electrical impedance spectroscopy (18). Among 262 CSF samples with positive cultures, the most frequently isolated agent was *S. pneumoniae* with 23%, which was followed by CoNS (21%), *A. baumannii* (10%), Neisseria meningitidies (9%), Enterobacteriaceae strains (9%), *Pseudomonas* spp. (7%), *Staphylococcus aureus* (5%), *Haemophilus influenzae* (3%), *Candida* spp. (2%), and *Enterococcus* spp. (2%) (19). Based on this study, we used the three most frequently isolated agents to compare with the sterile CSF in our research.

In studies that used PA to differentiate tissues, it was shown that a low PA was associated with tumor development, cell death or decreased cell integrity; however, high PA was associated with a healthy cell or cell membrane (20). Similar to these results, non-sterile CSF PA values were low and sterile PA values were high in our study.

Table 2. Pairwise comparison of phase angle (PA) and impedance values for cerebrospinal fluid (CSF) samples at different colony numbers

	p–values of PA/impedance at different colony numbers				
	A. baumannii	CoNS	*S. pneumoniae*	Sterile	Colony
A. baumannii	–				1
	–				2
	–				5
	–				10
CoNS	0.008/0.008	–			1
	1/0.008	–			2
	0.008/0.008	–			5
	0.003/0.003	–			10
S. pneumoniae	0.008/0.008	0.008/0.056	–		1
	0.052/0.004	0.004/0.537	–		2
	0.003/0.003	0.03/0.003	–		5
	0.001/0.001	0.0001/0.0001	–		10
Sterile	0.931/0.247	0.004/0.004	0.004/0.004	–	1
	0.004/0.004	0.004/0.004	0.002/0.002	–	2
	0.004/0.004	0.004/0.004	0.001/0.001	–	5
	0.004/0.004	0.001/0.001	0.0001/0.0001	–	10

We performed a rapid and straightforward bioimpedance method to differentiate sterile from non-sterile CSF with different colony numbers. In this study, we found that sterile and non-sterile CSF with different colony numbers resulted in various electrical Z spectral responses. However, the comparison of sterile CSF with CSF inoculated with *A. baumannii* did not yield any significant difference in Z spectra and PA in response to one colony due to its thin cell wall composition. The diameter cell membrane of *A. baumanni* is small due to its thin cell wall composition, and thus, the capacitive effect of its membrane is low. This situation may explain the fact that the cell membrane does not exhibit insulating properties and therefore has similar R results with the measurement from sterile CSF. Further, we observed that when colony numbers of *A. baumannii* were increased in CSF, the differentiation of sterile and non-sterile CSF became significant since the applied current interacted better with the inner structure of the *A. baumannii*. Similarly, its cell membrane did not exhibit insulating properties and therefore it had similar Z results with the measurement from sterile CSF. These results indicate that the bioimpedance probe differentiates the sterile from non-sterile CSF samples with different colony numbers.

CONCLUSION

Today, molecular tests have become prominent as rapid diagnostic methods in the diagnosis of meningitis. However, these tests are expensive and thus are not being used in routine practice. Bioimpedance spectroscopy technique can provide a predictive approach for quantifying bacterial cells in an inexpensive, simple, and time-saving way in CSF samples with different colony numbers. Besides, the probe has the potential to be used in the rapid detection of bacteria in CSF during real-time examinations.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of SANKO University (2018/10-07).

Informed Consent: Due to the design of the study, informed consent was not taken.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.D.; Design - T.D., C.A.; Supervision - T.D.; Resources - H.D., M.E.; Materials - T.D., C.A.; Data Collection and/or Processing - T.D., C.A., M.E.; Analysis and/or Interpretation - T.D., P.G.K.; Literature Search - T.D., M.E.; Writing Manuscript - T.D., C.A.; Critical Review - T.D.

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REFERENCES

1. Benninger F, Steiner I. CSF in acute and chronic infectious diseases. Handb Clin Neurol 2017: 187-206. [Crossref]

2. Lukaski HC. Biological indexes considered in the derivation of the bioelectrical impedance analysis Am J Clin Nutr 1996; 64: 397S-404S. [Crossref]
3. Kalvøy H, Frich L, Grimnes S, Martinsen ØG, Hol PK, Stubhaug A. Impedance-based tissue discrimination for needle guidance Physiol Meas 2009; 30: 129-40. [Crossref]
4. Trebbels D, Fellhauer F, Jugl M, Haimerl G, Min M, Zengerle R. Online Tissue Discrimination for Transcutaneous Needle Guidance Applications Using Broadband Impedance Spectroscopy. IEEE Trans Biomed Eng 2012; 59: 494-503. [Crossref]
5. Kalvøy H, Sauter AR. Detection of intraneural needle-placement with multiple frequency bioimpedance monitoring: a novel method. J Clin Monit Comput 2016; 30: 185-92. [Crossref]
6. Mishra V, Schned AR, Hartov A, Heaney JA, Seigne J, Halter RJ. Electrical property sensing biopsy needle for prostate cancer detection. Prostate 2013; 73: 1603-13. [Crossref]
7. Kari J, Annala K, Annus P, Seppä VP, Kronström K. A thin needle with bio-impedance measuring probe: tissue recognition performance assessed in in vivo animal study. Injeq Oy Ltd., Tech Rep 2015.
8. Released IC, IBM SPSS Statistics for Windows, Version 23.0. 2013, IBM Corp.: Armonk, NY.
9. Vaneechoutte M, Dijkshoorn L, Nemec A, Kämpfer P, Wauters G. Acinetobacter, Chryseobacterium, Moraxella, and other nonfermentative Gram-negative rods. in Manual of Clinical Microbiology, 10th Edition. American Society of Microbiology, 2011: 714-38. [Crossref]
10. Zhou X, Li Y. Supragingival Microbes. in Atlas of Oral Microbiology, Academic Press: Oxford, 2015: 41-65. [Crossref]
11. Gisch N, Peters K, Zähringer U, Vollmer W. The Pneumococcal Cell Wall. in Streptococcus Pneumoniae, J. Brown, S. Hammerschmidt, and C. Orihuela, Editors. Academic Press: Amsterdam, 2015: 145-67. [Crossref]
12. Parija SC. Textbook of Microbiology and Immunology, 2/e. Elsevier India, 2012.
13. Van der AW, Minor M, Norde W, Zehnder AJB, Lyklema J. Conductivity and Dielectric Dispersion of Gram-Positive Bacterial Cells. J Colloid Interface Sci 1997; 186: 71-9. [Crossref]
14. Yang L. Electrical impedance spectroscopy for detection of bacterial cells in suspensions using interdigitated microelectrodes. Talanta 2008; 74: 1621-9. [Crossref]
15. Halonen S, Annala K, Kari J, Jokinen S, Lumme A, Kronstrom K, et al. Detection of spine structures with Bioimpedance Probe (BIP) Needle in clinical lumbar punctures. J Clin Monit Comput 2017; 31: 1065-72. [Crossref]
16. Halonen S, Kankaanpaa E, Kari J, Parmanne P, Relas H, Kronstrom K, et al. Synovial fluid detection in intra-articular injections using a bioimpedance probe (BIP) needle-a clinical study. Clin Rheumatol 2017; 36: 1349-55. [Crossref]
17. Chen Q, Wang D, Cai G, Xiong Y, Li Y, Wang M, et al. Fast and sensitive detection of foodborne pathogen using electrochemical impedance analysis, urease catalysis and microfluidics. Biosens Bioelectron 2016; 86: 770-6. [Crossref]
18. Ward AC, NP Tucker, and P Connolly. Development of a diagnostic device to detect different Pseudomonas aeruginosa phenotypes in medically relevant contexts. Conf Proc IEEE Eng Med Biol Soc 2014; 2014: 2757-60. [Crossref]
19. Pehlivanoğlu F, Şengöz G. Microorganisms Isolated from Cerebrospinal Fluid and their Antibiotic Resistance. ANKEM Derg 2011; 25: 1-5. [Crossref]
20. Norman K, Stobaus N, Zocher D, Bosy-Westphal A, Szramek A, Scheufele R, et al. Cutoff percentiles of bioelectrical phase angle predict functionality, quality of life, and mortality in patients with cancer. Am J Clin Nutr 2010; 92: 612-9. [Crossref]

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Original Research

Evaluation of the demographic and clinical features of skin cancers: a single-center experience

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ABSTRACT

Objective: This study aimed to present the clinical and demographic features of patients who underwent skin cancer surgery at our center during the last 10 years. Localization, age, sex, and subtype mapping of head and neck cancers were particularly evaluated.

Methods: A retrospective evaluation was conducted of the patient data recorded over the last 10 years in the information processing system of our institute. Age, sex, tumor localization, and subtype, if indicated, were recorded.

Results: The data of 455 patients were obtained from the our hospital archive scan. Of these, 342 (75.1%) patients had basal cell carcinoma (BCC), 99 (21.8%) had squamous cell carcinoma (SCC), 6 (1.3%) had basosquamous cell carcinoma (BSCC), and 8 (1.8%) had malignant melanoma (MM). BCC was most commonly found in men (M/F: 175/167) and the nasal region. SCC was seen more frequently in men (M/F: 52/47) and the cheeks. BSCC was most common on the cheek (3 patients, 50%) and MM (4 patients, 50%) on the scalp.

Conclusion: There are very few epidemiological studies on skin cancers throughout the world and especially in Turkey. This study showed that SCC was more common in men and located on the cheek, whereas MM was found to be more common on the scalp. Residents of the Çukurova region, an eastern Mediterranean region, are exposed to high levels of sunlight. We believe that the difference in demographic and clinical features of skin cancers in this region may be due to this.

Keywords: Basal cell carcinoma, malignant melanoma, squamous cell carcinoma

INTRODUCTION

Malignant skin cancers are generally classified as keratinocytic and melanocytic tumors. Keratinocytic or non-melanocytic skin cancers (NMSC) comprise basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and more rarely, basosquamous cell carcinoma (BSCC). These are the most seen tumors worldwide, especially in white races. The frequency of BCC in the USA has been reported to be 33%–39% in men and 23%–28% in women (1, 2). Although NMSC are widespread, the morbidity and mortality rates are not high. Despite significant developments in the diagnostic parameters of melanoma, the same developments have not been seen for the diagnosis of NMSC. For these types of tumors, the most important data are obtained clinically, and diagnosis is made according to these data.

BCC is more often encountered and originates from germinative cells found in the hair root follicles in the epidermis. The most significant factor playing a role in the etiology of this tumor is UV light. BCC is usually seen in white-skinned people and the geriatric population. SCC forms because of the malignant transformation of epithelial keratinocytes in the skin and mucosal surfaces (3). Chronic inflammation, chronic wounds, and wound scars are thought to play a role in the etiology of SCC. While acute and intense exposure to sunlight have a role in the etiology of BCC, long-term chronic exposure plays a role in the etiology of SCC (3, 4). Malignant melanoma (MM) melanocytes are thought to develop with intense UV exposure and other etiological reasons (5). The etiology of skin cancers varies according to geographical region, and no study in literature was found for the Çukurova region in south-east Turkey.

This study aimed to develop a demographic map of skin cancers in the Çukurova region.

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METHODS

A retrospective examination was made of the data of patients who presented with a skin tumor at our center in the Çukurova region between 2009 and 2019. Approval for the study was granted by the Ethics Committee of Adana City Training and City Hospital (2019/550). Informed consent was obtained from all the study participants. A retrospective scan was made of patient records from September 2009 to December 2019. The patients included in the study were those who were histopathologically diagnosed with NMSC (BCC, SCC, BSCC) and MM. Data related to age, sex, and tumor type and localization were analyzed. Only patients with sufficient data were considered in our analysis. The study excluded patients with multiple skin lesions, primary tumors in any tissue, and chronic autoimmune diseases.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (Version 16.0. SPSS Inc.; Chicago, IL, USA). Conformity of the data to normal distribution was checked using histograms and the Shapiro-Wilk Test. Descriptive data were given as mean ± standard deviation (SD) values or number (n) and percentage (%).

RESULTS

A total of 455 patients with complete data were included in the study. Because of unavailable necessary data, 23 patients were excluded from the study. Diagnoses were determined as BCC in 342 cases, SCC in 99, BSCC in 6, and MM in 8.

Demographic Data of Patients with BCC

Of the total 455 patients included in the study, BCC was diagnosed in 342 (75%), of which 175 (51%) were men and 167 (49%) were women with a mean age of 66.03±13.90 years. BCC was seen most often in the sixth and seventh decades of life (Figure 1).

Findings regarding the localization of the lesions are summarized in Figure 2. Localization had not been recorded in 38 patients. BCC was found most often in the nasal region (n: 107, 31%) and least often in the mentum (n: 6, 0.2%) and the lips (n: 2, 0.05%). Of the 107 lesions located on the nose, 14 (13%) were on the left side; 23 (21%) on the right side; and the remaining 70 were located on the nasal tip, radix, and dorsum. Subgroup classification could only be made in 38 patients. Subtypes were mostly nodular (n: 27), followed by the infiltrative type (n: 6). Of the 63 patients with BCC on the cheeks, approximately half were located on the right side (n: 31) and half on the left side (n: 32). Of these patients, subtypes could be identified in only 26. Of 17 (27%) tumors identified, 4 (0.6%) were ulcer type. Forty-five (13%) patients had periorbital localization, of which 27 (60%) were left-sided and 18 (40%) were right-sided. Subtype identification could be made in 17 patients, with nodular being the most common type (n: 13, 28%), followed by the superficial type (n: 2, 0.4%).

Main Points:

- This study aimed to present the clinical and demographic features of patients who underwent skin cancer surgery at our center during the last 10 years
- This study showed that SCC was more common in men and located on the cheek, whereas MM was found to be more common on the scalp
- Residents of the Çukurova region, an eastern Mediterranean region, are exposed to high levels of sunlight.
- We believe that the difference in demographic and clinical features of skin cancers in this region may be due to high levels of sunlight.

Demographic Data of Patients with SCC

The patients diagnosed with SCC comprised 52 (58%) men and 47 (44%) women with a mean age of 72.12±12.80 years. These tumors were seen mostly in the seventh decade of life (Figure 3). The lesion was mostly seen on the cheek region (n.24, 25%) and least frequent in the frontal (n: 5, 0.5%) and periorbital region (n: 4, 0.4%). The tumors seen on the cheeks were on the right and left sides at equal rates. Of the lesions observed on the lips, 19 (86%) were located on the lower lip (Figure 4).

Demographic Data of Patients with BSCC

BSCC was extremely rare and diagnosed only in six patients. These lesions were located on the left cheek in four cases (66%), the nose and ear in one patient each (16%). Extensive data analysis could not be performed because of few cases.

Figure 1. Demographic features in basal cell carcinoma

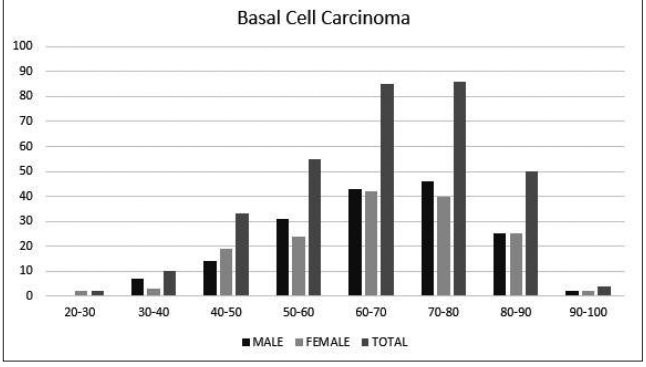


Figure 2. The localization of basal cell carcinoma lesions

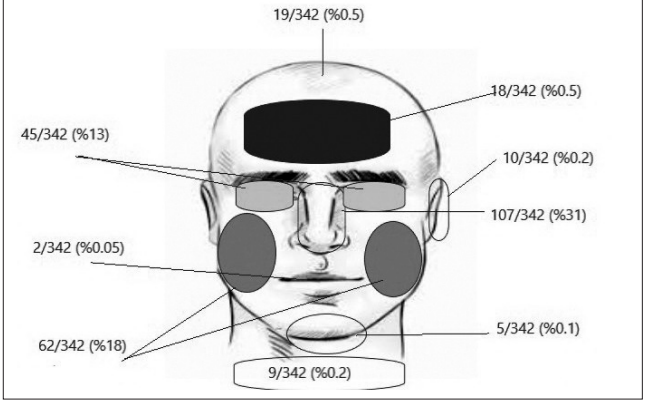


Figure 3. Demographic features in squamous cell carcinoma

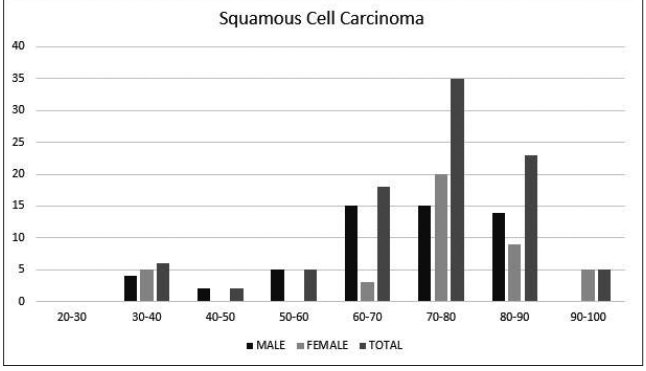
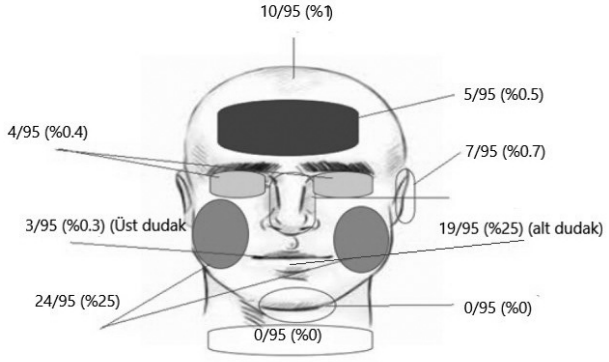


Figure 4. The localization of squamous cell carcinoma lesions



Demographic Data of Patients with MM

MM was diagnosed in eight patients, comprising 6 (75%) men and 2 (25%) women with a mean age of 73.8 years (range, 47–94 years). It was located in the scalp in 4 (50%) patients, on the cheek in 2 (25%, on the forehead in 1 (12.5%), and in the submandibular region in 1 (12.5%). Subtypes were observed most as nodular (n: 4, 50%) followed by lentigo maligna (n: 2, 25%). Extensive data analysis could not be performed because of few cases.

DISCUSSION

Sunlight and radiation are the two most important factors in the etiology of skin cancers. In addition, geographical region and advanced age are of significant importance in this etiology (6). With the implementation of the Cancer Monitoring System in Turkey in 2003 and the clear determination of early diagnostic criteria, the real incidence of the disease has been revealed. Thus, the disease can be diagnosed early before metastasis. Moreover, there has been a reduction in the incidence of mortality caused by diseases such as melanoma. However, there are very few epidemiological studies on skin cancer in Turkey.

Findik et al. (5) analyzed the demographic data of 400 patients who presented with NMSC at Konya Meram University Medical Faculty Hospital. The most frequent tumor was BCC (263 patients, 65%) located most frequently in the nasal region (82 patients, 31%). These results overlap with the findings of tumors seen in the current study region. In the same study by Findik et al, SCC cases accounted for 28% (114 cases). Lesions were located most often in the lower lip, eye region, and extremities and were more often seen in women (5). However, in our study, they were seen more in men, and most often found on the cheek. These data were not consistent with the findings of previous studies (5, 6). As the Çukurova region is in the eastern Mediterranean region of Turkey, it is exposed to more sunlight for most of the year because of the geographical characteristics and its proximity to the equator. In addition, the geographical conditions and employment in agriculture may explain the sex-related difference. The same reasons could explain the difference in the localization of the lesions. As there were very few MM and BSCC tumors in our study, detailed data analysis could not be performed.

The results in the current study are supported by the findings of other studies in Turkey (5, 7). MM subtypes show different responses with UV exposure. The vast majority of lentigo Malign Melanoma (LMM) are seen in white-skinned individuals with a tendency to actinic damage and long-term exposure to intense sunlight. The cumulative dose of UV light to which a person is exposed throughout life is thought to play a role in the formation of LMM (8). Nodular malignant melanoma (NMM) is seen in relatively younger patients, and exposure to intermittent but severe UV light at an early age plays a role in its development (8). The frequent observation of NMM in the Çukurova region can be explained by exposure to intermittent but intense UV light due to the geography and climate. However, the low number of cases in this study prevented the presentation of a detailed view. The results of the current study are consistent with those reported by other studies on MM in Turkey and throughout the world (9).

This study's limitations included its retrospective design, the absence of histological subgroup analysis, and the low sample size of the BCC and MM groups.

CONCLUSION

In conclusion, just as the demographic data of skin cancer cases vary from country to country, a difference may also be seen from region to region. When it is considered that except for MM, the diagnosis of skin cancer is generally made according to clinical data, studies such as ours can provide information that can be of guidance in early diagnosis. More studies with a larger number of cases and longer follow-up periods are recommended.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Adana City Training and Research Hospital. / (2019/550)

Informed Consent: Informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Fahradyan A, Howell AC, Wolfswinkel EM, Tsuha M, Sheth P, Wong AK. Updates on the management of non-melanoma skin cancer (NMSC). Healthcare (Basel) 2017; 5: 82. [Crossref]
2. Lear W, Dahlke E, Murray CA. Basal cell carcinoma: review of epidemiology, pathogenesis, and associated risk factors. J Cutan Med Surg 2007; 11: 19-30. [Crossref]
3. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol 2012; 166: 1069-80. [Crossref]
4. Barton V, Armeson K, Hampras S, Ferris LK, Visvanathan K, Rollison D, et al. Nonmelanoma skin cancer and risk of all-cause and cancer-related mortality: a systematic review. Arch Dermatol Res 2017; 309: 243-51. [Crossref]
5. Findik S, Uyanik O, Altuntas M, Altuntas Z. Epidemiological and localization characteristics of non-melanoma skin cancers: Retrospective analysis of 400 cases. Turk J Plast Surg 2019; 27: 19. [Crossref]
6. Rigel DS. Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. J Am Acad Dermatol 2008; 58: S129-S32. [Crossref]
7. Kasap S, Pektaş M, Dere Y, Altıparmak M. Retrospective Evaluation of Skin Cancer Patients in Muğla. Medical Journal of Muğla Sıtkı Koçman University 2015; 2: 34-7. [Crossref]
8. Elwood JM, Jopson J. Melanoma and sun exposure: an overview of published studies. International Journal of Cancer 1997; 73: 198-203. [Crossref]
9. Baykal C, Ergun T, Kavak A. Dermatoloji polikliniklerinin 5 yıllık malign melanom olgularının değerlendirilmesi. Türkderm 1996; 30: 190-6.

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Original Research

Intraneural Vascular Resistive Index of the Median Nerve as a Predictor of Severity of Carpal Tunnel Syndrome

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ABSTRACT

Objective: There is a limited data about resistive index (RI) of median nerve (MN) in patients with carpal tunnel syndrome (CTS). In our study, we aimed to evaluate the relationship between CTS severity and MN-RI.

Methods: A total of 115 CTS patient wrists, and 49 wrists of control subjects without CTS, were examined on ultrasonography (US) and color Doppler US (CDUS), pulsed Doppler ultrasonography (PDUS), and by electrophysiological evaluations. MN peak-systolic velocity (MN-PSV), MN end-diastolic velocity, MN-RI and MN pulsatility index (MN-PI) were measured by PDUS. Patients were divided into 3 groups according to electrophysiological examinations severity findings of CTS as mild (Group-I), moderate (Group-II), and severe (Group-III).

Results: MN-PSV, MN-PI and MN-RI increased significantly from Group-I to Group-III and these parameters were significantly higher in Group-III than other two groups. MN-RI independently determines the patients to have severe CTS. Increased MN-RI (per-0.1) was found to increase the risk of having severe CTS by 3.45-times. In the ROC analysis, the area under the curve was 0.846 for MN-RI. When the MN-RI cut-off value was taken as 0.80, it determines patients to be severe CTS with 85.2% sensitivity and 78.2% specificity.

Conclusion: The increase in MN-RI in CTS patients is independently associated with disease severity and may be used in the clinical follow-up of these patients.

Keywords: Carpal tunnel syndrome, median nerve, pulsed doppler ultrasounography, resistive index

INTRODUCTION

Carpal tunnel syndrome (CTS) is the most predominant entrapment neuropathy of the median nerve (MN) constricted in the wrist. It is more common in middle-aged women (1). CTS affects 1% of the general population, and the prevalence is 5.8% for females and 0.6% for males (2). The MN is located tightly packed in the carpal tunnel (CT) with 9 tendons and synovial membranes. CTS may occur idiopathically, as well as in rheumatoid arthritis, hyperthyroidism, acromegaly, and diabetes mellitus (3). Pathologic changes occur in CTS due to MN pressure (4). Anatomic variations such as bifid median nerve anomaly and persistent median artery synovitis and cyst, ganglion, aberrant muscle, a tumor that may cause nerve compression in the CT can be detected by imaging methods (5). Ultrasonography (US) is the most commonly used imaging technique for this purpose. The fact that patients are not exposed to X-rays or contrasts, being a cheap, well-tolerated, noninvasive, ready-for-intervention, easily accessible, and easy-to-implement process, are the significant advantages of US.

In addition to anatomical parameters, MN epineural and intra-neural blood flow can be revealed by Doppler US (6). These Doppler US are color Doppler US (CDUS), pulsed Doppler US (PDUS), and superb microvascular imaging (SMI) examinations (6, 7). Intra-neural blood flow and density or vascularity can be visualized with color Doppler US. MN evaluation with US started with B-mode US, and in the last decade, in addition to B-mode, USG, PDUS, CDUS, and SMI are available for MN evaluation. As a result of these evaluations, in CTS patients; increased MN cross-sectional area (CSA), vascularity, hypoechogenity, and peak

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systolic velocity (PSV) can be revealed (7-13). There are studies that investigate CTS severity with intraneural PSV (1, 8). There is limited information about the change of MN resistive index (RI) and its clinical significance in CTS patients (14).

Magnetic resonance imaging (MRI) and US are two important techniques for the diagnosis and severity assessment of CTS. MN CSA diffusion tensor imaging (DTI) findings obtained from different levels with MRI have been shown to be closely associated with CTS severity (15, 16). In a study by Ikeda et al. (15), the CSA in the affected hand at the scaphoid body level was significantly larger rather than that in the unaffected hand. The CSA at the scaphoid body level positively correlated with distal motor latency in the affected hand. In a study by Wang et al. (16), the fractional anisotropy and apparent diffusion coefficient values obtained by DTI were reported to be closely associated with the diagnosis and severity of CTS. However, MRI for CTS evaluation is more expensive and difficult to reach than US examination. It was shown that MN CSA, MN vascularity increase, and MN-PSV and SMI were significant in order to determine the diagnosis and severity of CTS obtained by B-mode, CDUS, and PDUS (6, 7, 10, 11). However, other studies have reported no clear association between these findings and CTS severity (7, 12, 13). US is easily accessible, cheaper than the MRI, but is still not recommended as an objective and clear parameter. In most studies, MN vascularity has been shown to increase in CTS patients.

It has been shown that RI obtained from the renal artery, hepatic artery, splenic artery, and carotid artery determines the damage to the endothelium in these organs or related diseases (17-20). We think that MN compression, that occurs in patients with CTS, may also increase the RI and associated PDUS parameters in the arteries within this nerve by pressurizing the vasculature. There is limited information about the clinical use of the MN-RI value obtained by PDUS in CTS patients (14). In only one study, MN-RI values obtained by PDUS were reported to be significantly higher in patients with MN involvement than in those without MN involvement. However, this study did not give us any information on the relationship between the MN-RI value and the severity of CTS disease (14).

In this study, we aimed to investigate the relationship between PDUS findings of MN and CTS severity in patients diagnosed CTS with clinical and electrophysiological examinations.

METHODS

Patients and Study Design

One hundred and fifteen wrists of 82 patients (12 males, 70 females, mean age 51.9±10.9 years) with CTS, who were referred to our physical medicine and rehabilitation outpatient clinic between April 2014 and April 2015, were evaluated on US, CDUS, and PDUS. CTS diagnosis was confirmed by both clinical and electrophysiological examinations. All patients had signs of paralysis, pain, and/or vasomotor symptoms of MN injury. For clinical assessment, provocative tests, including Allen's test, Tinnel test, and carpal compression test, were used. Patients with at least one positive provocative test were given preliminarily diagnosed with CTS. Electrophysiological examinations were performed using a four-channel Medelec Synergy (Oxford Instruments Medical, Surrey, UK) electromyography device. Neurophysiological tests included nerve conduction studies and needle electromyography for the median and ulnar nerves. Nerve conduction studies included measurement of the distal sensory and distal motor latencies and sensory/motor nerve conduction velocities. The exact diagnosis of CTS was made according to the most recently updated guidelines (21). CTS severity was classified, on the basis of electrophysiological results, as mild, moderate, severe, or extreme, according to the modified scoring system of Padua et al. (22). Patients were excluded if they had polyneuropathy, radiculopathy, brachial plexus injury, proximal median neuropathy, diabetes mellitus, hypothyroidism, rheumatoid arthritis, amyloidosis, chronic renal failure managed by hemodialysis, pregnancy, and a history of surgery for CTS.

Main Points:

- MN-RI increase is associated with disease severity in CTS patients.
- Increased MN-RI (>0.80) usually indicates increased CTS severity
- MN-RI may be used in the clinical follow-up of CTS patients.

Forty-nine clinically and electrophysiologically normal wrists were included in the control group.

CDUS and electrophysiological evaluations were performed in all controls.

The study protocol was prepared according to the principles of the Declaration of Helsinki. The ethics committee of Baskent University Faculty of Medicine approved the study protocol, and each participant provided written informed consent.

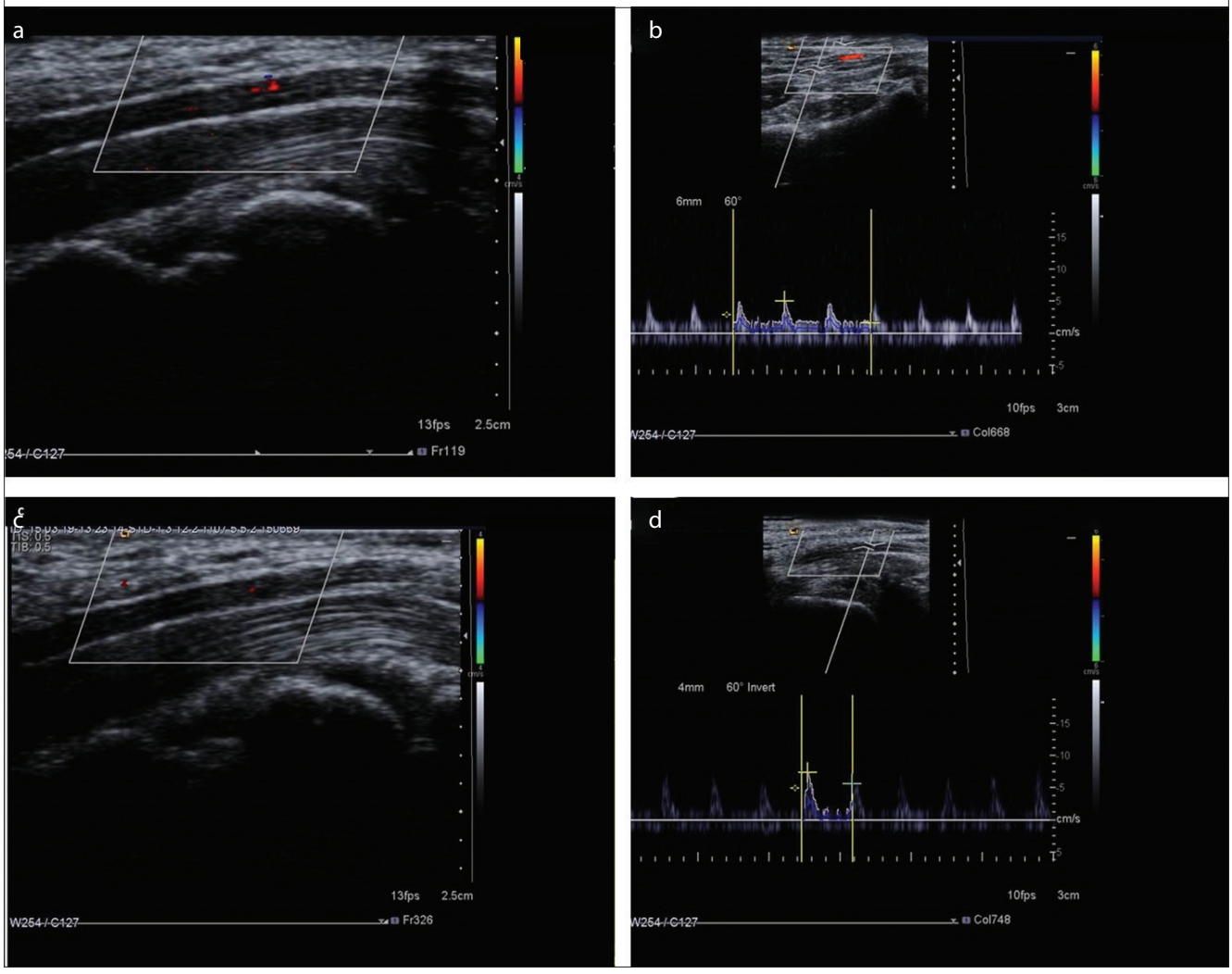
Median Nerve Ultrasonography

All subjects were examined on B-mode and CDUS using a 13-MHz linear array transducer (Sonoline Antares; Siemens Medical Solutions, Inc., Hoffman Estates, IL, USA) in the neutral supine position. All examinations were performed by a single radiologist who had 15 years of work experience. The MN from the distal forearm to the carpal tunnel outlet was assessed in the transverse and longitudinal planes. Three internal anatomic landmarks were used for the images. The images of the MN were obtained at the radial-ulnar junction immediately proximal to the flexor retinaculum, and at the level of the pisiform and the hook of the hamate (23, 24). MN vascularity was evaluated with CDUS, and flow velocity measurements were analyzed with PDUS. Repeated measurements and at least 8 waveforrms of intraneural arteries were obtained. PSV, end-diastolic velocity (EDV), pulsatility index (PI), and RI of intraneural arteries were measured on PDUS (Figure 1). MN-RI and MN-PI values were measured automatically by PSV-EDV/PSV formula, and PSV-EDV/MV (mean flow velocity) formula, respectively. The mean values of MN-PSV, MN-EDV, MN-PI, and MN-RI were recorded (Figure 1a-d).

Statistical Analysis

Continuous variables were expressed as mean±standard deviation (mean±SD), while categorical variables were reported as

Figure 1. a-d. Color Doppler ultrasonography (CDUS) and pulsed Doppler ultrasonography (PDUS) findings of median nerve (MN) in carpal tunnel syndrome (CTS). (a) In patients with severe CTS; significantly increased intraneural vascularity of MN is demonstrated by CDUS. (b) In patients with severe CTS; significantly increased MN resistive index (MN-RI) is demonstrated by PDUS. The MN-RI is measured as 0.80. (c) In patient with mild CTS; mildly increased MN intraneural vascularity is demonstrated by CDUS. (d) In patient with mild CTS; MN-RI is measured as 0.23 with PDUS



counts and percentages. Comparisons of continuous variables were performed by the One-way ANOVA or Kruskal-Wallis 1-way ANOVA tests according to the distribution. For normally distributed data, the Scheffe and Games-Howell tests were used for multiple comparisons of groups with respect to the homogeneity of variances. For non-normally-distributed data, the Bonferroni-adjusted Mann Whitney U test was used for multiple comparisons of groups. The Chi-Square Test was used to compare categorical variables. Univariate analysis revealed demographic and Doppler US parameters that were significantly different in patients with CTS severity. For independent determination of patients who had severe disease for CTS, multivariate logistic regression analysis was performed. ROC curve analysis was performed to reassess markers that were independent for identifying patients with severe disease for CTS and to determine the threshold values of these markers. The value of the area under the curve was used as the accuracy criterion of the test. The threshold for statistical significance was set at $p<0.05$. All analyses were performed with SPSS 20.0 (IBM SPSS Corp.; Armonk, NY, USA) statistical software package.

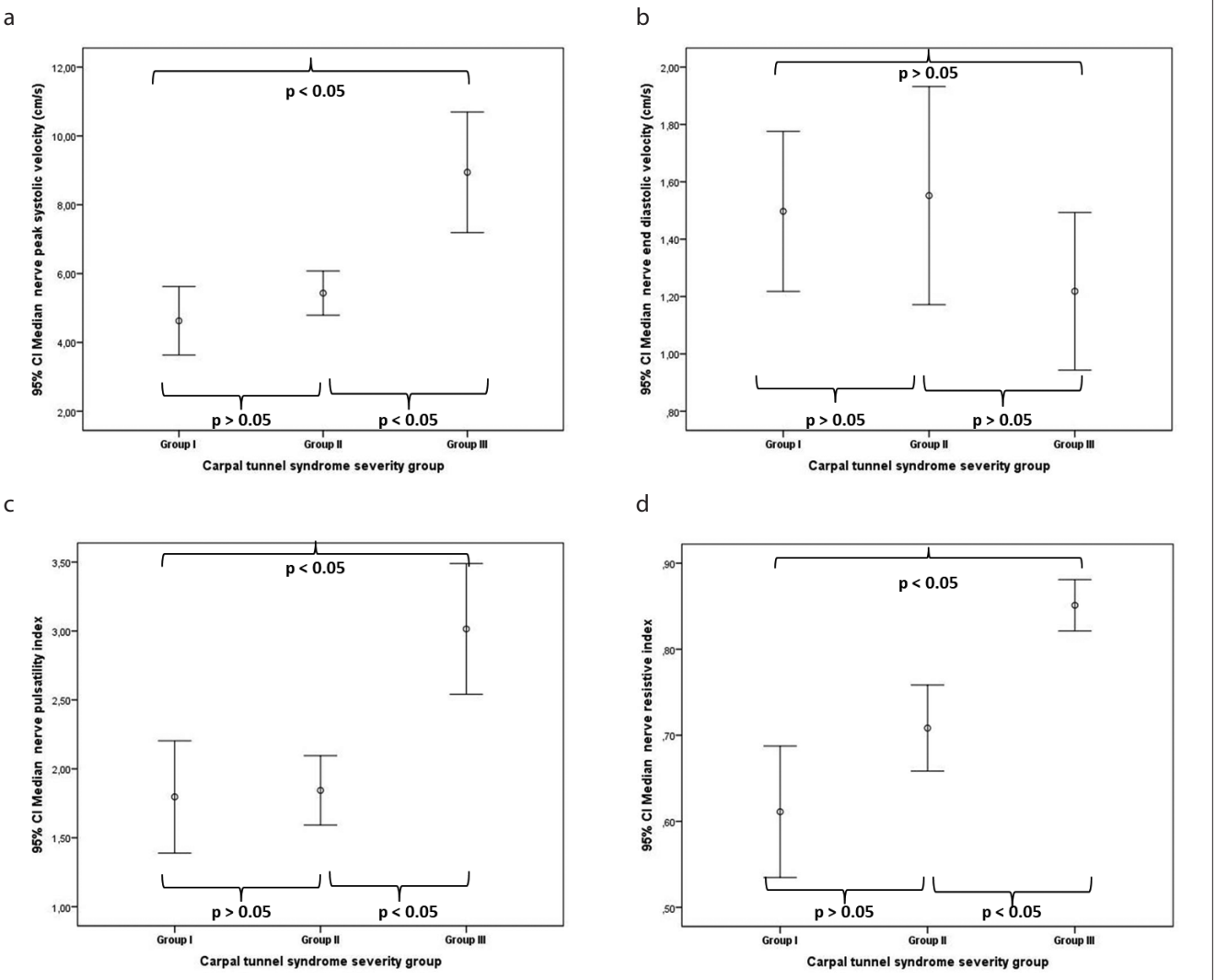
RESULTS

The median MN-PSV, MN-EDV, MN-PI, and MN-RI values were 5.3 cm/s, 1.2 cm/s, 2.0 cm/s, and 0.79 cm/s, respectively. The median values of the same parameters in patients with severe CTS were 7.2 cm/s, 1.1 cm/s, 2.71 cm/s, and 0.88 cm/s, respectively. The demographic findings and PDUS scores of the patients were evaluated according to the previously-mentioned CTS groups. In the control group, no hypervascularization was detected in any of the subjects.

Demographic and Ultrasonography Findings of the Study Groups

There was no significant difference between groups in terms of demographic (age and gender) parameters. MN- PSV, MN-PI,

Figure 2. a-d. Median nerve (MN) peak systolic velocity (a), MN end-diastolic velocity (b), MN pulsatility index (c) and MN resistive index (d) measurements of carpal tunnel syndrome severity groups



and MN-RI increased significantly from Group I to Group III, and these parameters were significantly higher in Group III than in the other two groups (Table 1, Figure 2 a-d). Also, MN-PSV, MN-PI, and MN-RI were higher in Group II than in Group I, though the difference was not statistically significant (Table 1). MN-EDV was similar among the groups (Table 2, Figure 2b).

Multivariate Logistic Regression Analysis for the Detection of Patients with Severe CTS

Multivariate logistic regression analysis was performed to determine the closest relationship between CTS severity and age, gender, MN-PSV, MN-EDV, MN-RI, and MN-PI. Upon multivariate logistic regression analysis, it was found that only MN-RI independently identified the patients with severe CTS (Odds Ratio=3.449, 95% Confidence Interval: 1.147–8.392, p<0.001; Table 2). According to this analysis, increased MN-RI (per 0.1) was found to increase the risk of patients having severe CTS by 3.45 fold (Table 2)

ROC Analysis for the Detection of Patients with Severe CTS

In the ROC analysis, the area under the curve was 0.846 for MN-RI for the prediction of severe CTS group (Area Under Receiver Operating Characteristic Curve=0.846, 95% Confidence Interval=0.762–0.930, p<0.001, p <0.05, Table 3 and Figure 3). An MN-RI value of 0.80 determined identified severe CTS with 85.2% sensitivity and 78.2% specificity.

DISCUSSION

The main finding in this study is that increased MN-RI obtained by PDUS independently predicts the severity of CTS patients. To our knowledge, this is the first study in the literature that inspects this relation. When the MN-RI cut-off value is taken as >0.80, it determines the risk of severe CTS development with acceptable sensitivity and specificity.

Electromyography is recommended as the gold standard with electrophysiological examinations in the evaluation of patients

Table 1. Demographic and Doppler ultrasonography findings of the study groups

Variable	Group I n=36	Group II n=52	Group III n=27	p
Age (year)	50.1±11.4	53.1±9.9	51.9±11.9	0.469
Gender (male/female)	5/31	9/43	6/21	0.692
Median nerve peak systolic velocity (cm/s)	4.63±2.94[α]	5.43±2.26[¥]	8.94±2.26	<0.001
Median nerve end diastolic velocity (cm/s)	1.50±0.82	1.55±1.34	1.22±0.69	0.407
Median nerve pulsatility index	1.80±1.19[α]	1.84±0.82[¥]	3.02±1.15	<0.001
Median nerve resistive index	0.61±0.23[α]	0.71±0.17[¥]	0.85±0.76	<0.001

The values were shown as mean±standard deviation or n (%), Group I=Control group, Group II=Medical treatment group and Group III=Planed surgery group
[α]the significant association between the Group I and Group III (p<0.05)
[β]the significant association between the Group I and Group II (p<0.05)
[¥]the significant association between the Group II and Group III (p<0.05)

Table 2. Variable regression analysis for the detection of patients with severe disease group

Variable	Odds Ratio	95% Confidence Interval	p
Age (year)	0.965	0.907–1.026	0.257
Gender (male/female)	0.588	0.113–3.052	0.528
Median nerve peak systolic velocity (cm/s)	1.194	0.972–1.466	0.091
Median nerve end diastolic velocity (cm/s)	0.325	0.039–2.728	0.300
Median nerve pulsatility index	0.927	0.372–2.310	0.870
Median nerve resistive index	3.449	1.147–8.392	<0.001

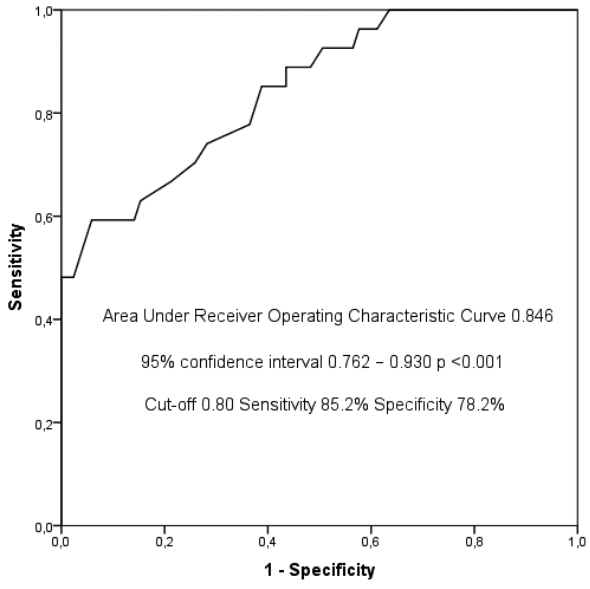
Table 3. Receiver Operating Characteristic analysis for the detection of patients with severe disease group

Variable	Area Under Receiver Operating Characteristic Curve	p	Cut-off value	Sensitivity	Specificity
Median nerve resistive index	0.846 (0.762–0.930)	<0.001	0.80	85.2%	78.2%

with CTS in daily practice and in determining disease severity (6). However, with electromyography, just electrophysiological properties of MN are shown. The MN entrapment status is shown indirectly. In addition, electromyography has electrode and needle placement problems (25). As a matter of fact, in order to complete these disadvantages and its deficiencies, MN US examination was recommended in the evaluation of CTS patients in 1990 guidelines (26). US examination was initially proposed as an adjunctive one rather than a diagnostic one and used as an adjunctive modality for detecting nerve abnormalities (such as bifid MN), and mass lesions, or guiding injection treatments. The B-mode US of MN provides information about the nerve anatomy and also can detect the transverse carpal tunnel ligament pressure (5, 25). MN CSA is also calculated with B-mode US, and the CSA increase is reported to be associated with the presence of CTS and CTS severity (6, 27). However, in some studies, CSA and CTS severity are not related (7, 12, 13). In the CTS guidelines, MN CSA has been recommended as a new study on this issue because of the lack of the precise location of the measurement site and having a very good cut-off value (21), because there are too many publications that have different threshold values (21).

Many studies have been carried out with the view that this increased vascularity is associated with direct nerve pressure may be associated with disease severity. Initially, CDUS was used, and in a large majority of these studies, MN vascularity was shown to be increased in CTS patients (6, 11, 28, 29) and reported to be important for diagnosis. However, the question as to whether increased vascularity is associated with disease severity is unclear and has conflicting results. In some of these studies, the severity of CTS was associated with increased MN vascularity (10, 11), although some studies did not correlate with CTS severity (12, 25, 26, 30). At the same time, the increase in vascularity is a relative and subjective measurement, resulting in a preference for flow velocity and pattern obtained with PDUS. The last published guideline also recommended more objective studies of vascularity enhancement (21). With the recent MN-PSV study (1, 8), there is currently limited MN-RI examination as far as we have investigated, and our study is second in the literature (14). A similar study was performed

Figure 3. Receiver operating characteristic curves with median nerve resistive index for the diagnosis of severe carpal tunnel syndrome



by Sayed et al. (14), and 28 patients with CTS were evaluated by electromyography. In patients with MN involvement, MN-RI values were reported to be significantly higher than those without MN involvement. The most important limitation in the Sayed et al. (14) study is that the number of patients evaluated is low, and the MN-RI value and its importance in patients with severe CTS is not stated. In our study, CTS severity in 115 wrists was evaluated according to electrophysiological examinations, and MN-RI levels were significantly higher in patients with severe CTS than in other groups. In addition, when we were taken as a 0.80 threshold value, we showed that patients with severe CTS were determined with acceptable sensitivity and specificity.

MN-PI was not observed directly in the MN vascularity increase region, PI was observed in arterial radialis indicis and arterial radialis palmaris from the 1st and 5th fingers while resting and provocation and it has been reported that the decline in PI value obtained by both resting and provocation from arterial radialis indicis passing through the carpal tunnel is greater in patients with moderate to advanced CTS (29). As a result of the study, patients with CTS were reported to have impaired vasomotor function with PDUS that was reported to be associated with the severity of CTS disease (29). In another study similar to the previous study, the MN intraneural flow pattern was used. This study by Evans et al. (1) investigated MN intraneural flow. The mean PSV obtained from the CT entry of patients with CTS was found to be significantly lower. However, the findings of this study are contradictory to those of previous CTS studies. In the study conducted by Evans et al. (1), MN-RI evaluation was performed in addition to MN-PSV in PDUS evaluation, even though MN-PSV evaluation was performed, but they did not give us any information about MN-RI importance. It is clear that MN-RI significantly increased in the prevalence of CTS. The most pertinent problem in this study was reported to be that MN-PSV values were lower in patients with CTS than in in asymptomatic cases (1). Although vascularity decreases in this study, increased MN vascularity has been reported in the majority of studies (6, 11, 28, 29). Wilder-Smith et al. (8) reported that the MN-PSV value obtained by Evans et al. (1) was significantly low. Our study value was very close to the value reported by Wilder-Smith Therimadasamy (8) In all patients with CTS, the mean median value was 5.3 cm/s; the MN-PSV value increased with disease severity and the median value in the severe CTS group was 7.2 cm/s. This value in our study is compatible with that reported by Wilder-Smith Therimadasamy (8) and there is no other threshold value in the literature to the best of our knowledge.

In 2010, inconsistencies in the studies on MN CSA enhancement (7, 13), and the subjectivity of data on increased vascularity and hypoechogenicity were directed us towards the PDUS, but several studies have also been discordant (1, 8). It is clear that there is a need for an US parameter that is different from these parameters in patients with CTS; this should be physiopathologically appropriate, more objective and acceptable. For this reason, the hypothesis that RI values obtained from intraneural arteries that have not previously been used in patients with CTS may be associated with the severity of CTS. It is certain that the increase in vascularity, that is the result of compressed MN, is encountered by an increased resistance. For this reason, MN-RI is thought to be increased. Evans et al. (1) demonstrated that there is an increase in MN-RI in CTS, but they did not emphasize this finding in their studies. This is because in those years there was not much data available on RI evaluation (especially renal RI), especially for other organs, and the importance was not as much as it is today. In our study MN-RI was found to increase with the increasing severity of patients with CTS. MN-RI also independently determines the severity of CTS better than the other PDUS parameters associated with CTS (including MN-PSV). When the MN-RI threshold value is taken as 0.80, it identifies patients with severe CTS with acceptable sensitivity and specificity. To the best of our knowledge, this is the first in the literature as far as we investigate these findings and we could not find a new threshold to compare. CTS severity is not associated with MN-EDV in this study. Intraneural MN-PSV and MN-PI values were associated only in univariate analysis, but no significant correlation was found in multivariate analyses. In conclusion, PDUS findings may contribute to the diagnosis of CTS, its severity, and its treatment plan. This method can be used as a noninvasive method in cases in that electromyography cannot be performed.

A major limitation of our study is that our study was performed in a single center, it had a cross sectional design, and included a limited number of patients. The results of MN-RI and other studies could not be compared due to the lack of sufficient data on MN-RI values and its clinical availability in patients with CTS in the literature. For this reason, more studies with bigger samples are needed. In our study, CSA and hypoechogenicity with B-mode US were not evaluated because of the conflicting results of many studies. A new review of MN vascularity was not evaluated in our study. Better and more meaningful results could be obtained if we had assessed the SMI in our study.

CONCLUSION

According to our results, the increase in the MN-RI in CTS patients is independently associated with disease severity and may be used in the clinical follow-up of these patients. We think that MN-RI evaluation should be a part of the assessment of CTS. Clinicians should consider patients with MN-RI >0.80, as serious CTS.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Başkent University School of Medicine (24.06.2015/ KA15/204).

Informed Consent: Written informed consent was obtained from participants who participated in this study.

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REFERENCES

1. Evans KD, Volz KR, Hutmire C, Roll SC. Morphologic Characterization of Intraneural Flow Associated With Median Nerve Pathology. J Diagn Med Sonogr 2012; 28: 11-9. [Crossref]
2. de Krom MC, Knipschild PG, Kester AD, Thijs CT, Boekkooi PF, Spaans F. Carpal tunnel syndrome: prevalence in the general population. J Clin Epidemiol 1992; 45: 373-6. [Crossref]
3. Porth CM. Essentials of Pathophysiology. 3. Philadelphia: Lippincott Williams & Wilkins 2011.
4. Bianchi, S, Martinoli C. Ultrasound of the Musculoskeletal System. Berlin: Springer-Verlag 2007. [Crossref]
5. Özçakar L, Kara M, Chang KV, Çarl AB, Akkaya N, Tok F, et al. Nineteen reasons why physiatrists should do musculoskeletal ultrasound: EURO-MUSCULUS/USPRM recommendations. Am J Phys Med Rehabil 2015; 94: 45-9. [Crossref]
6. Vanderschueren GA, Meys VE, Beekman R. Doppler sonography for the diagnosis of carpal tunnel syndrome: a critical review. Muscle Nerve 2014; 50: 159-63. [Crossref]
7. Karahan AY, Arslan S, Ordahan B, Bakdik S, Ekiz T. Superb Microvascular Imaging of the Median Nerve in Carpal Tunnel Syndrome: An Electrodiagnostic and Ultrasonographic Study. J Ultrasound Med 2018; 37: 2855-61. [Crossref]
8. Wilder-Smith EP, Therimadasamy A. Nerve blood flow. J Ultrasound Med 2013; 32: 187-8. [Crossref]
9. Ghasemi-Esfe AR, Khalilzadeh O, Mazloumi M, Vaziri-Bozorg SM, Niri SG, Kahnouji H, et al. Combination of high-resolution and color Doppler ultrasound in diagnosis of carpal tunnel syndrome. Acta Radiol 2011; 52: 191-7. [Crossref]
10. Ghasemi-Esfe AR, Khalilzadeh O, Vaziri-Bozorg SM, Jajroudi M, Shakiba M, Mazloumi M, et al. Color and power Doppler US for diagnosing carpal tunnel syndrome and determining its severity: a quantitative image processing method. Radiology 2011; 261: 499-506. [Crossref]
11. Mohammadi A, Ghasemi-Rad M, Mladkova-Suchy N, Ansari S. Correlation between the severity of carpal tunnel syndrome and color Doppler sonography findings. AJR Am J Roentgenol 2012; 198: 181-4. [Crossref]
12. Bayrak IK, Bayrak AO, Tilki HE, Nural MS, Sunter T. Ultrasonography in carpal tunnel syndrome: comparison with electrophysiological stage and motor unit number estimate. Muscle Nerve 2007; 35: 344-8. [Crossref]
13. Kwon HK, Kang HJ, Byun CW, Yoon JS, Kang CH, Pyun SB. Correlation between Ultrasonography Findings and Electrodiagnostic Severity in Carpal Tunnel Syndrome: 3D Ultrasonography. J Clin Neurol 2014; 10: 348-53. [Crossref]
14. Sayed Z, Hazim T, Neven Fa, Manal A. The value of power and pulsed Doppler in the diagnosis of CTS: Is a solution in sight. The Egyptian Journal of Radiology and Nuclear Medicine 2013; 44: 589-96. [Crossref]
15. Ikeda M, Okada M, Toyama M, Uemura T, Takamatsu K, Nakamura H. Comparison of Median Nerve Cross-sectional Area on 3-T MRI in Patients With Carpal Tunnel Syndrome. Orthopedics 2017; 40: 77-81. [Crossref]
16. Wang CK, Jou IM, Huang HW, Chen PY, Tsai HM, Liu YS, et al. Carpal tunnel syndrome assessed with diffusion tensor imaging: comparison with electrophysiological studies of patients and healthy volunteers. Eur J Radiol 2012; 81: 3378-83. [Crossref]
17. Ninet S, Schnell D, Dewitte A, Zeni F, Meziani F, Darmon M. Doppler-based renal resistive index for prediction of renal dysfunction reversibility: A systematic review and meta-analysis. J Crit Care 2015; 30: 629-35. [Crossref]
18. Tanaka K, Numata K, Morimoto M, Shirato K, Saito S, Imada T, et al. Elevated resistive index in the hepatic artery as a predictor of fulminant hepatic failure in patients with acute viral hepatitis: a prospective study using Doppler ultrasound. Dig Dis Sci 2004; 49: 833-42. [Crossref]
19. Zhang L, Duan YY, Li JM, Yin JK. Hemodynamic features of Doppler ultrasonography in patients with portal hypertension: intraoperative direct measurement of portal pressure in the portal venous system. J Ultrasound Med 2007; 26: 1689-96. [Crossref]
20. Frauchiger B, Schmid HP, Roedel C, Moosmann P, Staub D. Comparison of carotid arterial resistive indices with intima-media thickness as sonographic markers of atherosclerosis. Stroke 2001; 32: 836-41. [Crossref]
21. Graham B, Peljovich AE, Afra R, Cho MS, Gray R, Stephenson J, et al. The American Academy of Orthopaedic Surgeons Evidence-Based Clinical Practice Guideline on: Management of Carpal Tunnel Syndrome. J Bone Joint Surg Am 2016; 98: 1750-4. [Crossref]
22. Padua L, LoMonaco M, Gregori B, Valente EM, Padua R, Tonali P. Neurophysiological classification and sensitivity in 500 carpal tunnel syndrome hands. Acta Neurol Scand 1997; 96: 211-7. [Crossref]
23. Bayrak IK, Bayrak AO, Kale M, Turker H, Diren B. Bifid median nerve in patients with carpal tunnel syndrome. J Ultrasound Med 2008; 27: 1129-36. [Crossref]
24. Altınkaya N, Leblebici B. Prevalence of persistent median artery in carpal tunnel syndrome: sonographic assessment. Surg Radiol Anat 2016; 38: 511-5. [Crossref]
25. Öztürk GT, Malas FÜ, Yildizgören MT, Baki AE, İnal EE, Batmaz İ, et al. Ultrasonographic Assessment of the Femoral Cartilage Thickness in Patients with Pes Planus: A Multicenter Study by TURK-MUSCULUS. Am J Phys Med Rehabil 2015; 94: 568-72. [Crossref]
26. Cartwright MS, Hobson-Webb LD, Boon AJ, Alter KE, Hunt CH, Flores VH, et al. Evidence-based guideline: neuromuscular ultrasound for the diagnosis of carpal tunnel syndrome. Muscle Nerve 2012; 46: 287-93. [Crossref]
27. Akcar N, Ozkan S, Mehmetoglu O, Calisir C, Adapinar B. Value of power Doppler and gray-scale US in the diagnosis of carpal tunnel syndrome: contribution of cross-sectional area just before the tunnel inlet as compared with the cross-sectional area at the tunnel. Korean J Radiol 2010; 11: 632-9. [Crossref]
28. Ozcan HN, Kara M, Ozcan F, Bostanoglu S, Karademir MA, Erkin G, et al. Dynamic Doppler evaluation of the radial and ulnar arteries in patients with carpal tunnel syndrome. AJR Am J Roentgenol 2011; 197: 817-20. [Crossref]
29. Ghasemi-Esfe AR, Morteza A, Khalilzadeh O, Mazloumi M, GhasemiEsfe M, Rahmani M. Color Doppler ultrasound for evaluation of vasomotor activity in patients with carpal tunnel syndrome. Skeletal Radiol 2012; 41: 281-6. [Crossref]
30. Yoshii Y, Tanaka T, Ishii T. Correlations of median nerve area, strain, and nerve conduction in carpal tunnel syndrome patients. Hand 2016; 11: 161-7. [Crossref]

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Original Research

Comparison of Urinary Incontinence Subgroups according to Possible Risk Factors

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ABSTRACT

Objective: Urinary incontinence (UI) is involuntary urine flow that causes social and hygienic problems. The association between risks factors and UI in women was assessed. We compared the risk factors based on UI subtypes.

Methods: The study included 470 women with different UI types (320 urge UI [UUI], 80 stress UI [SUI], and 70 mixed UI [MUI]). Age, educational level, urban/rural residence, parity, delivery type, diabetes mellitus (DM), and hypertension status, any neurological abnormality, menopausal status, surgical history, and body mass index (BMI) were obtained.

Results: Of all women, 320, 80, and 70 had UUI, SUI, and MUI, respectively. The groups did not differ significantly in terms of age, hypertension status, neurological abnormality rate, smoking status, or surgical history (all $p>0.05$). Parity, episiotomy, DM status, delivery type, menopause status, hysterectomy history, and BMI differed significantly among the groups (all $p<0.05$).

Conclusion: Our study found that parity, episiotomy, DM status, delivery type, menopause status, hysterectomy history, and BMI may be independent risk factors for different UI types.

Keywords: Stress, urge, urinary incontinence

INTRODUCTION

The lower urinary system consists of the bladder and urethra. However, urine filling and discharging occurs in harmony with the pelvic floor and neurological system that affect the working mechanism of the vesicourethral unit formed by these two anatomical structures. Urinary incontinence is involuntary urine flow that causes social and hygienic problems.

The International Continence Society describes urinary incontinence (UI) as any involuntary urine leakage; UI is subdivided into stress, urge, and mixed UI (MUI). Stress UI (SUI) is defined as intra-abdominal involuntary urine flow that accompanies increased intraabdominal pressure due to urethral hypermobility, bladder neck and insufficient support, and/or proximal urethra arising from failure in the intrinsic sphincter (1, 2). SUI is involuntary urination that occurs while exercising, laughing, sneezing, or coughing. Involuntary loss of urine associated with an urgent need to urinate constitutes UI; MUI is a combination of SUI and urge UI (UUI) (3, 4). Although its incidence increases with age, it affects approximately 20% of all women. Current epidemiological data showed that 17% of women older than 20 years and 38% of women older than 60 years were affected (5, 6).

Previous studies showed that smoking, old age, female sex, familial predisposition, number of births, menopause, poor general health, diabetes mellitus, asthma, high body mass index (BMI)/obesity, chronic constipation, previous urogynecological surgery, cognitive decline, decreased physical function, and other medical and social conditions (pulmonary diseases, neurological diseases, and spinal cord injuries) were UI risk factors (7-10). Here, we evaluated the risk factors in our patients with different UI types. We compared the risk factors based on UI subtypes.

METHODS

This was a prospective case–control study conducted in the department of urology of a tertiary research and education hospital from January 2014 to January 2015. All patients provided informed written consent, and the Sanko University Ethics Committee (November 21, 2018/number: 2018/11-08) approved the protocol. We recorded patient Age, parity, urban/rural residence, educational status, delivery type, diabetes mellitus and hyper-

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Table 1. Comparison of the clinical parameters between the different types of urinary incontinence

Variables	Urge UI group (n=320)	Mix UI group (n=70)	Stress UI group (n=80)	p
Age (years)	49.48±19.45	52.82±7.05	44.88±9.23	0.127
BMI (kg/m^2)	27.62±6.57	23.07±0.86	22.28±1.22	0.006*
Parity	2.95±2.3	3.54±1.1	2.5±0.88	0.020*
Episiotomy	40 (0.13)	0 (0)	0 (0)	0.017*
Diabetes mellitus	57 (0.18)	35 (0.50)	27 (0.34)	0.001*
Hypertension	102 (0.32)	30 (0.43)	15 (0.19)	0.128
Delivery type				
Cesarean	112 (0.35)	0 (0)	22 (0.28)	0.001*
Vaginal	167 (0.48)	70 (0.93)	57 (0.63)	
No birth	40 (0.13)	0 (0)	0 (0)	
Neurological abnormalities	47 (0.15)	12 (0.18)	5 (0.06)	0.362
Smoking	20 (0.06)	2 (0.04)	0 (0)	0.317
Menopause	137 (0.43)	30 (0.43)	12 (0.16)	0.015*
Previous surgery	135 (0.42)	42 (0.61)	30 (0.38)	0.143
Previous cesarean	75 (0.23)	5 (0.07)	22 (0.28)	0.106
Hysterectomy	20 (0.06)	35 (0.50)	5 (0.06)	0.001*

*: p<0.05: Two-sided p values were considered statistically significant, BMI: body mass index.

tension status, any neurological abnormality, smoking status, and history of surgery, episiotomy, or cesarean section were obtained. The hospital discharge records described maternal conditions based on ICD-10 diagnoses. Incontinence type was evaluated based on the International Continence Society. Exclusion criteria were presence or history of inferior genital tract cancer, previous treatment with pelvic radiotherapy, pregnancy, and previous urogynecological surgery (e.g., sling, anterior/posterior colporrhaphy, Burch operation).

Statistical Analysis

The Shapiro–Wilk test was used to confirm the normality of distribution of continuous variables. The Kruskal–Wallis and Dunn's multiple comparisons tests were used to compare non-normally distributed data among the three groups. The chi-squared test was used to compare pairs of categorical variables, and the Bonferroni correction was used to adjust for multiple comparisons when the chi-squared test result was significant. The Firth logistic regression model of the R ver. 3.5.1 brglm package to reduce bias caused by the low prevalence of certain categories when a binomial-response general linear model is used (11). All univariate statistical analyses were performed using SPSS for Windows ver (IBM SPSS Corp.; Armonk, NY, USA). 24.0; p<0.05 was accepted as statistically significant.

Main Points:

- Although UI is a treatable condition, many women experience psychological, social, and physical problems due to this disorder.
- Parity, episiotomy, DM status, delivery type, menopause status, hysterectomy history, and BMI may be independent risk factors for different UI types.

RESULTS

The study included 470 women with UI, of which 320 (68.1%), 80 (17%), and 70 (14.9%) had UUI, SUI, and MUI, respectively. Table 1 shows the comparison of demographic and clinical characteristics. No significant among-group difference was found for age, hypertension, smoking status, neurological abnormality rate, or surgical history (all p>0.05). Parity, episiotomy, DM status, delivery type, menopause, hysterectomy status, and BMI differed significantly among the groups (all p<0.05). On subgroup analysis using the Dunn's test, BMI in the UUI group was significantly higher than that in the SUI group (p=0.001); significant differences were observed between the SUI and MUI (p=0.011) and between UUI and MUI (p=0.001) groups. Bonferroni correction showed that the incidence rates of episiotomy and DM were significantly higher in the UUI group (p=0.017 and 0.001, respectively). Vaginal delivery was more common in the MUI group (p=0.001), menopause in the UUI group (p=0.015), and previous hysterectomy in the MUI group (p=0.001). Table 2 summarizes the outcomes of logistic regression. Vaginal delivery (OR=82.66) and menopause (OR=32.43) were risk factors for SUI compared with UUI; episiotomy (OR=20.82) and DM (OR=4.32) were risk factors for UUI compared with MUI; and hysterectomy

Tablo 2. Odds ratios of the clinical parameters between the subgroups

Variable groups	OR (95% CI)	p
Urge UI group vs Mix UI		
BMI	0.870 (0.801–0.945)	0.001*
Episiotomy	20.824 (0.992–436.917)	0.003*
Diabetes mellitus	4.326 (1.334–14.028)	0.015*
Delivery type		
No vaginal	0.03 (0.001–0.681)	0.027*
Menopause	0.12 (0.034–0.421)	0.001*
Hysterectomy	0.153 (0.042–0.551)	0.004*
Stress UI group vs Urge UI group		
BMI	0.84 (0.77–0.92)	0.001*
Episiotomy	0.017 (0.001–0.472)	0.017*
Diabetes mellitus	0.179 (0.05–0.64)	0.008*
Delivery type		
No vaginal	82.664 (3.529–1936.155	0.006*
No vaginal no cesarean	47.391 (1.493–1504.326)	0.029*
Menopause	32.434 (7.378–142.584)	0.001*
Stress UI group vs Mix UI group		
BMI	0.48 (0.275–0.846)	0.011*
Hysterectomy	17.112 (3.893–75.228)	0.002*

*: p<0.05: Two–sided p values were considered statistically significant, BMI: body mass index; UI: urinary incontinence

(OR=17.11) and menopause (OR=0.48) were risk factors for SUI compared with MUI. No other significant differences were found among the groups.

DISCUSSION

UI is an important medical and social public health problem due to its incontinence, family, and healthcare costs. Although one-third of women have UI, most women do not consult a physician for this symptom. It is especially important to direct patients with risk factors.

The risk factors were evaluated for UI by UI subtypes. On regression analysis, vaginal delivery (OR=82.66) and menopause (OR=32.43) were risk factors for SUI compared with UUI; episiotomy (OR=20.82) and DM (OR=4.32) were risk factors for UUI compared with MUI; and hysterectomy (OR=17.11) and menopause (OR=0.48) were risk factors for SUI compared with MUI. Previous studies have found that obesity was a risk factor for UI (12, 13). In a cross-sectional study of the Women's Health Australia project, obese women with BMI of 30–40 kg/m^2 were at a two-fold higher risk of UI than women with BMI<20 kg/m^2 (14). Obesity was also found as a risk factor in all subgroups. Basak et al. (10) found that women attending a outpatient urology department tended to have MUI and more than risk or associated factors, including obesity and DM (10).

Childbearing may cause UI in women. The delivery mode is considered a major risk factor for UI; most UI complaints are associated with pregnancy, childbirth, or postpartum issues. However, the impact of birth mode on incontinence and the possible protective role of cesarean section remain debatable. Singh et al. (15) found that UI was more common in women with a history of vaginal delivery compared with nulliparous women and those who underwent cesarean section. However, Parazzini et al. (16) found no increased risk of UUI after vaginal delivery. In another study, the prevalence of both SUI and UUI was lower among nulliparous women and higher among women with 5–6 deliveries (17). This may be associated with impairment in pelvic muscle nerves during birth, development of atrophy in muscles, and development of prolapse over time (18). We found that vaginal delivery was a risk factor for SUI. By contrast, some studies showed that delivery with a birth weight of >4 kg affected UI (19).

Any association between episiotomy and UI remains unclear. In two Turkish studies, no significant correlations were evident between vaginal episiotomy, age at first childbirth, and UI (20, 21). However, Chang et al. (22) found that UI was significantly more common in women who had episiotomy compared with those who did not. Episiotomy was a risk factor in the UI group compared with the MUI group. According to the literature, episiotomy can effectively prevent anterior perineal laceration, but not perineal damage, as well as urinary and anal incontinence and pelvic floor relaxation (23).

DM is also associated with UI. Kılıc et al. (24) considered that DM triggered UI by causing glycosuria, detrusor muscle overactivity, recurrent urinary infections, and diabetic cystopathy. Although some studies found correleations between DM status and UI, other studies did not (25, 26). We found that DM was a risk factor in the UI group compared with the MUI group.

Hysterectomy violates the integrity of the pelvic floor musculature and connective tissue, and denervates the bladder, all of which are associated with UI (27). Two long-term follow-up studies showed significant associations between hysterectomy and UI (28, 29). Similarly, we also found a strong association between hysterectomy and SUI compared with MUI. Previous studies found that menopause affected the UI rate. De Boer et al. reported that menopausal women reported significantly more UI and required more pelvic organ prolapse (POP)/incontinence surgeries than did other women Another study found that menopause predisposed women to POP (30).

CONCLUSION

Although UI is a treatable condition, many women experience psychological, social, and physical problems due to this disorder. Many studies have been conducted on UI risk factors. In our study, we investigated the risk factors among UI subtypes. Such analyses could improve treatment outcomes.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sanko University School of Medicine (November 21, 2018/number, 2018/11-08).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Abrams P, Andersson KE, Birder L, Brubaker L, Cardozo L, Chapple C, et al. Fourth International Consultation on Incontinence Recommendations of the International Scientific Committee: Evaluation and treatment of urinary incontinence, pelvic organ prolapse, and fecal incontinence. Neurourol Urodyn 2010; 29: 213-40. [Crossref]
2. Wein AJ. Pathophysiology and categorization of voiding dysfunction. In Walsh PC, Retik AB, Vaughan ED (eds)Campbell's Urology. Philadelphia: WB Saunders 2002; 887-98.
3. Minassian V. Drutz H. Al-Badr A. Urinary incontinence: a worldwide problem. Int J Gynecol Obstet 2003; 82: 327-38. [Crossref]
4. Hayek S. Abrams P. Women's lower urinary tract function and dysfunction: definitions and epidemiology. Minerva Gynecol 2004; 56: 311-25.
5. Goode PS, Burgio KL, Redden DT, Markland A, Richter HE, Sawyer P, et al. Population based study of incidence and predictors of urinary incontinence in black and white older adults. J Urol 2008; 179: 1449-53. [Crossref]
6. Lukacz ES, Santiago-Lastra Y, Albo ME, Brubaker L. Urinary Incontinence in Women: A Review. JAMA 2017; 318: 1592-604. [Crossref]
7. Markland AD, Goode PS, Burgio KL, Redden DT, Richter HE, Sawyer P, et al. Correlates of urinary, fecal, and dual incontinence in older African-American and white men and women. J Am Geriatr Soc 2008; 56: 285-90. [Crossref]
8. Smith AL, Wang PC, Anger JT, Mangione CM, Trejo L, Rodríguez LV, et al. Correlates of urinary incontinence in community-dwelling older Latinos. J Am Geriatr Soc 2010; 58: 1170-6. [Crossref]
9. Tennstedt SL, Link CL, Steers WD, McKinlay JB. Prevalence of and risk factors for urine leakage in a racially and ethnically diverse population of adults: The Boston Area Community Health (BACH) Survey. Am J Epidemiol 2008; 167: 390-9. [Crossref]
10. Basak T, Uzun S, Arslan F. Incontinence features, risk factors, and quality of life in Turkish women presenting at the hospital for urinary incontinence. J Wound Ostomy Continence Nurs 2012; 39: 84-9. [Crossref]
11. Firth D. Bias reduction of maximum likelihood estimates. Biometrika 1993; 80: 27-38. [Crossref]
12. Tsai Y-C, Liu CH. Urinary incontinence among Taiwanese women: An outpatient study of prevalence, co morbidity, risk factors, and quality of life. Int Urol Nephrol 2009; 41: 795-803. [Crossref]
13. Onur R, Deveci SE, Rahman S, Sevindik F, Acik Y. Prevalence and risk factors of female urinary incontinence in eastern Turkey. Int J Urol 2009; 16: 566-9. [Crossref]
14. Chiarelli P, Brown W, McElduff P. Leaking urine: prevalence and associated factors in Australian women. Neurourol Urodyn 1999; 18: 567-77. [Crossref]
15. Singh U, Agarwal P, Verma ML, Dalela D, Singh N, Shankhwar P. Prevalence and risk factors of urinary incontinence in Indian women: A hospital-based survey. Indian J Urol 2013; 29: 31-6. [Crossref]
16. Parazzini F, Chiaffarino F, Lavezzari M, Giambanco V. Risk factors for stress, urge or mixed urinary incontinence in Italy. BJOG 2003; 110: 927-33. [Crossref]
17. Shakhatreh F. Epidemiology of urinary incontinence in Jordanian women. Saudi Med J 2005; 26: 830-5.
18. Kaşıkçı M, Kılıç D, Avşar G, Şirin M. Prevalence of urinary incontinence in older Turkish women, risk factors, and effect on activities of daily living. Arch Gerontol Geriatr 2015; 61: 217-23. [Crossref]
19. Oliveira E, Zuliani L, Ishicava J, Silva SV, Albuquerque SS, Souza AMB, Barbosa CP. Evaluation of factors related to the occurrence of female urinary incontinence. Rev Assoc Med Bras 2010; 56: 688-90. [Crossref]
20. Bilgili N, Belgin A, Emel E, Sultan A. Incidence of the urinary incontinence and affecting risk factors in women Turkiye Klinikleri. J Med Sci 2008; 28: 487-93.
21. Karaçam Z, Eroğlu K. Effects of episiotomy on bonding and mothers' health. J Adv Nurs 2003; 43: 384-94. [Crossref]
22. Chang SR, Chen KH, Lin HH, Chao YM, Lai YH. Comparison of the effects of episiotomy and no episiotomy on pain, urinary incontinence, and sexual function 3 months postpartum: a prospective follow-up study. Int J Nurs Stud 2011; 48: 409-18. [Crossref]
23. Hartmann K, Viswanathan M, Palmieri R, Gartlehner G, Thorp J, Lohr KN. Outcomes of routine episiotomy: a systematic review. JAMA 2005; 293: 2141-8. [Crossref]
24. Kılıç M. Incidence and risk factors of urinary incontinence in women visiting Family Health Centers. Springerplus 2016; 5: 1331. [Crossref]
25. Song YF, Zhang WJ, Song J, Xu B. Prevalence and risk factors of urinary incontinence in Fuzhou Chinese women. Chin Med J 2005; 118: 887-92.
26. Zhu L, Lang J, Wang H, Han S, Huang J. The prevalence of and potential risk factors for female urinary incontinence in Beijing, China. Menopause 2008; 15: 566-9. [Crossref]
27. Skorupska KA, Miotła P, Kubik-Komar A, Rechberger E, Adamiak-Godlewska A, Rechberger T. Urinary incontinence after hysterectomy-does type of surgery matter? Ginekol Pol 2016; 87: 94-7. [Crossref]
28. Altman D, Granath F, Cnattingius S, Falconer C. Hysterectomy and risk of stress-urinary-incontinence surgery: nationwide cohort study. Lancet 2007; 370: 1494-9. [Crossref]
29. Allahdin S, Harrild K, Warraich QA, Bain C. Comparison of the long - term effects of simple total abdominal hysterectomy with transcervical endometrial resection on urinary incontinence. BJOG 2008; 115: 199-204. [Crossref]
30. De Boer TA, Slieker-Ten Hove MC, Burger CW, Kluivers KB, Vierhout ME. The prevalence and factors associated with previous surgery for pelvic organ prolapse and/or urinary incontinence in a cross-sectional study in The Netherlands. Eur J Obstet Gynecol Reprod Biol. 2011; 158: 343-9. [Crossref]

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Original Research

Strain Wave Elastography Imaging for the Evaluation of Pancreas in Healthy Volunteers

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ABSTRACT

Objective: The objective of this study is to evaluate the normal elastography values of the three anatomical regions (head, corpus, and tail) of the pancreas in the normal adult population using strain elastography (SE) imaging.

Methods: The study included 93 (35 males and 58 females) healthy volunteers. In the healthy volunteers, we semi-quantitatively assessed the pancreatic elasticity by measuring the SE images based on age and gender in the healthy individuals. We also compared the elasticity measurements with respect to gender and age. A threshold value was derived for the healthy volunteers.

Results: In the healthy volunteers, the strain ratio (SR) values were compared with respect to gender and age (before and after 40 years). The elastography values were determined separately for each region of the pancreas. Then, the elastography values before and after the age of 40 years were determined. Importantly, we compared the pancreatic elastography values between the genders, pancreatic areas, and before and after the age of 40 years. The significance value of p was taken at 0.05.

As a result, there was no significant difference between males and females. The average SR values in women and men were 1.86±0.98 (0.26–4.54) and 1.76±1.20 (0.43–5.26), respectively. There was no significant difference between the SR values measured with respect to age before and after 40 years (p=0.293). The average SR value did not differ between woman and men (p=0.751). Only the measurements of pancreas corpus were slightly different before and after the age of 40 years (p=0.018).

Conclusion: SE imaging can be used as an efficient technique for the evaluation of pancreatic elasticity. This study determined the normal elasticity values of the pancreas in healthy volunteers. Information obtained from the healthy adults can serve as a baseline against which pancreatic diseases can be examined in clinical practice.

Advances in knowledge: Designing the value of SR of pancreas parenchyma in healthy volunteers will lead to further elastography studies that can be used in the differential diagnosis of pathological tissues in the pancreatic tissue, leading to future monitoring of other pathologies.

Keywords: Elastography, pancreas, strain index, strain ratio

INTRODUCTION

The pancreas is an organ that has both exocrine and endocrine secretory functions. In total, 85% of the entire pancreas comprises the exocrine portion, 2% is the endocrine portion, and the rest is the extracellular matrix and vascular structure (1, 2). The pancreas shares a close relationship with many organs and structures in terms of localization (2).

The pancreas is an organ in which many malignant and benign diseases can occur (3). Many diseases ranging from diabetes mellitus to pancreatic carcinoma can be observed in the pancreas (1). Sonographic elastography (USE), which is used in the diagnosis of diseases by measuring tissue stiffness, especially for the distinguishing malignant and benign diseases, has witnessed a recent and frequent usage for the diagnosis of may pancreatic diseases.

USE is a new procedure that shows the stiffness of the tissue under examination. This procedure assists in obtaining information about the stiffness of the observed lesion or tissue and revealing the difference of the examined tissue from the normal tissue (1,4-5). Many studies have been conducted on elastography so far, with several of them examining the usefulness of elastography. It is not only used in the examination of superficial organs, but also in the examination of many organ tissues (6-9). Although there are many elastography studies on organs, there are very few publications and research on the USE studies of the pancreas. Because the location of the pancreas and its smaller size as compared to other organs are some of the reasons that led to only a few studies in this region (7-9). However, new studies report that USE is technically more useful. Especially, the use of elastography in the pancreatic tissue, where the biopsy material is difficult to obtain, is more important (7-9)

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There are two importantly known USE modalities. The first one is strain elastography (SE), which can perform a qualitative or semi-quantitative measurement. This technique is performed by applying and compressing the tissues from the body surface with the help of a probe and obtaining information about tissue's hardness. The tissue is compressed by the practitioner who apply rhythmic pressures at the intervals of every two seconds with the aid of a probe. B-mode, color map, and compression–decompression wave appear on the same screen. Then, a color image indicating the stiffness of the examined tissue is shown and the semi-quantitative strain index (SI) is determined. After the SI of neighboring tissues is taken as a reference, the indices of the target tissue and adjacent tissue are proportioned for obtaining the strain ratio (SR). Hence, information about the stiffness of the tissue is secured (9, 10).

The second method is sharewave elastography (SHE). In this method, independent of the user, the velocity of the wave (m/s), or the pressure value (Kpa) of the wave, which is obtained by the parallel movement of the waves sent to the tissue, is measured. Therefore, the faster travel of the wave in the tissue indicates the respective hardness in the tissue, and the information about this tissue is numerically obtained (9, 10).

In the light of all these information and references, we determined the normal elastographic values of the three anatomical regions (head, corpus, tail) of the pancreas in the normal adult population using SE technique in our study. Moreover, we aimed to guide the further elastography studies that can be later used in the differential diagnosis of pathological tissues in the pancreatic tissue, along with the monitoring of many other pathologies.

Main Points:

- Sonoelastography is a new imaging modality that canquantitatively measure tissue elasticity with the use of sonography. Strain elastography assesses tissue elasticity by comparing local tissue displacements before and after the application of a compressive force. Basically hard tissues show less deformation than soft tissues under transducer compression (strain).
- Elastography is a useful, quick, non invasive method inthe diagnosis of tissue and organs lesions but it needs specifictraining as well as acknowledging technical andpathological factors which may influence it. Elastography is to be considered as an additional tool tocomplete ultrasound evaluation in all the organs studied such as Thyroid, Breast, Renal and pancreas.
- The pancreas is an organ in which many malignant and benign diseases can ocur. Sonographic elastography (SE), which is used in the diagnosis of diseases by measuring tissue stiffness, especially for distinguishing malignant and benign diseases, has recently been used in pancreatic diseases. Strain Elastography imaging can be used as an efficient technique for the evaluation of pancreas elasticity. This study determined normal elasticity values of the pancreas in healthy volunteers. Information obtained from healthy adults can serve as a baseline against which pancreatic diseases can be examined.

METHODS

Informed consent form was obtained from all the patients, and we performed this study was performed in accordance with the ethical guidelines of the Helsinki Declaration. We received no financial support for this study.

Pancreatic SE was performed on the healthy volunteers (35 males and 58 females) who were free from any known diseases. The average age and height of the participants were 36.13±17.27 years (18–81) and 166.81±9.50 cm (150–190 cm), respectively. Additionally, the average weight and body mass index averages of the participants were 61.75±11.47 kg (40–90 kg) and 22.36±3.44 cm/m^2 (14.30–29.30), respectively.

After the clear and careful evaluation of pancreas of each volunteer on the gray scale under ultrasonography, SE was performed on the head, trunk, and tail regions of the pancreas. The procedures were performed by a radiologist with at least five years of experience in this field. The procedure was performed with a 3.5–5 MHz convex probe using a Toshiba Aplio 500 device (Toshiba Medical Systems, Co., Ltd., Otawara, Japan) with two presses per second to the three areas of the pancreas. While taking the samples, the probe was placed in the tissue in a parallel position. The screen is divided into three main regions. On the left, it shows the degree of stiffness of the tissue and the tissue is observed in color with the gray scale on the right. The lower part displays the waveform showing compression–decompression and supporting our adjustment of the rhythm (Figure 1). Then, the three samples were separately taken from the pancreatic head, trunk, and tail regions. While taking the samples, the SR values were

Figure 1. Shows the elastography US image of the pancreas. The monitor is divided into three windows. The right window shows the gray scale US image, left window shows the color-coded US elastography image and the bottom window shows the sinusoidal wave of compression–decompression. The circles indicate the ROIs. The upper ROI is on the parapancreatic tissue and the lower ROI is on the pancreas. The vertical white line on the sinusoidal wave indicates the point of measurement

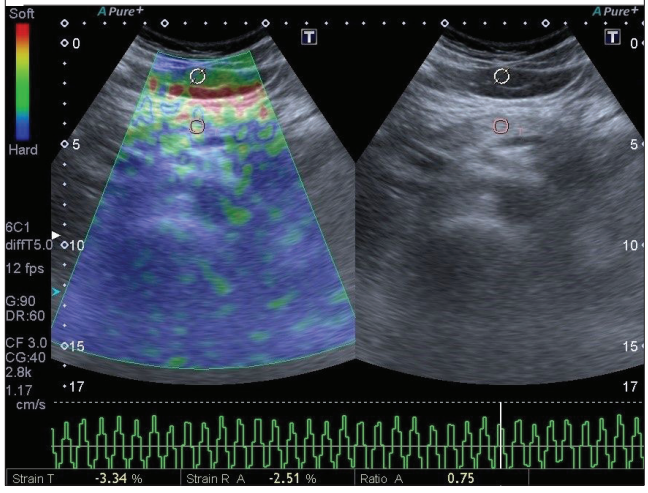

Table 1. Baseline description in the healthy volunteers who underwent strain elastography. Average values are expressed as mean±standard deviation, and range is shown as minimum–maximum. A threshold value was derived for the detection of changes in the pancreatic elasticity between the men and women on (Mann–Whitney U test was used because the data are non–parametric). The significance of P value was defined at 0.05

	Men (Average value)	Women (Average value)	p
Head	2.10±1.97 (0.15–6.95)	1.85–1.87 (0.00–9.06)	0.337
Corpus	1.56±1.45 (0.15–6.40)	1.60±1.66 (0.20–7.59)	0.555
Tail	1.93±1.39 (0.13–5.83)	1.84±1.57 (0.26–7.67)	0.523
Average	1.86±0.98 (0.26–4.54)	1.76±1.20 (0.43–5.26)	0.293

Table 2. Pancreatic SR measurements by age groups (Mann–Whitney U test was used because the data is non–parametric). P significance value was taken as 0.05

	<40 years (58 volunteers)	≥40 years (35 volunteers)	p
Head	1.93±1.84 (0.12–9.06)	1.97±2.02 (0.00–6.52)	0.629
Corpus	1.43±1.64 (0.15–7.59)	1.84±1.45 (0.46–6.53)	0.018
Tail	1.68±1.28 (0.13–5.87)	2.19±1.78 (0.31–7.67)	0.490
Average	1.68±1.04 (0.26–5.26)	2.00±1.22 (0.43–4.97)	0.279

found by placing the target ROI in the tissue and the reference ROI of the adjacent connective tissue. The SR values were recorded and calculated for all the patients (Figure1).

Statistical Analysis

We used IBM SSPS 2.1 software program for statistical analyses in the study (SPSS Inc.; Chicago, IL, USA). We separately wrote gender, age, and weight of all patients and recorded their values. We took three samples from the pancreatic head, trunk, and tail sections, and compared the SR values with respect to gender and age (before and after 40 years). We separately determined the values for each region of the pancreas. Thereafter, the values before and after the age of 40 years were found. Finally, we compared the pancreatic elastography values between the genders, among the pancreatic areas, and before and after the age of 40 years. The significant p value was taken at 0.05 for this study.

RESULTS

The average SR values in women and men were 1.86±0.98 (0.26–4.54) and 1.76±1.20 (0.43–5.26), respectively. There was no significant difference between the SR values measured with respect to age before and after 40 years (p=0.293) and between male and female genders. The average SR value did not differ between woman and men (p=0.751).

There was only a slight difference in the pancreas corpus measurements between before and after the age of 40 years (p=0.018) (Tables 1, 2).

DISCUSSION

There has been an increase in the popularity of the sonographic elastography technique, which is used in both superficial and deep tissues as well as organs such as breasts, thyroid, liver, other digestive organs, and kidney tissues from deep organs (6-9). However, there are only fewer number of studies on pancreas (8, 9).

Many studies have been conducted with different UES techniques. UES, which basically has two different types, is performed by SHE and can make quantitative measurements. Other semi-quantitative technique is SE. Although both principles have their own differences, both these techniques provide us with valuable results about tissue stiffness. However, SE depends on the person and is semi-quantitative and difficult to be performed in very deep tissues. Although SHE is independent of the individual, it also shows anisotropy in the heterogeneous tissue structure, which is its main disadvantage (5, 7-10). Our study was performed with a SE technique, which has been used in a relatively few studies. Due to the measurements in the three separate areas of the pancreas, other studies that can be performed in the normal population will be able to shed light on this topic. Moreover, our study would make the comparison process easier.

There are only a few studies related to the pancreas. As in other tissues, due to fibrosis and changes in tissue in chronic inflammatory processes and malignancies, tissue elasticity disappears, thereby hardening the tissue. In those cases, the tissue will be hard whenever elastography is performed (9-13). Also, it can be difficult to distinguish between pancreatic cancer and pancreatitis with multi-slice computed tomography and magnetic resonance imaging. This situation causes the need for biopsy. However, a biopsy of an organ such as pancreas is not always easy. There have been many publications showing that SE is used in the differential diagnosis of many tissues. SE has been used successfully in the differential diagnosis of malignant lesions of the breast, and fibroadenomas have been found to be softer than breast cancer (13-15). It has been shown that elastography significantly reduces the number of fine needle aspiration biopsy in the detection of thyroid diseases (8, 14-16). Additionally, the importance of elastography has been demonstrated in showing the degree of musculoskeletal diseases and liver fibrosis (17, 18). Therefore, diseases such as pancreatitis and pancreas can be recognized with the help of elastography. Thus, a differential diagnosis of focal pancreatitis and tumors that can provide mass images has been attempted. The studies were conducted with USE to develop a noninvasive method according to endoscopic sonography. Thus, a study emphasized that USE is a usable method (14).

Some studies show that the diagnosis increased above 90% in the B-mode combination of UES and sonography (14, 19). A study with a capacity of 121 patients found high specificity and sensitivity of UES in the differential diagnosis of malign and benign lesions (14, 20).

In addition, USE has been used in other diseases of the pancreas, diabetes mellitus, and similar diseases (21).

CONCLUSION

In our study, we found the normal elastography values of the pancreas that can shed light on the USE studies that can save the patients with pancreatic disorders from biopsy, which is difficult and sometimes impossible to perform. With the increase in new studies, it appears to be certain that elastography will provide a promising avenue in the future.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine Ethics Committee (14.09.2015/01).

Informed Consent: Informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Saisho Y. Pancreas volume and fat deposition in diabetes and normal physiology: con sideration of the interplay between endocrine and exocrine pancreas. Rev Diabet Stud 2016; 13: 132-47. [Crossref]
2. Çağlar V, Gönül Y, Songur A. Pankreas Anatomisi ve Varyasyonları. Uluslararası Klinik Araştırmalar Dergisi 2014; 2: 77-5.
3. Chen N, Unnikrishnan I R, Anjana RM, Mohan V, Pitchumoni CS. The complex exocrine-endocrine relationship and secondary diabetes in exocrine pancreatic disorders. J Clin Gastroenterol 2011; 45: 850-61. [Crossref]
4. Klintworth N, Mantsopoulos K, Zenk J, Psychogios G, Iro H, Bozzato A. Sonoelastography of parotid gland tumours: initial experience and identification of characteristic patterns. Eur Radiol 2012; 22: 947-56. [Crossref]
5. Onur MR, Goya C. Ultrasound Elastography: Abdominal Applications. Turkiye Klinikleri J Radiology Special Topics 2013; 6: 59-69.
6. Menzilcioglu MS, Çitil S, Akman Y, Tüten F. Strain Index Values in the Ultrasonographic Evaluation of Psoriasis. Medical Science and Discovery 2019; 6: 96-9. [Crossref]
7. Kawada N, Tanaka S. Elastography for the pancreas: Current status and future perspective. World J Gastroenterol 2016; 22: 3712-24. [Crossref]
8. Hirooka Y, Kuwahara T, Irisawa A, Itokawa F, Uchida H, Sasahira N, et al. Erratum to: JSUM ultrasound elastography practice guidelines: pancreas. J Med Ultrason 2015; 42: 175. [Crossref]
9. Dietrich CF, Hocke M. Elastography of the Pancreas, Current View. Clin Endosc 2019; 52: 533-40. [Crossref]
10. Menzilcioglu MS, Duymus M, Citil S, Gungor G, Saglam M, Gungor O, et al. The comparison of resistivity index and strain index values in the ultrasonographic evaluation of chronic kidney disease. Radiol Med 2016; 12: 681-7. [Crossref]
11. Zechner D, Knapp N, Bobrowski A, Radecke T, Genz B, Vollmar B. Diabetes increases pancreatic fibrosis during chronic inflammation. Exp Biol Med 2014; 239: 670-6. [Crossref]
12. Ghosh AK, Quaggin SE, Vaughan DE. Molecular basis of organ fibrosis: potential therapeutic approaches. Exp Biol Med 2013; 238: 461-81. [Crossref]
13. Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. Diabetes 2014; 63: 3880-90. [Crossref]
14. Metin MR, Tahtacı M. Sonoelastrografi ile fokal pankreas kitleleri; fokal pankreatit mi? Pankreatik adonakanser mi? Akademik gastroenteroloji dergisi 2018; 17: 104-9. [Crossref]
15. Ophir J, Garra B, Kallel F, Konofagou E, Krouskop T, Righetti R, et al. Elastographic imaging. Ultrasound Med Biol 2000; 26: 23-9. [Crossref]
16. Bojunga J, Herrmann E, Meyer G, Weber S, Zeuzem S, Friedrich-Rust M. Real-time elastography for the differentiation of benign and malignant thyroid nodules: a meta-analysis. Thyroid 2010; 20: 1145-50. [Crossref]
17. Ooi CC, Malliaras P, Schneider ME, Connell DA. "Soft, hard, orjustright?" Applications andlimitations of axial-strain sono elastography and shear-wave elastography in theassessment of tendon injuries. Skeletal Radiol 2014; 43: 1-12. [Crossref]
18. Menzilcioglu MS, Duymus M, Gungor G, Citil S, Sahin T, Boysan SN, et al. The value of real-time ultrasound elastography in chronic autoimmune thyroiditis. Br J Radiol 2014; 87: 20140604. [Crossref]
19. Uchida H, Hirooka Y, Itoh A, Kawashima H, Hara K, Nonogaki K, et al. Feasibility of tissue elastography using transcutaneous ultrasonography for the diagnosis of pancreatic diseases. Pancreas 2009; 38: 17-22. [Crossref]
20. Giovannini M, Botelberge T, Bories E, Pesenti C, Caillol F, Esterni B, et al. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. World J Gastroenterol 2009; 15; 1587-93. [Crossref]
21. Öztürk M, Citil S, Menzilcioglu MS. Assessment of the Pancreas with Strain Elastography in Healthy Children: Correlates and Clinical Implications. Pol J Radiol 2017; 82: 688-92. [Crossref]

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Original Research

Do Patients with Differentiated Thyroid Cancer Face the Risk of Hyponatremia at the Expense of Preparation for Radioactive Iodine Treatment?

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ABSTRACT

Objective: Differentiated thyroid cancer (DTC) is the most common endocrine cancer. The main therapeutic strategies are surgery and radioactive iodine (RAI) treatment in selected intermediate- and high-risk patients. Hyponatremia is the most frequent form of electrolyte imbalance and few studies have assessed the frequency and clinical impact of hyponatremia in patients with thyroid cancer. In this study, we aimed to determine the prevalence and severity of hypo¬natremia among hypothyroid patients in the peri-ablation period. The secondary objective was to assess the correlation between Sodium (Na) level and hypothyroidism severity, age, and RAI dosage.

Methods: A total of 51 patients with DTC who were referred to our Nuclear Medicine Department for RAI ablation/treatment were enrolled. Serum Na, thyroid-stimulating hormone (TSH), and free triiodothyronine and thyroxine levels were measured three times during the study (under LT4 suppression, when the patient was hypothyroid before and after receiving RAI). Baseline, pre-, and post-RAI mean serum Na and other hormonal parameters were compared. The number of patients with hyponatremia and possible related symptoms were noted. Correlation of serum Na levels with age, RAI dosage, and hypothyroidism severity was determined.

Results: The number of patients with hyponatremia did not differ significantly in the baseline, pre-, and post-RAI periods. None of the patients experienced moderate-to-severe hyponatremia. There was no significant correlation between serum Na levels and age, serum TSH, or the hormone levels.

Conclusion: In conclusion, preparation for RAI treatment with LT4 withdrawal and or a low-iodine diet is not a common etiological factor for the development of hyponatremia in patients with DTC.

Keywords: Hyponatremia, radioactive iodine, thyroid neoplasms

INTRODUCTION

Differentiated thyroid cancer (DTC) is the most common endocrine cancer and its incidence is increasing worldwide (1). Mortality rates are low, and it has an excellent prognosis in the absence of high-risk factors such as distant metastasis (2). The cornerstones of therapy are surgery and radioactive iodine (RAI) in selected intermediate and high-risk patients (3). RAI can be given for ablative, adjuvant, or treatment purposes. It decreases regional recurrences and disease specific mortality (4). RAI treatment has acute and chronic complications such as sialadenitis, transient testicular or ovarian dysfunction, and secondary malignancies (2).

Before RAI treatment, high levels of TSH is essential to increase the uptake of iodine and for effective ablation of the remnant tissue. High TSH can be achieved either with withdrawal of levothyroxine for 4–6 weeks after total or near total thyroidectomy or with the highly purified, recombinant form of TSH (rhTSH).

Hyponatremia is the most frequent electrolyte imbalance in routine clinical practice. To date, few studies have assessed the frequency and clinical impact of electrolyte imbalance on patients with thyroid cancer (5). It can be caused by lowered water clearance and inappropriately high concentration of anti-diuretic hormone (ADH) due to iatrogenic hypothyroidism, which is required for the maximum uptake of RAI within the remnant tissue and for effective ablation (6). In the setting of hypothyroidism, a decreased glomerular filtration rate is shown to directly lower the free water excretion by decreasing the delivery of water to the diluting segment of the renal tubules, which may contribute

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to the further dilution and worsen the hyponatremia (6). Another possible explanation for hyponatremia is the low-iodine diet (LID) that is started two to four weeks prior to RAI treatment to eliminate dietary iodine interference and facilitate the uptake of RAI (7). Such a LID protocol usually ends up with low dietary salt intake, and rarely severe hyponatremia cases.

In this study, we aimed to determine the prevalence and severity of hyponatremia in hypothyroid patients during the peri-ablation period. In order to show the effect of hypothyroidism on the occurrence of hyponatremia, we compared the sodium levels measured on and off LT4 treatment. We also compared the pre and post-RAI hyponatremia prevalence to see if RAI treatment itself had a separate effect on the serum sodium levels. Secondary purposes were to describe the demographic profile of hypothyroid DTC patients, to determine the correlation of TSH, age, fT3 and T4 levels and RAI dosage with serum sodium levels in the pre- and post-RAI period.

METHODS

We prospectively enrolled 51 patients who were admitted to our tertiary center's endocrinology outpatient clinics and diagnosed with DTC after total thyroidectomy with or without lymph nodes dissection and referred to the Nuclear Medicine Department for RAI ablation between September 2019 and February 2020. Before starting the study, we obtained the local ethical board approval in accordance with principles of the Declaration of Helsinki. RAI ablation decision was made by a multidisciplinary team including endocrinologists, surgeons, nuclear medicine, and pathology specialists. Informed consent was obtained for each patient before the treatment. To minimize the confounding factors that could cause hyponatremia, patients with comorbid conditions such as congestive heart failure, using diuretics including thiazides, chronic or acute kidney failure or cirrhosis were excluded. Demographic and clinical data of the patients such as age, gender, presence of hypertension or diabetes, the dosage of the RAI received, tumor subtype, duration of LID, and indication for RAI were recorded.

The standard protocol for RAI treatment in our institution is L-thyroxine withdrawal for 21 days and LID for 14 days before the treatment. Serum Na, TSH, free T3 and T4 levels were measured (Siemens, Atellica®) three times during the study; at the time when the patient was seen in the multidisciplinary council while using L-thyroxine (8–12 weeks after the operation) (baseline), the day before RAI ablation, and five to seven days after RAI at the time of post treatment whole body scanning. Mean sodium concentrations and the prevalence of mild hyponatremia (130–134 mEq/L), moderate hyponatremia (120–129 mEq/L), and severe hyponatremia (<120 mEq/L) were determined. The normal reference range for serum sodium concentrations is 135–145 mEq/L. Clinical symptoms of hyponatremia that may be associated with hyponatremia were questioned if hyponatremia was detected.

Main Points:

- Postoperative RAI treatment is widely recommended in DTC for remnant ablation and treatment of residual or metastatic disease. Before RAI treatment, LID and high levels of TSH are essential to increase the uptake of iodine.
- Hyponatremia is the most frequent electrolyte imbalance and hyponatremia could be present in patients with hypothyroidism; however, its frequency and severity has not been well documented in the pre-ablation and post-RAI period.
- Our results present that; preparation for RAI treatment with LT4 withdrawal and or LID is not a common etiological factor for the development of hyponatremia in patients with DTC and clinicians should not be greatly concerned about rare, life-threatening hyponatremia during preparation for RAI treatment

Statistical Analysis

The Statistical Package for the Social Sciences, version 25 (IBM SPSS Corp.; Armonk, NY, USA) was used for the statistical analysis. Descriptive analysis was performed in order to identify the baseline characteristics of the hypothyroid patients. Mean, standard deviation, and range were used to describe continuous variables (serum sodium levels), while Median was used to describe non-parametric variables (age and TSH concentration). Baseline Na (under LT4 treatment), pre-RAI and post-RAI Na, TSH, and free T3 and T4 levels were compared with the Non-parametric Friedman test. Sex was described using number and percentage. Correlations were analyzed using Spearman test. A P value of less than 0.05 was statistically significant.

RESULTS

A total of 51 patients with DTC who underwent total/near total thyroidectomy and were determined to receive RAI treatment (ablative/adjuvant or therapeutic) were enrolled. Of these, 38 (74.5%) were females and 13 (25.5%) were male. The mean age was 47.9±12.5 years (min–max; 20–77). As a comorbid condition, four patients had asthma, seven had diabetes, and eleven had hypertension. Median RAI dosage was 50 mCi (min–max; 30–200 mCi). Forty-seven patients had papillary thyroid carcinoma (4 with columnar cell, 2 with tall cell 1 with hobnail, and 40 with classical variant), whereas four had follicular carcinoma (one was minimally invasive and three had vascular invasion). The indications for RAI were large tumor size, presence of lymph node or distant metastasis, extrathyroidal extension, presence of poor prognostic variant or inappropriately high thyroglobulin (Tg) level after the surgery (1). Demographic and clinical characteristics of patients are presented in Table 1.

Median TSH concentration under L-thyroxine treatment before withdrawal was 1.3 (min–max; 0.01–15) (mU/L). Median free T3 and T4 levels at the baseline under LT4 treatment were 3.0 ng/L (0.2–5.4) and 1.14 ng/dL (0.4–1.7), respectively. Mean baseline serum Na level was 140.2±0.7 mEq/mL. The minimum Na level was 135 mEq/L and none of the patients had hyponatremia (Table 2).

Median TSH concentration measured the day before RAI treatment was 103 (mU/L) (min–max; 0.5–150). Median free T3 and T4 levels were 1.3 ng/L (0.2–3.5) and 0.5 (0.09–0.98), respectively. Pre-RAI mean Na was 139±2.4 and only one patient had hyponatremia (134 mEq/L) (Table 2).

Table 1. Demographic and Clinical Characteristics of the Patients with DTC

Mean age (years)	47.9±12.5
Sex (female/male) (no/%)	38 (74.5)/13 (25.5)
Median RAI dosage (mCi)(min–max)	50 (30–200)
Pathologic subtype (no) (papillary/follicular)	Papillary: 47
	Classical variant: 40
	Columnar cell:4
	Tall cell: 2
	Hobnail: 1
	Follicular Cancer:4
	Minimally invasive :1
	Extensive vascular invasion:3
Comorbid condition	Asthma:4
	DM:7
	HT:11
Duration of low–iodine diet (days)	14 days
Indications for RAI treatment (no)	Extrathyroidal extension :3
	Distant metastasis:2
	Poor prognostic variant:7
	Vascular invasion:3
	Lymph node metastasis:9
	Inappropriately high postoperative Tg:9
	Large Tumor size:8
	Anti Tg positivity:2
	Patient's choice: 2
	Multifocality:6

Table 2. Comparison of baseline, pre and post–RAI laboratory parameters

	Baseline (under LT4)	Pre–RAI	Post–RAI	p
TSH (mU/L) (median; min–max)	1.3 (0.01–15)	103(0.5–150)	87(4–150)	<0.001
f T4 (ng/dL) (median; min–max)	1.14 (0.4–1.7	0.5 (0.09–0.98)	0.29(0.021.13)	<0.001
f T3 (ng/L) (median; min–max)	3.0 (0.2–5.4)	1,3 (0.2–3.5)	1.38 (0.2–3)	<0.001
Na (mean) mEq/L	140.2±0.7	139±2.4	140±2.5	0.28

Median TSH concentration measured 5–7 days after RAI was 87 (mU/L) (min–max; 4–150). Median free T3 and T4 levels were 1.38 (0.2–3) and 0.29 ng/dl (0.02–1.13), respectively. Mean Na was 140±2.5 and minimum Na was 131 mEq/L (Table 2). Two patients had mild hyponatremia (134 and 131 mEq/L)

Baseline TSH under LT4 treatment was significantly lower than the pre- and post-RAI TSH levels as expected (p<0.001). There was no significant difference between the pre-RAI and post-RAI TSH levels. Free T3 level was significantly lower in pre-and post-RAI measurements compared to the baseline (under LT4) (p<0.001). Baseline Free T4 was significantly higher than pre-and post-RAI fT4 (p<0.001). There was also no significant difference between the pre- and post-RAI groups regarding ft4, with higher levels in the post-RAI measurements (p=0.01). There was no statistically significant difference between baseline, pre-RAI, and post-RAI Na levels (p=0.28). The number of patients with hyponatremia did not differ significantly in baseline, pre- and post-RAI periods. None of the patients experienced moderate-to-severe hyponatremia or related symptom.

In Spearman test, there was no significant correlation between serum Na and age, Serum TSH and free hormone levels (Table 3).

Table 3. Correlation of serum Na with age, f T3, T4 andRAI dosage

	Pre–RAI Sodium		Post–RAI Sodium	
	Correlation Coefficient	p	Correlation Coeeficient	p
TSH	0.084	0.57	0.48	0.74
f T4	–0.15	0.92	0.109	0.44
f T3	0.40	0.68	–0.68	0.63
Age	–0.91	0.54	–0.93	0.52
RAI dose	–	–	0.22	0.12

DISCUSSION

Thyroid cancer constitutes the 3.0% of all new cancer cases in the U.S.A and based on the national cancer database, there were 52,070 newly diagnosed cases with 2170 deaths from the disease in 2019 (8). Papillary and follicular carcinoma make up 95% of all DTC cases. RAI after total/near total thyroidectomy are well established treatments in DTC. RAI treatment is now being reserved for high and selected intermediate risk patients since there should be a balance between therapeutic efficacy and unwanted side effects (9).

In patients who undergo RAI treatment, the uptake depends on the adequate stimulation with TSH that can be obtained either by LT4 withdrawal (THW) or administration rhTSH (10). LT4 withdrawal is associated with side effects such as fatigue, constipation, emotional disturbance, decreased intellectual functions, and worsening of heart failure symptoms. Hyponatremia is one of possible complications of RAI (11). Hyponatremia developing due to hypothyroidism may occur as a result of several mechanisms involving renal, cardiovascular and hypothalamo-adrenal systems (12). Hypothyroidism also has effects on almost all components of the renin-angiotensin-aldosterone system, including renin generation, production of angiotensinogen in the liver, secretion and inactivation of aldosterone in the adrenal glands (5).

In our study, the aim was to determine the frequency of hyponatremia during the peri-ablation period in the absence of other confounding etiological factors. We also aimed to determine the association between hypothyroidism severity, RAI dosage, and age with the serum Na levels. In our study, severe or moderate hyponatremia or associated symptoms were not detected. There was mild hyponatremia in pre- and post-ablation measurements in two cases, but the frequency was not different from that of the baseline measurements taken under LT4 suppression. The rate of hyponatremia in our study group was lower than that of other cancers, which may be attributed to the patients' characteristics such as young age, short hospital stay, and less comorbid conditions (13). There are several case reports of severe and symptomatic hyponatremia in the literature after thyroid hormone withdrawal that mostly occurred in elderly patients using diuretics (14). Our results are compatible with the two previous studies such that none of the patients prepared for RAI with both THW and LID had Na levels of <130 mEq/L (8, 15). To determine the effect of RAI itself on the development of hyponatremia, we compared Na levels for the day before and 5–7 days after RAI treatment. The frequency of hyponatremia was similar in pre- and post-RAI measurements in our study. However in a previous report, hyponatremia after RAI and isolation was more common at a prevalence of 26.7% compared with that at 7% pre-RAI treatment (16). The possible explanations for that difference were nausea and vomiting after RAI treatment and patient anxiety that potentiated the ADH secretion. In our study, we did not detect any correlation between serum Na level and hypothyroidism severity, which was consistent with that reported by two previous studies (17, 18).

LID was administered to the patients 2 weeks before RAI treatment at our center. Such an LID is usually accompanied with low salt intake, and reports of severe symptomatic hyponatremia developed due to LID are scarce. In those reports, it was suggested that a prolonged LID, low salt intake, and the use of thiazide diuretics in elderly patients are risk factors for the development of severe hyponatremia (19). We advise our patients to take non-iodinated salt in the diet, which may have reduced the incidence of hyponatremia. Moreover, exclusion of patients using medications that may worsen hyponatremia might have contributed to the low incidence of Na imbalance.

This study had some limitations, including the limited number of patients. Another limitation is that patient-related factors such as the amount of hydration performed post-RAI treatment and adherence to low salt intake and LID pre-RAI therapy that may affect results of this study were not evaluated.

CONCLUSION

Preparation for RAI treatment with LT4 withdrawal and or LID is not a common etiological factor for the development of hyponatremia in patients with DTC. Hyponatremia was neither prevalent nor severe or symptomatic during preparation for RAI treatment when it occurred. Clinicians should not be greatly concerned about rare, life-threatening hyponatremia during preparation for RAI treatment and should not exaggerate the possibility of the development of severe hyponatremia.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara City Hospital (E1- 20-428).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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REFERENCES

1. Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. Nat Rev Endocrinol 2016; 12: 646-53. [Crossref]
2. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016; 26: 1-133. [Crossref]
3. Orosco RK, Hussain T, Noel JE, Chang DC, Dosiou C, Mittra E, et al. Radioactive iodine in differentiated thyroid cancer: a national database perspective. Endocr Relat Cancer 2019; 26: 795-802. [Crossref]
4. Twining CL, Lupo MA, Tuttle RM. Implementing Key Changes in the American Thyroid Association 2015 Thyroid Nodules/Differentiated Thyroid Cancer Guidelines Across Practice Types. Endocr Pract 2018; 24: 833-40. [Crossref]
5. Niri T, Horie I, Ando T, Kawahara H, Ueda M, Eto M, et al. Renal Function and Plasma Renin Activity as Potential Factors Causing Hyperkalemia in Patients with Thyroid Carcinoma Undergoing Thyroid Hormone Withdrawal for Radioactive Iodine Therapy. Endocr Pract 2020; 26: 197-206. [Crossref]
6. Vannucci L, Parenti G, Simontacchi G, Rastrelli G, Giuliani C, Ognibene A, et al. Hypothyroidism and hyponatremia: data from a series of patients with iatrogenic acute hypothyroidism undergoing radioactive iodine therapy after total thyroidectomy for thyroid cancer. J Endocrinol Invest 2017; 40: 49-54. [Crossref]
7. Nozu T, Yoshida Y, Ohira M, Okumura T. Severe hyponatremia in association with I(131) therapy in a patient with metastatic thyroid cancer. Intern Med 2011; 50: 2169-74. [Crossref]
8. Kim J, Cho SG, Kang SR, Kwon SY, Cho DH, Cho JS, et al. Preparation for radioactive iodine therapy is not a risk factor for the development of hyponatremia in thyroid cancer patients. Medicine (Baltimore) 2017; 96: e6004. [Crossref]
9. Tuttle RM, Ahuja S, Avram AM, Bernet VJ, Bourguet P, Daniels GH, et al. Controversies, Consensus, and Collaboration in the Use of (131) I Therapy in Differentiated Thyroid Cancer: A Joint Statement from the American Thyroid Association, the European Association of Nuclear Medicine, the Society of Nuclear Medicine and Molecular Imaging, and the European Thyroid Association. Thyroid 2019; 29: 461-70. [Crossref]
10. Tang CYL, Thang SP, Zaheer S, Kwan CK, Ng DC. Recombinant human thyrotropin versus thyroid hormone withdrawal in an Asian population. Endocrine 2020; 69: 126-32. [Crossref]
11. Silva MC, Furlanetto TW. Another possible contributor to hyponatremia after radioactive iodine. Thyroid 2014; 24: 923. [Crossref]
12. Ota K, Kimura T, Sakurada T, Shoji M, Inoue M, Sato K, et al. Effects of an acute water load on plasma ANP and AVP, and renal water handling in hypothyroidism: comparison of before and after L-thyroxine treatment. Endocr J 1994; 41: 99-105. [Crossref]
13. Doshi SM, Shah P, Lei X, Lahoti A, Salahudeen AK. Hyponatremia in hospitalized cancer patients and its impact on clinical outcomes. Am J Kidney Dis 2012; 59: 222-8. [Crossref]
14. Shakir MK, Krook LS, Schraml FV, Hays JH, Clyde PW. Symptomatic hyponatremia in association with a low-iodine diet and levothyroxine withdrawal prior to I131 in patients with metastatic thyroid carcinoma. Thyroid 2008; 18: 787-92. [Crossref]
15. Baajafer FS, Hammami MM, Mohamed GE. Prevalence and severity of hyponatremia and hypercreatininemia in short-term uncomplicated hypothyroidism. J Endocrinol Invest 1999; 22: 35-9. [Crossref]
16. Dayrit JC, Cunanan EC, Kho SA. Prevalence of Hyponatremia in Hypothyroid Patients during Radioactive 131I Ablation for Differentiated Thyroid Cancer: Single Institution Experience. Endocrinol Metab (Seoul) 2016; 31: 410-5. [Crossref]
17. Hammami MM, Almogbel F, Hammami S, Faifi J, Alqahtani A, Hashem W. Acute severe hypothyroidism is not associated with hyponatremia even with increased water intake: a prospective study in thyroid cancer patients. BMC Endocr Disord 2013; 13: 27. [Crossref]
18. Tyack PL, Calambokidis J, Friedlaender A, Goldbogen J, Southall B. Formal Comment on Schorr GS, Falcone EA, Moretti DJ, Andrews RD. First Long-Term Behavioral Records from Cuvier's Beaked Whales (Ziphius cavirostris) Reveal Record-Breaking Dives. PLoS ONE 2014; 9: e92633. [Crossref]
19. Al Nozha OM, Vautour L, How J. Life-threatening hyponatremia following a low-iodine diet: a case report and review of all reported cases. Endocr Pract 2011; 17: e113-7. [Crossref]

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Original Research

Evaluation of Stress Distribution in Mandibular Donor Site After Harvesting Bone Grafts of Different Sizes from the Symphysis

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ABSTRACT

Objective: The purpose of this study is to investigate the stress distribution in the mandibular donor site after harvesting bone grafts of different sizes from the symphysis by applying three different occlusal loads.

Methods: First, we constructed 16 experimental mandible models after harvesting the block grafts. We harvested rectangular-shaped and cylindrical block grafts of different sizes, localizations, and depths from the symphysis region. Three different occlusal loads were applied on the models. After the application of three occlusal loads on the models, we analyzed the von Mises stress distribution in the surface of the donor sites in the symphysis.

Results: In all the mandible models, the highest von Mises stress values were detected under incisal loads. Under the ipsilateral load of 300 N, the maximum von Mises stress was similar between R1 (unilateral one cylindrical graft) and R2 (unilateral two cylindrical grafts) models, and also between R1+1 (bilateral two cylindrical grafts) and R2+2 (bilateral four cylindrical grafts) models. The maximum von Mises stress under incisal load in the models measuring 20×10×4 mm (width, height, and depth, respectively) and 20×10×6 mm were 2.46 and 2.79 MPa, respectively. Among the unilateral models, the lowest maximum von Mises stress value was found in the R4 model (cylindrical graft: diameter=10 mm, depth=4 mm), and the highest value was found in the model measuring 15×10×6 mm under all the loads.

Conclusion: The application of incisal load led to a higher stress as than that of the bilateral and ipsilateral loads. The stress distribution in the symphysis donor site varies according to the localization, shape, and dimensions of the harvested grafts. Cylindrical grafts led to lower stress than the rectangular grafts.

Keywords: Block graft, finite element analysis, stress, symphysis

INTRODUCTION

The quantity of the dentoalveolar bone at the recipient site is a crucial factor for a successful insertion of endosteal implants and ensuring long-term survival rates. Trauma, tooth loss, and pathological entities such as cyst, tumor, and periodontal diseases may decrease the dimensions of the dentoalveolar bone. When the quantity of the dentoalveolar bone is insufficient in the dental implant surgery, several techniques can be used to increase the dimensions of the dentoalveolar bone for supporting the dental implants. These techniques include socket/dentoalveolar ridge preservation, distraction osteogenesis, bone splitting, on-lay graft, and guided bone regeneration (1). Previously, autologous bone graft was accepted as the gold standard for oral reconstruction, and intraoral and extraoral autologous bone grafts were used for the reconstruction of oral cavity defects. However, intraoral bone grafts are easily harvested in the present times for correcting limited and small defects. They provide various advantages such as the proximity between the donor and recipient sites. Intraoral donor sites include zygomatic arch, coronoid process, maxillary tuberosity, buccal aspect of the third molar region, anterior mandibular ramus, lateral aspect of the ramus, and symphysis (2-6).

Symphysis is an intraoral donor site that facilitates an easy and rapid surgical intervention. However, there may be some complications in the donor sites of symphysis during the intra- and/or post-operative period. These complications may include hemorrhage, infection, flap dehiscence, hematoma, pulp necrosis following damage to the dental root, hypoesthesia or anesthesia due to the injury of the mental nerve, insensitivity of the anterior teeth because of the proximity of the donor site to the roots of the teeth, pain, and swelling.

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After the harvesting of intraoral bone graft, biomechanical forces may lead to an increased risk of fracture. Although maxillofacial trauma is the primary cause of mandibular fractures, harvesting bone graft from the ramus increases the risk of mandibular fractures because of the weakness of the mandible (7, 8).

The assessment of stress distribution to the mandible is highly crucial for analyzing the risk of fracture after harvesting the autologous bone grafts. Finite element analysis (FEA) is a numerical method that provides solutions for the complicated mechanical problems by dividing the problem into smaller and basic components (9). Our study employed a three-dimensional (3D) FEA to analyze the stress distribution in the donor site of symphysis after harvesting different bone grafts of varying sizes from the symphysis under different occlusal loads.

METHODS

Ethics approval was not required for this *in vitro* study. We used a cone-beam computed tomography (CBCT) image of a patient with no tooth loss to form a 3D solid model of the mandible that comprised the dense trabecular and cortical bones. CBCT images of bones and teeth were taken in the DICOM format, and these images were combined layer by layer via the 3D Slicer® program to obtain a 3D mesh format (Figure 1). The mesh file obtained in the STL (stereolithography) format was converted into a solid model by Rhinoceros 4.0 software (Robert McNeel & Associates, Seattle, USA) (Figure 2). Cavity operations were performed on the solid model via Rhinoceros 4.0® and Solidworks® software, and then the models were prepared for analysis (Figures 3, 4). The models prepared for analysis were exported in the parasolid format and transferred to ANSYS 14.0 (ANSYS Inc., Canonsburg, PA, USA) software.

The thickness of the cortical bone in the lingual and vestibular/buccal surfaces was 2 mm at the interforaminal region and 2.5 mm at the site between the mental foramen and ramus. At the base of the mandible, the thickness of the cortical bone was 4 mm. Table 1 presents the elasticity modulus and Poisson's ratio of materials (10).

The boundaries of the block graft harvested from the symphysis were as follows:

- Superiorly 5 mm below from the apexes
- Inferiorly 4 mm superior to the inferior border of the mandible
- Laterally 5 mm anterior to the mental foramens
- Posteriorly at the lingual cortex of the mandible regeneration, (1)

Main Points:

- The highest stress values after harvesting the grafts from the symphysis were detected under the incisal loads.
- Harvested cylindrical grafts lead to a lower stress than the rectangular grafts in the symphysis.
- The fracture risk increases under the bilateral posterior loads because of the excessive depth of the harvested block graft from the midline symphysis.
- All of the drawings belong to us.

Figure 1. Mesh image of the mandible

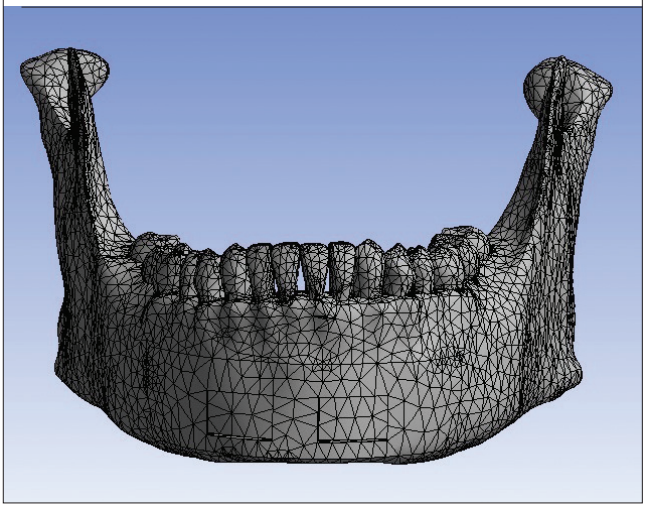


Figure 2. 3D solid model of the mandible

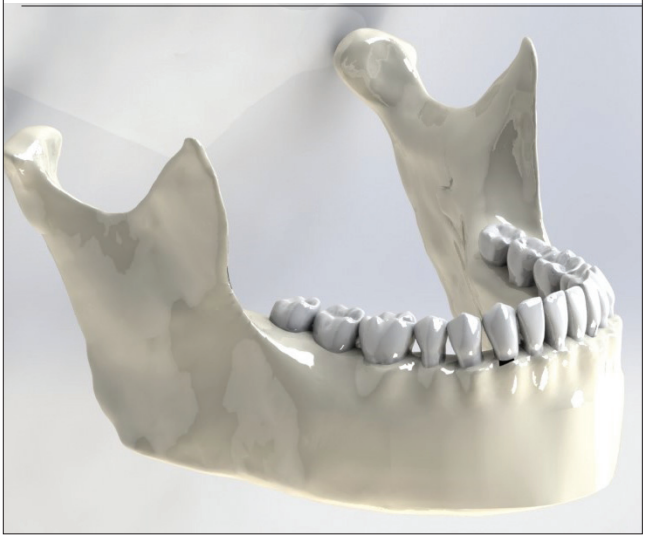
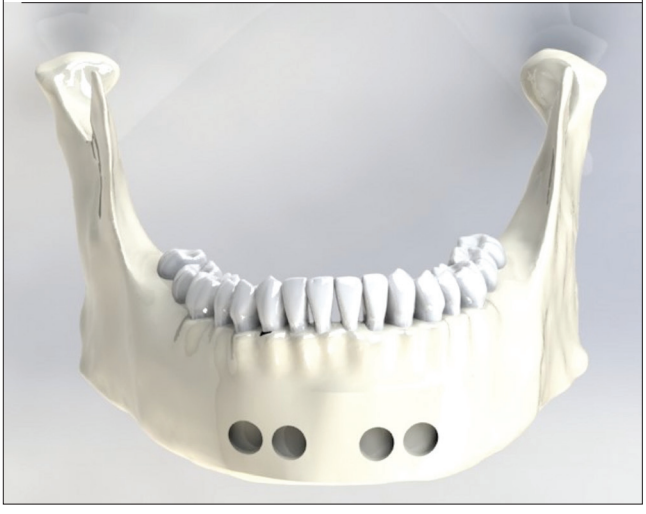


Figure 3. Mandible model with the harvested cylindrical grafts (diameter: 10 mm)



The grafts were harvested at the depths of 4 mm and 6 mm following the mandible external curvature, of which 4-mm-deep grafts included 2-mm cortical and 2-mm trabecular bone and 6-mm-deep grafts included 2-mm cortical and 4-mm trabecular bone. The block grafts were harvested from unilateral, bilateral, and midline symphysis, among which the unilateral grafts were harvested from the right side of the symphysis in all the models and bilateral bock grafts were harvested symmetrically from the midline of the mandible (Figures 3, 4). The dimensions of graft models were as follows:

- Cylindrical graft (R4: diameter=10 mm, depth=4 mm)
- Cylindrical graft (R6: diameter =10 mm, depth=6 mm)
- 15×10×4 mm
- 15×10×6 mm
- 20×10×4 mm
- 20×10×6 mm
- 30×10×4 mm
- 30×10×6 mm, (Figures 3, 4).

The localization of the cylindrical grafts was defined as follows:

- R1: Only one cylindrical graft on the right site of the symphysis (unilateral one cylindrical graft)
- R2: Two cylindrical grafts on the right site of the symphysis (unilateral two cylindrical grafts)
- R1+1: One cylindrical graft on the right and one cylindrical graft on the left site of the symphysis (bilateral two cylindrical grafts)
- R2+2: Two cylindrical grafts on the right and two cylindrical grafts on the left site of the symphysis (bilateral four cylindrical grafts) (Figure 3).

All the mandible models were termed according to the size of the harvested grafts. Table 2 enlists all the mandible models of this study. After harvesting the grafts, three loads were applied on the central fossa of the first molar teeth and on the midline of the incisal teeth.

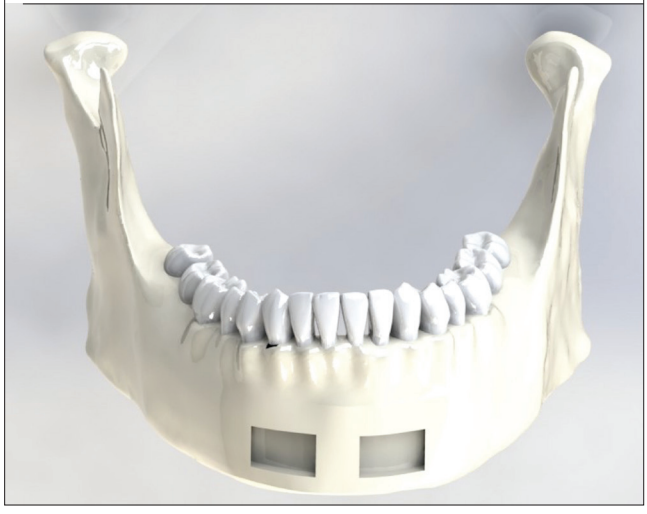
The occlusal forces applied on the models were as follows:

1. Incisal load of 300 N (on the midline of the incisal teeth)
2. Ipsilateral load of 300 N (on the right first molar)
3. Bilateral (ipsilateral and contralateral) loads (on the right and left first molars) of 600 N.

We conducted the 3D FEA using the Rhinoceros 3-D modeling software with ANSYS 14.0 (ANSYS Inc., Canonsburg, PA, USA) analysis program. Moreover, we used von Mises stress, which is the initial value of deformation energy indicating the stress distribution in FEA, as the indicator of stress in the analysis (11). All the materials used in this study were assumed to be homogenous, isotropic, and linearly elastic. While establishing the limit conditions, the condyle of the mandible was fixed at the x, y, and z axes, and the other node points were allowed to move in all the axes. Vertical movement of the incisal and molar teeth on which the occlusal force was applied were fully constrained in the vertical direction by the displacement of boundary conditions (12).

After applying three occlusal loads on the models, we analyzed the von Mises stress distribution on the surface of the donor sites in the symphysis. In all the graft models, we only analyzed the stress distribution in the donor site and did not assess stress distribution on the other regions of the mandible (Figures 5, 6).

Figure 4. Mandible model with the harvested rectangular-shaped grafts



RESULTS

All the harvested grafts had the depths of either 4 mm or 6 mm and were either cylindrical or rectangular in shape. All the grafts were harvested from the three different locations including unilateral, bilateral, and midline symphysis. After applying three different occlusal loads, we analyzed the von

Figure 5. Finite element analysis of the mandible model with harvested cylindrical grafts

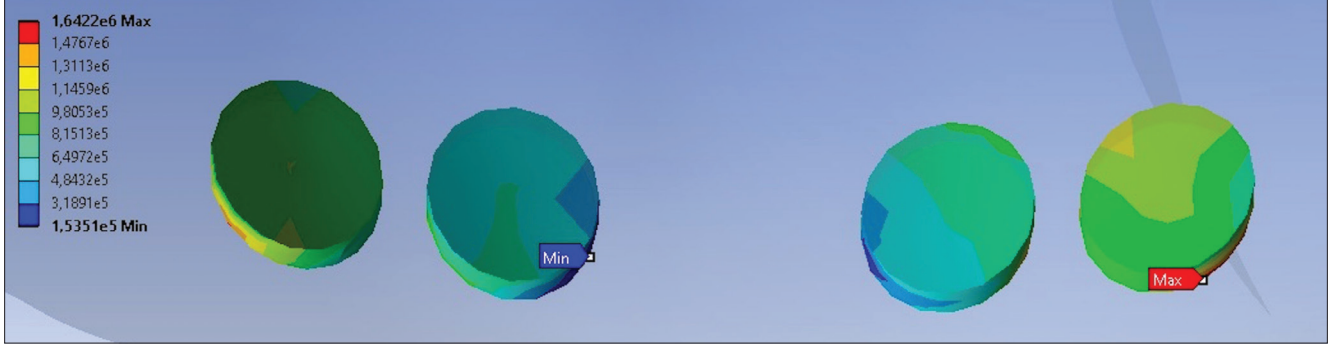


Figure 6. Finite element analysis of the mandible model with harvested rectangular-shaped grafts

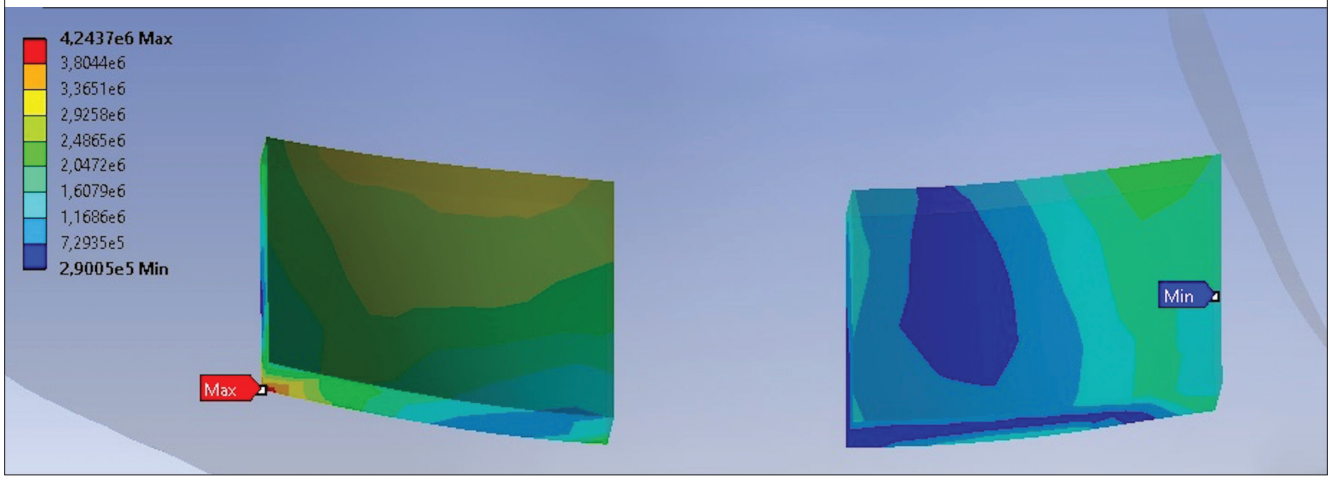


Table 1. Material properties

Material	Elasticity modulus (Gpa)	Poisson's ratio
Cortical bone	13.7	0.3
Dense trabecular bone	1.37	0.3

Mises stress distribution of each donor site. In all the mandible models, we detected the highest von Mises stress values under incisal loads (Figures 3, 4).

In the mandibular models with harvested cylindrical grafts, the highest von Mises stress was detected under incisal load. Under an ipsilateral load of 300 N, the maximum von Mises stress was similar between the R1 (unilateral one cylindrical graft) and R2 (unilateral two cylindrical grafts) models, and also between R1+1 (bilateral two cylindrical grafts) and R2+2 (bilateral four cylindrical grafts) models. However, the maximum von Mises stress in the R1+1 and R2+2 models was higher (approximately two times) than in the R1 and R2 models under ipsilateral load. Under the ipsilateral and contralateral loads of 600 N, the maximum von Mises stress was higher in the R2 and R2+2 models than in the R1 and R1+1 models. Under the ipsilateral load, the maximum von Mises stress was similar in the mandibular models with harvested 4- and 6-mm cylindrical grafts than under the incisal and bilateral loads (Figure 7, 8).

Four different block grafts with the dimensions of 20×10×4 mm, 20×10×6 mm, 30×10×4 mm, and 30×10×6 mm were harvested from the midline symphysis. The maximum von Mises stress under incisal load in the models measuring 20×10×4 mm and 20×10×6 mm were 2.46 and 2.79 MPa, respectively. The maximum von Mises stress in the model measuring 20×10×6 mm was higher than in the models measuring 20×10×4 mm, 30×10×4 mm, and 30×10×6 mm under incisal loads. However, the maximum von Mises stress under ipsilateral load was lowest in the model measuring 20×10×4 mm (1.72 MPa) and highest in the model measuring 30×10×6 mm (2.38 MPa). Under bilateral loads, the von Mises stress values were similar in the models measuring 30×10×4 mm (1.52 MPa) and 30×10×6 mm (1.50 MPa). The lowest maximum von Mises stress under bilateral load (600 N) was in the model measuring 20×10×4 mm (0.92 MPa) (Figure 9).

Among unilateral models, the lowest and highest maximum von Mises stress values under incisal load were in the R4 model (2.30 MPa) and in the model measuring 15×10×6 mm (3.03 MPa), respectively. Under ipsilateral load, the maximum von Mises stress values were similar in all the models. In both the R4 and R6 models, the maximum von Mises stress distribution under the bilateral load were similar to the values under incisal load; however, they were lower than those under the incisal load (Table 6). The maximum von Mises stress values were higher under the incisal loads than under the ipsilateral and bilateral loads in all the harvested grafts. The lowest maximum von Mises stress value was in the R4 model, and the highest value was in the model measuring 15×10×6 mm under all the loads (Figure 10).

The maximum von Mises stress values were detected in the bilateral models measuring 15×10×4 mm, and the lowest maximum von Mises stress values were detected in the unilateral models measuring 15×10×4 mm and 15×10×6 mm under all the three loads (Figure 11).

DISCUSSION

The clinical investigation and analysis of stress distribution after harvesting the bone graft is not possible. Therefore, numerical methods such as FEA can facilitate the analysis of the stress distribution after harvesting the grafts from the mandibular symphysis. A previous theoretical and biomechanical study used various materials (cortical, trabecular bone and teeth) that were assumed to be isotropic, homogenous, and linearly elastic to standardize the harvested mandible models and accurately compare the models; however, the cortical and trabecular bones of the mandible does not have a clinically homogenous and isotropic structure (10).

Table 2. Experimental groups and conditions

Analysis model	Depth (mm)	Maximum von Mises values (MPa)			Cycle time (s)	Load direction (deg.)
		Load localization and magnitude (N)				
		Incisal 300 N	Unilateral 300 N	Bilateral 600 N		
R1	4	2.30	1.06	1.32	1.80	90°–Vertical
	6	2.45	1.08	1.49		
R2	4	2.65	1.08	1.67	1.80	90°–Vertical
	6	2.82	1.07	1.75		
R1+1	4	2.67	2.00	1.32	1.80	90°–Vertical
	6	2.74	1.98	1.47		
R2+2	4	2.62	1.98	1.64	1.80	90°–Vertical
	6	2.98	2.16	1.88		
15×10×4 unilateral		2.49	1.15	1.63	1.80	90°–Vertical
15×10×4 bilateral		4.24	3.55	3.68	1.80	90°–Vertical
15×10×6 unilateral		3.03	1.20	1.83	1.80	90°–Vertical
15×10×6 bilateral		3.28	2.71	2.21	1.80	90°–Vertical
20×10×4		2.46	1.72	0.92	1.80	90°–Vertical
20×10×6		2.79	1.98	1.26	1.80	90°–Vertical
30×10×4		2.63	2.21	1.52	1.80	90°–Vertical
30×10×6		2.45	2.38	1.50	1.80	90°–Vertical

Figure 7. Four different cylindrical grafts with a depth of 4 mm harvested from the symphysis and distribution of the maximum von Mises stress values (MPa)

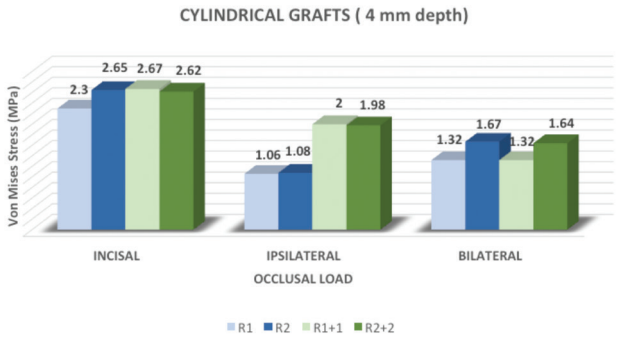
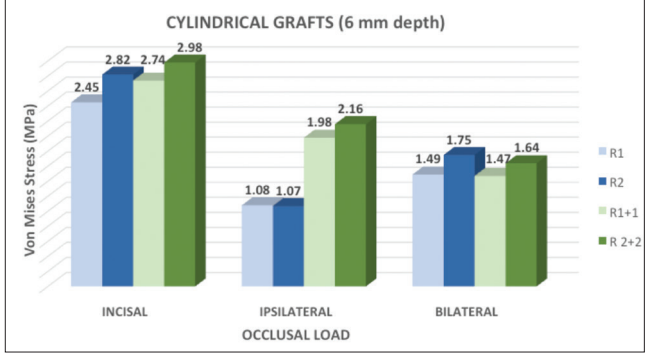


Figure 8. Four different cylindrical grafts with a depth of 6 mm harvested from the symphysis and the distribution of the maximum von Mises stress values (MPa)



Möhlhenrich et al. (8) investigated the stress distribution of bone grafts of various sizes harvested from the ascending ramus of a dentate mandible using FEA. The authors reported that the location of the force application significantly affected the resulting stress within the donor site and also noted that the lowest stress was detected under the incisal load, whereas the occlusal load in the molar region particularly increased the stress under the contralateral load (8). However, Möhlhenrich et al. (8) only analyzed the donor sites of the bone grafts harvested from the ascending ramus and did not evaluate the curved osteotomies or cylindrical grafts. In other study, Ertem et al. (13) investigated the stress distribution of two different osteotomies in the mandibles, which had a rectangular shape with angled borders and an elliptical shape with curved design osteotomies. The authors indicated that the curved osteotomy model had lower stress and a wider stress distribution than the angled model (13). In

Figure 9. Two harvested grafts of different dimensions with the depths of 4 mm and 6 mm in the midline of the symphysis and the distribution of the maximum von Mises stress values (MPa)

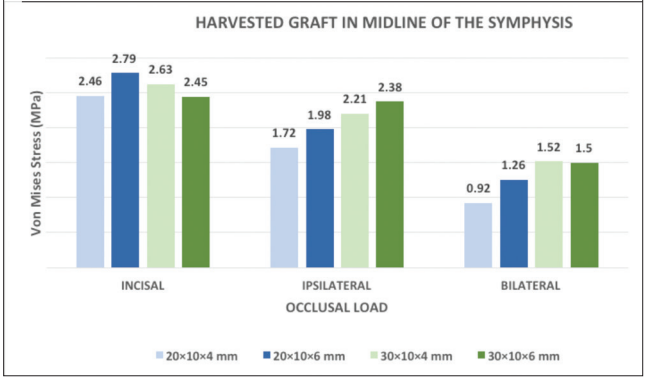


Figure 10. Stress distribution and maximum von Mises stress values when the unilateral block grafts were harvested (MPa)

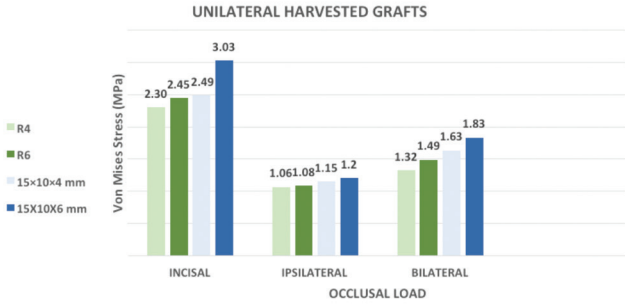
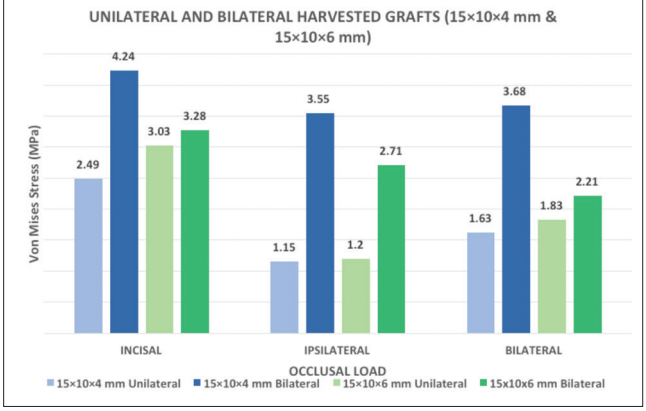


Figure 11. Comparison of the unilateral and bilateral harvested grafts with the depths of 4 and 6 mm



our study, the maximum von Mises stress values were higher under the incisal load than under the ipsilateral and bilateral loads, mainly because the incisal load was closer to the donor site. In block grafts that were harvested from the midline symphysis models, the maximum von Mises stress was 37% higher in the model measuring 20×10×6 mm than in the model measuring 20×10×4 mm under the bilateral load. The mandibular model harvested a graft measuring 20×10×4 mm from the midline symphysis, wherein the fracture risk increased under bilateral posterior loads with the increase in the depth of the harvested block graft (Figure 9). Cylindrical grafts had lower stress than the rectangular grafts with angled borders, and these findings were similar to the findings of Möhlhenrich et al. and Ertem et al. (13) (Figure 10). Furthermore, the location, dimension, and laterality (unilateral or bilateral) of the models affected the resultant stress in the grafts. Based on these findings, we consider that cylindrical grafts reduce the stress and if the rectangular grafts need to be harvested in the symphysis, then the localization of the grafts and the pattern of the occlusal load should be considered in terms of stress levels.

CONCLUSION

The application of incisal load led to a higher stress than that of bilateral and ipsilateral loads. The stress distribution in the symphysis donor site varies according to the localization, shape, and dimensions of the harvested grafts. Cylindrical grafts lead to a lower stress than rectangular grafts.

Ethics Committee Approval: Ethics approval was not required for this in vitro study.

Informed Consent: Due to the study was experimental, informed consent was not taken.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.Y.; Design - M.Y., E.A.; Supervision - M.Y., E.A; Materials - E.A.; Data Collection and/or Processing - E.A.; Analysis and/or Interpretation - E.A.; Literature Search - M.Y.; Writing Manuscript - M.Y.; Critical Review - M.Y., E.A.

Conflict of Interest: The authors have no conflicts of interest to declare.

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REFERENCES

1. Möhlhenrich SC, Heussen N, Ayoub N, Hölzle F, Modabber A. Three-dimensional evaluation of the different donor sites of the mandible for autologous bone grafts. Clin Oral Investig 2015; 19: 453-8. [Crossref]
2. Misch CM, Misch CE, Resnik RR, Ismail YH. Reconstruction of maxillary alveolar defects with mandibular symphysis grafts for dental implants: a preliminary procedural report. Int J Oral Maxillofac Implants 1992; 27: 360-6.
3. Khoury F, Buchmann R. Surgical therapy of peri-impant disease: a 3-year follow-up study of cases treated with 3 different techniques of bone regeneration. J Periodontol 2001; 72: 1498-508. [Crossref]
4. Misch CM. Comparison of intraoral donor sites for onlay grafting prior to implant placement. Int J Oral Maxillofac Implants 1997; 12: 767-76.
5. Jensen J, Sindet-Pedersen S. Autogenous mandibular bone grafts and osseointegrated implants for reconstruction of the severely atrophied maxilla: a preliminary report. J Oral Maxillofac Surg 1991; 49: 1277-87. [Crossref]
6. Khoury F. Augmentation of the sinus floor with mandibular bone block and simultaneous implantation: a 6-year clinical investigation. Int J Oral Maxillofac Implants 1999; 14: 557-64.

7. Goodday RH. Management of fractures of the mandibular body and symphysis. Oral Maxillofac Surg Clin North Am 2013; 25: 601-16. [Crossref]
8. Möhlhenrich SC, Kniha K, Szalma J, Ayoub N, Hölzle F, Wolf M, et al. Stress distribution in mandibular donor site after harvesting bone grafts of various sizes from the ascending ramus of a dentate mandible by finite element analysis. Clin Oral Investig 2019; 23: 2265-71. [Crossref]
9. Geng JP, Tan KB, Liu GR. Application of finite element analysis in implant dentistry: A review of the literature. J prosthet Dent 2001; 85: 585-98. [Crossref]
10. Yalçın M, Kaya B, Laçin N, Arı E. Three-Dimensional Finite Element Analysis of the Effect of Endosteal Implants with Different Macro Designs on Stress Distribution in Different Bone Qualities. Int J Oral Maxillofac Implants 2019; 34: e43-e50. [Crossref]
11. Singley JE, Mishke CR. Mechanical Engineering Design, ed 5. NewYork: McGraw-Hill Book Company; 1989.
12. Korioth TW, Hannam AG. Deformation of the human mandible during simulated tooth clenching. J Dent Res 1994; 73: 56-66. [Crossref]
13. Ertem SY, Uckan S, Ozden UA. The comparison of angular and curvilinear marginal mandibulectomy on force distribution with three dimensional finite element analysis. J Craniomaxillofac Surg 2013; 41: e54-8. [Crossref]

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Original Research

Subclinical Coronary Atherosclerosis in Patients Undergoing Catheter Ablation for Idiopathic Premature Ventricular Complexes

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ABSTRACT

Objective: Idiopathic premature ventricular complexes (PVC) occur in the absence of clinically apparent structural heart disease (SHD) and are treated effectively with catheter ablation. We aimed to evaluate the association of PVC characteristics with subclinical coronary artery disease (CAD) during catheter ablation.

Methods: A total of 116 patients (age: median 55 years; sex: 58.6% men), without SHD, in whom PVC ablation and coronary angiography had been performed simultaneously were enrolled. PVC localizations were categorized into 4 groups as; right ventricular outflow tract (RVOT), left ventricular outflow tract (LVOT), left ventricle (LV)-body, and right ventricle (RV)-body. PVC frequency was also classified as moderate (5,000–10,000 PVCs/day) and frequent (≥10,000 PVCs/day). Coronary artery stenoses were categorized as normal, non-critical (<50%), and critical (≥50%).

Results: Co-incidental CAD was more frequent among patients with LV-body originated PVCs (non-critical, 51.6 % and critical, 22.6 %); while most of the patients with LVOT, RVOT, and RV-body originated PVCs had normal coronary arteries (58.8%, 56%, and 55.6%, respectively; p=0.019). There was no significant association between PVC frequency and coronary artery lesion severity (p=0.080) or between PVC recurrence and PVC frequency (p=0.748), PVC localization (p=0.188), coronary artery lesion severity (p=0.080), number of involved coronary artery segments (p=0.566), and number of involved coronary artery vessels (p=0.729).

Conclusion: Subclinical CAD was more frequent among patients with LV-body originated idiopathic PVCs. Thus, its routine pre-procedural assessment may be considered for LV-body originated PVCs.

Keywords: Coronary artery disease, premature ventricular complex, radiofrequency ablation

INTRODUCTION

Premature ventricular complexes (PVCs) develop due to automaticity, micro-re-entry, or triggered activity with a prevalence of 1%–4% on 12-lead electrocardiography (ECG) and 40%–75% on Holter monitorization (1-3). Although, PVCs generally have a benign course, they can be associated with more serious conditions, such as ventricular tachycardia and ventricular fibrillation, causing sudden cardiac death, particularly in patients with SHD or ionic channel disorders. PVC-induced cardiomyopathy is another manifestation that responds well to either antiarrhythmic medications or catheter ablation (4). Among several landmark studies that evaluated the association between PVC and survival after acute coronary syndrome, the GISSI trial (5) demonstrated that more than 10 PVCs per hour, detected by Holter monitorization, were associated with an increased mortality risk after a myocardial infarction. It has also been reported that implantable cardioverter-defibrillator prolongs survival in patients with coronary heart disease and asymptomatic non-sustained ventricular tachycardia (VT) (6). However, data regarding the relationship between subclinical coronary atherosclerosis and PVC characteristics in patients without structural heart disease (SHD) who underwent catheter ablation is limited.

Catheter ablation offers safe and effective treatment for AAD resistant symptomatic PVCs in patients with and without SHD (7, 8). We aimed to evaluate the association between PVC characteristics and coincidentally detected coronary atherosclerosis, and predictors of PVC recurrence, in patients without known SHD.

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METHODS

Study Population

Patients who underwent catheter ablation due to frequent and symptomatic PVCs between May 2015 and June 2019 were screened. Those who concurrently underwent diagnostic coronary angiography (CAG) were included in the study. Coronary angiography indications during catheter ablation were as follows: presence of any risk factor for ischemic heart disease, moderate-to-severe mitral regurgitation, reduced left ventricular ejection fraction (HFrEF), and previous history of coronary artery disease (CAD). Patient data including demographic, clinical, laboratory, procedural, and follow-up parameters were obtained from the electronic database of our university hospital. Exclusion criterias were: age <40 years, lack of CAG assessment, history of myocardial infarction, presence of polymorphic PVCs, ischemic signs and/or symptoms, and previous diagnosis of cardiomyopathy; including, arrhythmogenic right ventricle cardiomyopathy, non-compaction cardiomyopathy, hypertrophic or dilated cardiomyopathy, etc. Patients with systolic blood pressure of ≥140 mmHg and/or diastolic BP (DBP) of ≥90 mmHg, or those receiving antihypertensive medications were considered as hypertensive (9). Diabetes mellitus was defined by the presence of one of the following conditions: HbA1c levels≥6.5%, fasting plasma glucose levels≥126 mg/dL, random plasma glucose levels≥200 mg/dL, plasma glucose levels 2 hours after oral glucose loading≥200 mg/dL, or administration of antidiabetic medications (10). Chronic kidney disease was defined as an estimated glomerular filtration rate (eGFR)<60 mL/min/1.73 m^2 in at least two measurements at three months interval. PVCs were categorized into 4 groups according to the anatomic localization: 1) PVCs originating from the right ventricular outflow tract (RVOT), 2) PVCs originating from the left ventricular outflow tract (LVOT) including outflow tract and aortic cusps, 3) LV-body originating PVCs including papillary muscle, aortico-mitral continuity region, and LV summit, and 4) RV-body originating PVCs including free wall, moderator band, and papillary muscle. PVC frequency was classified as moderate (5,000-10,000 PVCs/day) and frequent (≥10,000 PVCs/day). Ethical approval was received from the Local Ethical Committee of Hacettepe University, School of Medicine. All patients were hospitalized and signed a hospitalization form that included the permission to use their clinical data for future clinical studies. Due to the retrospective design of the study no additional informed consent was obtained from the patients.

Mapping and Ablation Procedure

Antiarrhythmic drugs were stopped for a duration of five half-lives of the drugs before the intervention. Anticoagulant and antiplatelet drugs (P2Y12 receptor antogonists), except aspirin, were discontinued before the procedure for the recommended time interval.

Main Points:

- There is not any relationship between the frequency of PVC and coronary artery lesion severity.
- LV-body originated PVCs are associated with the presence and severity of CAD in patients with a structurally normal heart
- PVC frequency and localization is not a predictor of recurrence after cathether ablation.

Since PVC suppression may occur under general anesthesia, electrophysiological studies and catheter ablation were performed under conscious sedation using intravenous midazolam. Clinical PVC templates were recorded at the beginning of the procedure. If spontaneous PVCs were not seen, isoproterenol infusion was administered to induce them. After femoral access, a thorough electrophysiological study was performed in all patients using coronary sinus, RV, and His catheters. In all patients, three-dimensional electroanatomical mapping system was used (Ensite Precision, Abbott or CARTO, Biosense Webster). If PVCs were of RV origin on the surface ECG, activation mapping of RV and RVOT was performed initially; while, for those of LV origin and/or without any early site at the RV, activation mapping of the LV including aortic cusps was performed. LV mapping was performed via the retrograde transaortic approach. Heparin was administered to maintain an activated clotting time between 300 and 350 seconds in patients who underwent LV mapping. Intravenous heparin (50 IU/kg) was also administered to all patients if only right-sided mapping and ablation were performed. Additionally, pace-mapping was also used to localize the PVC exit site and a pace-map score ≥90% was applied for an appropriate ablation site. Radiofrequency (RF) current ablation using irrigated tip catheters (maximum energy: 30–40 W; duration: 60–120 s) was performed at the site of earliest local bipolar activation (≥30 ms from the onset of the QRS) and with the presence of a QS pattern in the unipolar signal. After ablation at the appropriate site, procedural success was defined as the complete elimination of the PVCs after a 30 minute waiting period, including isoproterenol infusion, and the absence of clinical PVCs in the 24-hour post-ablation period.

Coronary Angiography

Coronary angiography was performed using the Judkins technique via the femoral approach. Coronary arteries were visualized by injecting contrast medium in the right and left oblique positions at the cranial and caudal angles. Patients were grouped into three groups according to the coronary lesion presence and severity. These included normal coronary arteries (no visible atherosclerotic plaque), non-critical stenotic lesions (atherosclerotic plaque with <50% stenosis), and critical stenosis (atherosclerotic plaque with ≥50% stenosis).

Follow-up

Patients with normal coronary arteries were discharged and prescribed aspirin for a month. Patients with non-critical lesions were discharged with aspirin and statin therapy if their LDL-cholesterol levels were above recommended values. Patients with critical coronary artery stenosis were referred to the interventional cardiology team for optimal medical therapy, percutaneous coronary intervention, or coronary artery by-pass grafting. Patients were scheduled for outpatient clinic visits at 1, 3, and 6 months and yearly visits thereafter; wherein; resting 12-lead ECG, transthoracic echocardiography, and 24-hr Holter monitorization were performed. The PVC recurrence was defined as the detection of ≥5000 PVCs/day on 24-hr Holter monitorization on follow-up that had the same morphology as the previously ablated PVCs.

Statistical Analysis

Continuous variables were expressed as a mean with standard deviation or median with interquartile range (IQR). Categorical

Table 1. Baseline characteristics of the study population

Male gender	68 (58.6%)
Age, years	55 (14)
Comorbidities	
– Coronary artery disease	17 (14.6%)
– Diabetes mellitus	17 (14.7%)
– Hypertension	45 (38.8%)
– HFrEF	17 (14.6%)
– Chronic kidney disease	7 (6.0%)
Smoking	34 (29.3%)
Medications	
ACE inhibitor	20 (17.2%)
Anigotensin receptor blocker	28 (24.1%)
Beta–blocker	71 (61.2%)
Calcium channel blocker	6 (5.2%)
Statin	20 (17.2%)
Duration between symptom onset and ablation, months	6 (3–19.5)
Duration of follow–up, months	16.23 (8.75–26.00)
Recurrence during follow–up	11 (9.5%)
Re–do catheter ablation	9 (7.7%)
Recurrence free survival, months	15.43 (7.86–25.31)
LV end–diastolic diameter, mm	51.49 ± 7.14
LVEF, %	60 (50–62)
Mitral regurgitation	
–Mild	70 (60.3%)
–Moderate	37 (31.9%)
–Severe	4 (3.4%)
BNP, pg/mL	83.20 (28–176.75)
TSH, mIU / L	1.4 (0.8–2.4)
Hb, gr/dL	13.92 ± 1.45
Serum creatinine, mg/dL	0.8 (0.6–0.9)
Localization of VPBs	
– RVOT	25 (21.6%)
– LVOT	51 (44.0%)
– LV body	31 (26.7%)
– RV body	9 (7.8%)
PVCs frequency	
Moderate (5000–10 000 per day)	78 (67.2%)
Frequent (≥10 000 per day)	38 (32.8%)
Coronary artery stenosis severity	
– Normal	57 (49.1%)
– Non–critical	40 (34.5%)
– Critical	19 (16.4%)
Involved coronary segments	1 (4)
Coronary artery with severe stenosis	
– LAD	8 (6.8%)
– Cx	6 (5.1%)
– RCA	6 (5.1%)
Number of coronary artery with any plaque	
– One vessel	5 (4.3%)
– Two vessels	11 (9.4%)
– Three vessels	43 (37.0%)

ACE: Angiotensin converting enzyme; BNP: brain natriuretic peptide; Cx: circumflex artery; Hb: hemoglobin; HFrEF: heart failure with reduced ejection fraction; LAD: left anterior descending artery; LV: left ventricle; LVEF: left ventricular ejection fraction; LVOT: left ventricular outflow tract; PVC: premature ventricular complex; RCA: right coronary artery; RV: right ventricle; RVOT: right ventricular outflow tract; TSH: thyroid stimulating hormone

variables were expressed as a number (percentage). Independent groups were compared with chi-square, independent samples t-test, or one-way ANOVA as appropriate. The Cox proportional hazards model was performed to identify predictive factors for PVC recurrence. Predictors of PVC recurrence with $p<0.2$ in univariate analyses were further included as covariates in the multivariate model. Recurrence-free survival was performed using Kaplan-Meier curves and log-rank tests. Statistical analyses were performed using the SPSS statistical software (version 20; IBM SPSS Corp.; Armonk, NY, USA). All tests of significance were two-sided and $p<0.05$ was considered as statistically significant.

RESULTS

A total of 116 patients (median age, 55; sex, 58.6% men) were included in the final analysis. Hypertension was present in 45 (38.8%), diabetes in 17 (14.7%), and CKD in 7 (6.0%) patients. The median duration between the onset of PVC-related symptoms and catheter ablation was 6 (3–19.5) months and the median follow-up after catheter ablation was 16.23 (8.75–26.00) months. Baseline characteristics including demographic, comorbidities, medications, echocardiographic, and laboratory parameters are represented in Table 1. Most of the PVCs originated from LVOT (44.0%); while, other sites had a lower frequency in this

Table 2. PVC localization and coronary artery disease

	LVOT	LV body	RVOT	RV body	p
Coronary artery stenosis severity					
–Normal	30 (58.8%)	8 (25.8%)	14 (56%)	5 (55.6%)	0.019*
–Non–critical	11 (21.6%)	16 (51.6%)	10(40%)	3 (33.3%)	
–Critical	10 (19.6%)	7 (22.6%)	1 (4%)	1 (11.1%)	
Number of involved segments	0 (0–7)	4 (0–8)	0 (0–6)	0 (0–5)	0.027*
Number of involved vessel	0 (0–3)	3 (0–3)	0 (0–3)	0 (0–3)	0.062

PVC: premature ventricular complex

Table 3. PVC frequency and coronary artery disease

	Moderate–frequent	Very frequent	p
Lesion severity;			
–Normal coronary arteries	44 (56.4%)	13 (34.2%)	0.080
–Non–critical stenosis	23 (29.5%)	17 (44.7%)	
–Critical stenosis		11 (14.1%)	8 (21.1%)
Number of involved segments	0 (0–8)	3 (0–7)	0.049*
Number of involved vessel	0 (0–3)	2 (0–3)	0.041*

PVC: premature ventricular complex

study group (LV-body, 26.7%; RVOT, 21.6%; and RV-body, 7.8%). Thirty-eight (32.8%) patients had frequent PVCs, while others showed moderate PVCs.

Severe coronary artery stenosis was detected in 19 (16.4%) patients. Distribution of the severe coronary artery stenotic lesions was as follows: eight (6.8%) lesions were located in LAD, six (5.1%) in the circumflex artery, and six (5.1%) in the right coronary artery. The median number of coronary artery segments with atherosclerotic plaques was 1 (0–4). Atherosclerosis severity, localization, and burden are also shown in Table 1.

The presence and severity of coronary artery lesions were also assessed with respect to PVC localization, and are represented in Table 2. Most of the patients with LV-body originated PVCs had coronary artery stenosis (non-critical, 51.6% and critical, 22.6%), while most of the patients with LVOT, RVOT, and RV-body related PVCs had normal coronary arteries (58.8%, 56%, and 55.6%, respectively, p=0.019). Number of involved coronary artery segments was significantly higher in patients with LV-body originated PVCs (median, 4; range, 0–8) than in patients with PVCs originating from LVOT (median, 0; range, 0–7), RVOT (median, 0; range, 0–6), and RV-body (median, 0; range, 0–5) (p=0.027). Number of involved coronary vessels was higher in patients with LV-body originated PVCs (median, 3; range, 0–3) than in patients with PVCs originating from LVOT (median, 0; range, 0–3), RVOT (median, 0; range, 0–3), and RV-body (median, 0; range, 0–3); however, the difference was not statistically significant (p=0.062) (Table 2). Critical coronary artery stenosis was seen in 11 (14.1%) and 8 (21.1%) patients with moderate and frequent PVCs, respectively. PVC frequency and coronary artery lesion severity were not significantly associated (p=0.080). The number of the involved coronary artery segments and coronary artery vessels were significantly higher in patients with frequent PVCs than in patients with moderate PVCs (median 3 vs. 0; range 0–7 vs. 0–8; p=0.049 and median 2 vs. 0; range 0–3 vs. 0–3; p=0.041, respectively) (Table 3).

PVC recurrence occurred in 11 (9.5%) patients and nine of them underwent repeat catheter ablation. Median recurrence-free survival was 15.43 (7.86–25.31) months. No significant association was found between PVC recurrence and PVC frequency (p=0.748), PVC localization (p=0.188), coronary artery lesion severity (p=0.080), number of involved coronary artery segments (p=0.566), and number of involved coronary artery vessels (p=0.729).

DISCUSSION

In this study, the relationship between PVC characteristics and coronary atherosclerosis was evaluated in patients without SHD. LVOT was the most common site of PVC origin. PVCs originating from LV-body had higher coronary artery stenosis than in patients with other PVC localization. However, we did not find a significant relationship between coronary artery lesion severity and PVC frequency. Conversely, the burden of coronary atherosclerosis was higher in the frequent PVC group than in the moderate

PVC group. There was no association of PVC recurrence with PVC frequency, localization, and coronary arteriosclerotic lesion characteristics.

PVCs can be seen in 40%–75% of healthy individuals on 24–48 hr Holter recordings (1). The association of PVCs with long-term prognosis in patients without identifiable heart disease has been variably reported. While, some studies have shown that long-term risk is similar to that of healthy individuals (11, 12), according to another study PVCs and short-term NSVTs may increase the risk in individuals older than 30 years (13). In many studies, the frequency of PVCs was associated with LV dysfunction and dilatation (14, 15). In patients with outflow tract PVCs, catheter ablation is recommended in symptomatic patients and/or in patients with a failure of antiarrhythmic drug therapy (7). The ablation success rate is high in these patients and this rate reached up to 95% in RVOT-originated PVCs (16, 17). The recurrence rate of 9.5% and median recurrence-free survival of 15.43 (7.86–25.31) months in our study was in accordance with that in the literature. No association has been reported between the PVC frequency and the success of the ablation procedure (18). The same was observed in our study by the absence of a relationship between PVC recurrence and its frequency. Further, there was no significant association between PVC recurrence and coronary atherosclerotic lesion severity and extent. This showed that CAD and its characteristics in patients with a structurally normal heart did not affect the long-term success of ablation.

Ventricular outflow tracts are the most common origin of idiopathic PVCs (19, 20). Furthermore, 70% of them are reported to be of RVOT origin (21). In our study, most of the PVCs originated from LVOT. This may be attributed to several factors, including the inclusion of patients who underwent simultaneous coronary angiography and the enrollment of patients older than 40 years. Moreover, because of the proximity of the coronary artery ostium to the ablation areas in LVOT-originated PVCs, coronary imaging was almost always required to prevent possible injury to the coronary arteries during ablation.

One of the main objectives of our study was to investigate the relationship between PVC characteristics and CAD. Although, the association between PVCs and CAD has always been a subject of concern, data regarding the appropriate selection of patients who may benefit from coronary angiography is limited. In a previous study involving 343 patients with an intermediate-to-high probability of CAD who underwent single-photon emission computed tomography and a stress test, it was found that patients with stress-induced PVCs of right bundle-branch block morphology had higher rates of known CAD, ischemia, scar, and ST-segment changes (22). It was shown that exercise-induced PVCs were associated with a higher risk of cardiovascular mortality and all-cause mortality over long-term follow-up (23). In patients without SHD, PVC ablation is mostly performed in symptomatic patients refractory to medical therapy, or in those with systolic left ventricular dysfunction. The relationship between PVC characteristics and CAD in patients undergoing PVC ablation without SHD has not been assessed previously. CAD characteristics were associated with PVC localization in our study. CAD was significantly more frequent in patients with LV-body originated PVCs than in patients with LVOT, RVOT, and RV-body originated PVCs. Further, the extent of atherosclerosis was higher in patients with LV-body originated PVCs than at other sites. Consequently, those with LV-body originated PVCs may require assessment for CAD during the ablation procedure. Conversely, there was no relationship between PVC frequency and coronary artery stenosis severity in our study. Furthermore, the number of involved coronary segments and coronary artery vessels were significantly higher in patients with frequent PVCs than in patients with moderate PVCs. Thus, our findings have important implications regarding the relationship between PVC subtypes and CAD characteristics in patients with a structurally normal heart.

There were some limitations in our study. Firstly, the study was of a retrospective design with a small sample size. Secondly, it was conducted in patients in whom PVC ablation was primarily targeted. If ischemic symptoms were predominant, these patients may not have been enrolled in the study, despite the presence of PVCs. Thus, our findings cannot be generalized to the whole population with PVCs. Large scale long-term prospective studies are needed to clarify the relationship between CAD and PVC characteristics in such patient populations.

CONCLUSION

Our study showed that LV-body originated PVCs were associated with the presence and severity of CAD in patients with a structurally normal heart. There was no significant relationship between the frequency of PVC and coronary artery lesion severity. Furthermore, it was shown that PVC frequency and localization, and CAD characteristics were not predictors of PVC recurrence after catheter ablation. In patients with LV-body originated PVCs, a more detailed assessment of CAD during the ablation procedure may be required.

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REFERENCES

1. Ng GA. Treating patients with ventricular ectopic beats. Heart 2006; 92: 1707-12. [Crossref]

2. Enriquez A, Frankel DS, Baranchuk A. Pathophysiology of ventricular tachyarrhythmias : From automaticity to reentry. Herzschrittmacherther Elektrophysiol 2017; 28: 149-56. [Crossref]
3. Lerman BB. Ventricular tachycardia: mechanistic insights derived from adenosine. Circ Arrhythm Electrophysiol 2015; 8: 483-91. [Crossref]
4. Ahn MS. Current Concepts of Premature Ventricular Contractions. J Lifestyle Med 2013; 3: 26-33.
5. Maggioni AP, Zuanetti G, Franzosi MG, Rovelli F, Santoro E, Staszewsky L, et al. Prevalence and prognostic significance of ventricular arrhythmias after acute myocardial infarction in the fibrinolytic era. GISSI-2 results. Circulation 1993; 87: 312-22. [Crossref]
6. Moss AJ, Hall WJ, Cannom DS, Daubert JP, Higgins SL, Klein H et al. Improved survival with an implanted defibrillator in patients with coronary disease at high risk for ventricular arrhythmia. Multicenter Automatic Defibrillator Implantation Trial Investigators. N Engl J Med 1996; 335: 1933-40. [Crossref]
7. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J 2015; 36: 2793-867. [Crossref]
8. Cronin EM, Bogun FM, Maury P, Peichl P, Chen M, Namboodiri N, et al. 2019 HRS/EHRA/APHRS/LAHRS expert consensus statement on catheter ablation of ventricular arrhythmias. J Arrhythm 2019; 35: 323-484. [Crossref]
9. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J 2018; 39: 3021-104. [Crossref]
10. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes care 2011; 34: S62-9. [Crossref]
11. Engstrom G, Hedblad B, Janzon L, Juul-Moller S. Ventricular arrhythmias during 24-h ambulatory ECG recording: incidence, risk factors and prognosis in men with and without a history of cardiovascular disease. J Intern Med 1999; 246: 363-72. [Crossref]
12. Kennedy HL, Whitlock JA, Sprague MK, Kennedy LJ, Buckingham TA, Goldberg RJ. Long-term follow-up of asymptomatic healthy subjects with frequent and complex ventricular ectopy. N Engl J Med 1985; 312: 193-7. [Crossref]
13. Chiang BN, Perlman LV, Ostrander Jr LD, Epstein FH. Relationship of premature systoles to coronary heart disease and sudden death in the Tecumseh epidemiologic study. Ann Intern Med 1969; 70: 1159-66. [Crossref]
14. Niwano S, Wakisaka Y, Niwano H, Fukaya H, Kurokawa S, Kiryu M, et al. Prognostic significance of frequent premature ventricular contractions originating from the ventricular outflow tract in patients with normal left ventricular function. Heart 2009; 95: 1230-7. [Crossref]
15. Kanei Y, Friedman M, Ogawa N, Hanon S, Lam P, Schweitzer P. Frequent premature ventricular complexes originating from the right ventricular outflow tract are associated with left ventricular dysfunction. Ann Noninvasive Electrocardiol 2008; 13: 81-5. [Crossref]
16. Ling Z, Liu Z, Su L, Zipunnikov V, Wu J, Du H, et al. Radiofrequency ablation versus antiarrhythmic medication for treatment of ventricular premature beats from the right ventricular outflow tract: prospective randomized study. Circ Arrhythm Electrophysiol 2014; 7: 237-43. [Crossref]
17. Yamada T, Murakami Y, Yoshida N, Okada T, Toyama J, Yoshida Y, et al. Efficacy of electroanatomic mapping in the catheter ablation of premature ventricular contractions originating from the right ventricular outflow tract. J Interv Card Electrophysiol 2007; 19: 187-94. [Crossref]
18. Latchamsetty R, Yokokawa M, Morady F, Kim HM, Mathew S, Tilz R, et al. Multicenter Outcomes for Catheter Ablation of Idiopathic Premature Ventricular Complexes. JACC Clin Electrophysiol 2015; 1: 116-23. [Crossref]
19. Morady F, Kadish AH, DiCarlo L, Kou WH, Winston S, deBuitlier M, et al. Long-term results of catheter ablation of idiopathic right ventricular tachycardia. Circulation 1990; 82: 2093-9. [Crossref]
20. Tada H, Hiratsuji T, Naito S, Kurosaki K, Ueda M, Ito S, et al. Prevalence and characteristics of idiopathic outflow tract tachycardia with QRS alteration following catheter ablation requiring additional radiofrequency ablation at a different point in the outflow tract. Pacing Clin Electrophysiol 2004; 27: 1240-9. [Crossref]
21. Yamada T, McElderry HT, Doppalapudi H, Murakami Y, Yoshida Y, Yoshida N, et al. Idiopathic ventricular arrhythmias originating from the aortic root prevalence, electrocardiographic and electrophysiologic characteristics, and results of radiofrequency catheter ablation. J Am Coll Cardiol 2008; 52: 139-47. [Crossref]
22. Biere L, Mezdad TH, Dupuis JM, Vervueren L, Rakotonirina H, Prunier F, et al. Long-term prognostic significance of right bundle-branch morphology ventricular ectopy induced during stress test in patients with intermediate to high probability of coronary artery disease. Europace 2018; 20: 528-34. [Crossref]
23. Lee V, Perera D, Lambiase P. Prognostic significance of exercise-induced premature ventricular complexes: a systematic review and meta-analysis of observational studies. Heart Asia 2017; 9: 14-24. [Crossref]

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Original Research

The Neutrophil to Lymphocyte Ratio and In-Hospital All-Cause Mortality in Patients with COVID-19

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ABSTRACT

Objective: In December 2019, pneumonia associated with severe acute respiratory syndrome coronavirus 2 emerged in China, and has been spread worldwide eventuating the coronavirus disease 2019 (COVID-19) pandemic. As of June 27, 2020, 195,883 people have been diagnosed with COVID-19 in Turkey, among them 5082 are dead. Moreover, 9,999,606 people were infected worldwide. The neutrophil-to-lymphocyte ratio (NLR) has been reported as an inflammatory biomarker. This study aimed to evaluate the relationship between NLR on admission and in-hospital all-cause mortality in adult patients with COVID-19.

Methods: This retrospective cohort study included a total of 455 COVID-19 patients from Turkey. The diagnosis of COVID-19 was made according to the World Health Organization's interim guidance and confirmed by RNA detection of SARS-CoV-2. The NLR was calculated for each patient.

Results: The NLR on admission was found to be significantly higher in nonsurvivor COVID-19 patients than survivors (12.3 [0.8–137.3] vs. 3.2 [0.6–79.0], p<0.001). Forward stepwise logistic regression analysis was carried out to determine the independent predictors of in-hospital all-cause mortality of patients with COVID-19. The analysis demonstrated that age [odds ratio (OR)=1.203, 95% confidence interval (CI): 1.027–1.408, p=0.022], NLR (OR=1.261, 95% CI: 1.054–1.509, p=0.011), lactate dehydrogenase level (OR=1.013, 95% CI: 1.004–1.022, p=0.005), glomerular filtration rate (OR=0.920, 95% CI: 0.853–0.992, p=0.030), alanine transaminase level (OR=1.107, 95% CI: 1.011–1.212, p=0.028), and aspartate transaminase level on admission (OR=0.939, 95% CI: 0.888–0.993, p=0.027) were independent predictors of in-hospital all-cause mortality of patients with COVID-19. In the receiver operating characteristic curve analysis, the sensitivity and specificity of the NLR for predicting in-hospital all-cause mortality were found to be 92% and 53%, respectively, at the cut-off value of 3.

Conclusion: The NLR on admission predicts in-hospital all-cause mortality of patients with COVID-19.

Keywords: Coronavirus, lymphocyte, neutrophil

INTRODUCTION

In December 2019, pneumonia associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1) emerged in Wuhan, China, and has been spread worldwide eventuating the coronavirus disease 2019 (COVID-19) pandemic with emerging global concerns. New confirmed cases and deaths caused by COVID-19 are reported daily all over the world. As of June 27, 2020, 195,883 people have been diagnosed with COVID-19 in Turkey, among them 5082 are dead. Moreover, 9,999,606 people were infected worldwide. Risk stratification in such pandemics is extremely required. Early and effective predictors of clinical outcomes are urgently needed as there is no standardized treatment available currently.

The neutrophil-to-lymphocyte ratio (NLR), calculated by dividing absolute neutrophil count with absolute lymphocyte count, has been reported as an inflammatory biomarker that can be used as an indicator of systemic inflammation (2, 3). Prognostic value of the NLR in various diseases, such as community pneumonia (4, 5) and sepsis, have been reported in various studies (6). A recent

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study has exhibited that the NLR is independently associated with mortality in hospitalized COVID-19 patients (7). The present study aims to evaluate the impact of the NLR on in-hospital all-cause mortality in adult patients with COVID-19.

METHODS

Study Population and Design

This retrospective cohort study included a total of 455 adult COVID-19 patients from Turkey. The diagnosis of COVID-19 was made according to the World Health Organization's interim guidance and confirmed by RNA detection of SARS-CoV-2. All adult patients diagnosed with COVID-19 were screened, and those who died or discharged between March 10, 2019, and June 10, 2020, were enrolled in this study. Out of 455 patients, 145 were critically ill and were admitted to the intensive care unit. The study was conducted following the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Çukurova University (No: 99, 15 May 2020), as well as by the Ministry of Health. The need for written informed consent was waived due to the retrospective nature of the study.

Data Collection

Epidemiologic, demographic, clinical, laboratory, treatment, and outcome data were extracted from electronic medical records using admission numbers which were unique to each patient. In-hospital all-cause mortality outcomes were followed up till June 15, 2020.

Laboratory Procedures

RNA detection of SARS-CoV-2 in respiratory specimens was carried out by real-time polymerase chain reaction. The criteria for discharge were the absence of fever for at least 3 days, substantial improvement in both lungs detected by chest computed tomography, clinical remission of respiratory symptoms, and one negative sample of throat swab for SARS-CoV-2 RNA. Blood examinations included complete blood count and serum biochemical tests.

Statistical Analysis

Data analyses were performed using SPSS version 22.0 statistical software package (IBM SPSS Corp.; Armonk, NY, USA). The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous variables were expressed as mean±standard deviation or median (minimum-maximum). Categorical variables were expressed as number (percentage). The independent samples t-test or Mann–Whitney U-test was used to compare continuous variables based on whether statistical assumptions were fulfilled or not. The Chi-square test or Fisher's exact test was used to compare categorical variables based on whether statistical assumptions were fulfilled or not. All significant parameters in the univariate analysis were selected for the multivariable model, and a forward stepwise logistic regression analysis was used to determine the independent predictors of in-hospital mortality of COVID-19 patients. The odds ratio (OR) and 95% confidence interval (CI) of each independent variable were calculated. A receiver operating characteristic curve analysis was carried out to identify the optimal cut-off level of the NLR that predicts the in-hospital all-cause mortality. The area under the curve was calculated as a measure of the accuracy of the test. A two-tailed p-value of less than 0.05 was considered significant.

Main Points:

- This study evaluated the relationship between the neutrophil to lymphocyte ratio (NLR) on admission and in-hospital all-cause mortality in adult patients with the coronavirus disease 2019 (COVID-19).
- The NLR on admission was significantly higher in nonsurvivors COVID-19 patients than in survivors.
- Age, the NLR, lactate dehydrogenase level, glomerular filtration rate, alanine transaminase level, and aspartate transaminase level on admission were independent predictors of in-hospital all-cause mortality of patients with COVID-19.
- The sensitivity and specificity of the NLR for predicting in-hospital all-cause mortality were 92% and 53%, respectively, at the cut-off value of 3.

RESULTS

This retrospective cohort study enrolled a total of 455 hospitalized COVID-19 patients (217 males, median age: 56 [18–98] years). In the final analysis, 92 patients died during hospitalization, and 363 patients were discharged.

The most common comorbidity was found to be hypertension (37.4%), followed by diabetes mellitus (28.1%), and coronary artery disease (19.3%). The most common symptom found on admission was cough, followed by fever, and dyspnea. Leukocyte count, neutrophil count, mean platelet volume, red cell distribution width, the NLR, the platelet-to-lymphocyte ratio, C-reactive protein (CRP), procalcitonin, ferritin, lactate dehydrogenase, alanine transaminase, and aspartate transaminase levels on admission were found to be significantly higher in nonsurvivor COVID-19 patients than survivors. However, hemoglobin level, lymphocyte count, and glomerular filtration rate were significantly lower in the former. Table 1 shows the comparison of baseline characteristics of patients with COVID-19 according to in-hospital all-cause mortality.

Forward stepwise logistic regression analysis was carried out to determine the independent predictors of in-hospital all-cause mortality of patients with COVID-19. The analysis demonstrated the following factors as independent predictors: age (OR=1.203, 95% CI: 1.027–1.408, p=0.022), the NLR (OR=1.261, 95% CI: 1.054–1.509, p=0.011), lactate dehydrogenase level (OR=1.013, 95% CI: 1.004–1.022, p=0.005), glomerular filtration rate (OR=0.920, 95% CI: 0.853–0.992, p=0.030), alanine transaminase level (OR=1.107, 95% CI: 1.011–1.212, p=0.028), and aspartate transaminase level on admission (OR=0.939, 95% CI: 0.888–0.993, p=0.027) (Table 2).

The sensitivity and specificity of the NLR for predicting in-hospital all-cause mortality were found to be 92% and 53%, respectively, in the receiver operating characteristic curve analysis, at the cut-off value of 3, with the area under the curve being 0.842 (95% CI: 0.795–0.889, p<0.001) (Figure 1).

Table 1. Comparison of baseline characteristics of patients with COVID-19 according to in-hospital all-cause mortality

Variable	Total (n=455)	Survivor (n=363)	Nonsurvivor (n=92)	p
Demographic and Clinical Features				
Age (year)	56.0 (18.0–98.0)	52.0 (18.0–98.0)	71.0 (39.0–95.0)	<0.001
Gender, (male) n (%)	217 (47.7)	172 (47.4)	45 (48.9)	0.793
BMI (kg/m^2)	27.2 (18.4–49.1)	27.0 (18.4–49.1)	27.7 (18.7–47.8)	0.670
Current smoker, n (%)	101 (22.2)	77 (21.2)	24 (26.1)	0.315
ICU admission, n (%)	145 (31.9)	55 (15.2)	90 (97.8)	<0.001
Comorbidities				
DM, n (%)	128 (28.1)	90 (24.8)	38 (41.3)	0.002
HT, n (%)	170 (37.4)	113 (31.1)	57 (62.0)	<0.001
CAD, n (%)	88 (19.3)	53 (14.6)	35 (38.0)	<0.001
HF, n (%)	32 (7.0)	22 (6.1)	10 (10.9)	0.107
COPD, n (%)	45 (9.9)	28 (7.7)	17 (18.5)	0.002
Stroke, n (%)	14 (3.1)	9 (2.5)	5 (5.4)	0.171
Malignancy, n (%)	17 (3.7)	10 (2.8)	7 (7.6)	0.057
Symptoms				<0.001
Fever (temperature ≥37.3°C)	138 (30.3)	93 (25.6)	45 (48.9)	
Cough, n (%)	150 (33.0)	139 (38.3)	11 (12.0)	
Dyspnea, n (%)	51 (11.2)	24 (6.6)	27 (29.3)	
Fatigue, n (%)	21 (4.6)	16 (4.4)	5 (5.4)	
Headache, n (%)	6 (1.3)	6 (1.7)	0 (0.0)	
Loss of smell and taste, n (%)	18 (4.0)	17 (4.7)	1 (1.1)	
Other symptoms, n (%)	47 (10.3)	44 (12.1)	3 (3.3)	
Laboratory Findings				
Hemoglobin (g/dL)	12.5±1.2	12.8±2.0	11.1±2.3	<0.001
Leukocyte count, × 10^3/μL	7.8±4.5	7.0±3.4	11.5±6.1	<0.001
Platelet count, × 10^3/μL	216.0 (45.0–568.0)	217.0 (68.0–568.0)	215.0 (45.0–511.0)	0.711
Neutrophil count, × 10^3/μL	5.9±4.2	4.9±3.0	9.9±5.5	<0.001
Lymphocyte count, × 10^3/μL	1.4±1.0	1.5±0.9	1.0±1.3	<0.001
MPV (fL)	9.3 (6.5–13.3)	9.1 (6.5–12.3)	9.6 (7.4–13.3)	<0.001
RDW (%)	14.0±3.1	13.6±1.6	15.8±5.8	<0.001
MCV (fL)	85.9±7.3	85.6±7.2	87.0±7.9	0.126
NLR	3.7 (0.6–137.3)	3.2 (0.6–24.3)	12.1 (0.8–137.3)	<0.001
PLR	171.6 (21.5–1215.0)	164.7 (35.3–1215.0)	274.2 (21.5–1196.9)	<0.001
CRP (mg/L)	24.0 (0.1–437.0)	12.4 (0.1–356.0)	157.0 (1.8–437.0)	<0.001
Procalcitonin (ng/m)	0.12 (0.01–20.0)	0.10 (0.01–7.0)	1.3 (0.07–20.0)	<0.001

Table 1. Comparison of baseline characteristics of patients with COVID-19 according to in-hospital all-cause mortality (Continued)

Variable	Total (n=455)	Survivor (n=363)	Nonsurvivor (n=92)	p
Ferritin (ng/mL)	142.5 (5.8-25583.0)	117.5 (5.8-2000.0)	856.0 (69.5-25583.0)	<0.001
LDH (U/L)	284.5 (112.0-3970.0)	255.0 (117.0-1191.0)	484.5 (112.0-3970.0)	<0.001
GFR (mL/min per 1.73 m^2)	95.0 (4.0-139.0)	95.0 (4.0-138.0)	38.5 (5.0-139.0)	<0.001
ALT (U/L)	24.0 (10.0-627.0)	23.0 (10.0-270.0)	31.5 (10.0-627.0)	<0.001
AST (U/L)	30.0 (11.0-1983.0)	28.0 (11.0-397.0)	48.0 (20.0-1983.0)	<0.001
Imaging Features-				0.038
No features, n (%)	8 (1.8)	8 (2.2)	0 (0.0)	
Consolidation, n (%)	14 (3.1)	11 (3.0)	3 (3.3)	
Ground-glass opacity, n (%)	321 (70.5)	262 (72.2)	59 (64.1)	
Infiltration, n (%)	6 (1.3)	6 (1.7)	0 (0.0)	
Pleural effusion, n (%)	1 (0.2)	0 (0.0)	1 (1.1)	
Mixed features, n (%)	105 (23.1)	76 (20.9)	29 (31.5)	
Cause of Death				-
AKI, n (%)	4 (4.3)	-	4 (4.3)	-
ARDS, n (%)	7 (7.6)	-	7 (7.6)	-
MOF, n (%)	23 (25.0)	-	23 (25.0)	-
Sepsis, n (%)	51 (55.4)	-	51 (55.4)	-
Other, n (%)	7 (7.6)	-	7 (7.6)	-

Data are presented as number (%), mean±standard deviation or median (minimum-maximum).

p-value was calculated using the independent samples t-test or the Mann-Whitney U-test for continuous variables, and the Chi-square test or Fisher's exact test for categorical variables as appropriate. p-value<0.05 was considered significant.

AKI: acute kidney injury; ALT: alanine transaminase; ARDS: acute respiratory distress syndrome; AST: aspartate transaminase; BMI: body mass index; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; DM: diabetes mellitus; GFR: glomerular filtration rate; HF: heart failure; HT: hypertension; ICU: intensive care unit; LDH: lactate dehydrogenase; MCV: mean corpuscular volume; MOF: multiple organ failure; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; RDW: red cell distribution width.

DISCUSSION

The major findings of this study were the following independent predictors of in-hospital all-cause mortality of patients with COVID-19: age, the NLR, lactate dehydrogenase level on admission, and baseline glomerular filtration rate. The sensitivity and specificity of the NLR for predicting mortality were 92% and 53%, respectively, at the cut-off value of 3.

Recent studies have investigated the relationship between various baseline leukocyte counts and clinical outcomes in COVID-19 patients. Liu et al. (8) found that the NLR is a predictive factor for early-stage COVID-19 infection that may lead to a critical illness. Qin et al. (9) reported that patients with a severe form of the disease seem to have higher neutrophil count but lower lymphocyte count compared to patients with a nonsevere form of the disease, thus the NLR was found to be higher in the former. Similarly, Mo et al. (10) found that refractory patients had higher neutrophil counts as compared to general patients.

The NLR has been proposed as a novel and cost-effective inflammatory biomarker, considering both neutrophil and lymphocyte counts. High NLR results from the increased neutrophil count and decreased lymphocyte count. Inflammation may involve increased neutrophil release. Possible reasons for the COVID-19-associated lymphopenia may include a direct infection of lymphocytes by SARS-CoV-2, lymphocyte sequestration in the lung, cytokine-mediated lymphocyte trafficking, immune-mediated lymphocyte destruction, bone marrow, thymus suppression, or apoptosis. Dysregulated immune cell responses and consequently immunological abnormality play a remarkable role in the severity of viral infections (11). Previous studies have demonstrated that lymphopenia played a prominent role in severe acute respiratory syndrome coronavirus (SARS-CoV) infection, and lymphocyte counts could predict the severity and clinical outcomes (12). Immunological responses involving hematological changes of leukocytes were notably associated with the severity and clinical outcomes of the middle east re-

Table 2. Risk factors for in–hospital all–cause mortality in patients with COVID–19

	Univariate Analysis		Multivariate Analysis	
Variable	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Age (year)	1.090 (1.068–1.113)	<0.001	1.203 (1.027–1.408)	0.022
DM, n (%)	2.135 (1.323–3.445)	0.002		
HT, n (%)	3.603 (2.239–5.799)	<0.001		
CAD, n (%)	3.592 (2.153–5.992)	<0.001		
COPD, n (%)	2.712 (1.412–5.208)	0.003		
Hemoglobin (g/dL)	0.672 (0.597–0.756)	<0.001	–	–
Leukocyte count, × 10^3/μL	1.226 (1.160–1295)	<0.001	–	–
MPV (fL)	1.617 (1.298–2.016)	<0.001	–	–
RDW (%)	1.355 (1.202–1.526)	<0.001	–	–
NLR	1.213 (1.159–1.268)	<0.001	1.261 (1.054–1.509)	0.011
PLR	1.003 (1.002–1.004)	<0.001	–	–
Procalcitonin (ng/m)	1.119 (1.035–1.211)	0.005	–	–
Ferritin (ng/mL)	1.002 (1.001–1.003–)	<0.001	–	–
LDH (U/L)	1.007 (1.005–1.006)	<0.001	1.013 (1.004–1.022)	0.005
GFR (mL/min per 1.73 m^2)	0.960 (0.952–0.968)	<0.001	0.920 (0.853–0.992)	0.030
ALT (U/L)	1.007 (1.003–1.012)	0.002	1.107 (1.011–1.212)	0.028
AST (U/L)	1.014 (1.007–1.021)	<0.001	0.939 (0.888–0.993)	0.027

p–value<0.05 was considered significant.

ALT: alanine transaminase; AST: aspartate transaminase; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; GFR: glomerular filtration rate; HT: hypertension; LDH: lactate dehydrogenase; MPV: mean platelet volume; NLR: neutrophil–to–lymphocyte ratio; PLR: platelet–to–lymphocyte ratio; RDW: red cell distribution width.

spiratory syndrome coronavirus (MERS-CoV) disease (13). Lymphocytopenia was associated with poor prognosis in MERS-CoV infection (14). Similarly, recent studies on COVID-19 have shown that higher levels of inflammatory cytokines, chemokines, and NLR in infected patients were correlated with the severity of the disease suggesting the involvement of cytokine storm in disease severity (9, 15). These findings are consistent with the results of the present study. In this study, neutrophil count, CRP, and procalcitonin levels were found to be significantly higher in nonsurvivor COVID-19 patients than survivors. The possible reason for these findings would be patients with extremely dysregulated immune responses due to high viral burden are likely to get bacterial coinfections.

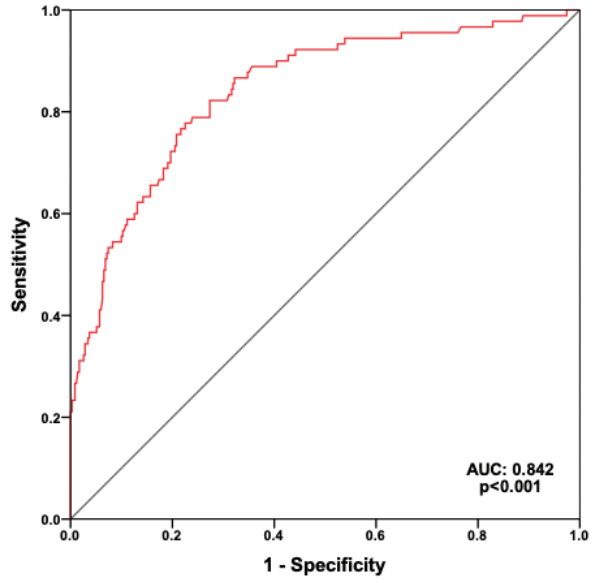
In the present study, out of the total 455 COVID-19 patients, 363 were discharged and 92 died during hospitalization. Zhou et al. (16) included a total of 191 patients in their study, of whom 137 were discharged and 54 died in hospital. Ruan et al. (17) included a total of 150 patients in their study, of whom 82 were discharged and 68 died. Wang et al. (18) and Zhang et al. (19) reported better clinical outcomes in their studies. This heterogeneity is probably due to the differences in the severity of illness of the enrolled patients. In this study, out of 455 patients, 145 were critically ill and were admitted to the intensive care unit.

The results of this study have several clinical implications. Physicians may identify high-risk COVID-19 patients at an early stage, assess admission to the intensive care unit for close monitoring, and modify treatments accordingly to reduce the in-hospital death as NLR could be quickly calculated based on a blood routine test on admission. Although treatment strategies were not investigated in this study, high-risk patients may be candidates to hydroxychloroquine, potent antibiotic and antiviral therapies, and corticosteroids.

Limitations of the Study

The present study has several limitations. First, because of its retrospective design, it might have a selection bias. Second, data regarding the time between the onset of illness and hospital admission could not be obtained. This might have influenced the findings that show the link between baseline NLR levels and mortality. Third, severity scores were not attainable. Thus, the relationship between NLR and disease severity could not be evaluated. Finally, as all subjects in this study were hospitalized Turk-

Figure 1. The receiver operating characteristic curve analysis of NLR for predicting in-hospital mortality in COVID-19 patients. AUC, area under the curve



ish patients diagnosed with COVID-19, the results of this study might not be directly applied to other ethnicities.

CONCLUSION

This retrospective cohort study, which was conducted among the Turkish population, revealed that NLR on admission is an independent risk factor for in-hospital all-cause mortality.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Çukurova University as well as by the Ministry of Health (No: 99, 15 May 2020).

Informed Consent: The need for written informed consent was waived due to the retrospective nature of the study.

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Author Contributions: Concept – S.A., A.Q.,G.A., A.Y.; Design - A.Q.,G.A, I.H.K., Ö.G. ; Supervision – A.Q., Ö.G., I.H.K.; Resources – S.A., H.H., G.A., Ö.G., A.Y.; Materials - S.A., H.H., G.A., Ö.G., A.Y.; Data Collection and/or Processing - S.A., H.H., G.A., Ö.G., A.Y.; Analysis and/or Interpretation – A.Q., Ö.G.; Literature Search - S.A., H.H., G.A., Ö.G., A.Y.; Writing Manuscript – A.Q.; Critical Review – A.Q., I.H.K.

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REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020; 382: 727-33. [Crossref]
2. Faria SS, Fernandes Jr PC, Silva MJ, Lima VC, Fontes W, Freitas-Junior R, et al. The neutrophil-to-lymphocyte ratio: a narrative review. Ecancermedicalscience 2016; 10: 702. [Crossref]
3. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med 2012; 5: 2. [Crossref]
4. de Jager CP, Wever PC, Gemen EF, Kusters R, van Gageldonk-Lafeber AB, van der Poll T, et al. The neutrophil-lymphocyte count ratio in patients with community-acquired pneumonia. PLoS One 2012; 7: e46561. [Crossref]
5. Curbelo J, Rajas O, Arnalich B, Galvan-Roman JM, Luquero-Bueno S, Ortega-Gomez M, et al. Neutrophil Count Percentage and Neutrophil-Lymphocyte Ratio as Prognostic Markers in Patients Hospitalized for Community-Acquired Pneumonia. Arch Bronconeumol 2019; 55: 472-7. [Crossref]
6. Kaushik R, Gupta M, Sharma M, Jash D, Jain N, Sinha N, et al. Diagnostic and Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Early and Late Phase of Sepsis. Indian J Crit Care Med 2018; 22: 660-3. [Crossref]
7. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect 2020; 81: e6-e12. [Crossref]
8. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med 2020; 18: 206. [Crossref]
9. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis 2020; 71: 762-8. [Crossref]
10. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin Infect Dis 2020. [Crossref]
11. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol 2017; 39: 529-39. [Crossref]
12. He Z, Zhao C, Dong Q, Zhuang H, Song S, Peng G, et al. Effects of severe acute respiratory syndrome (SARS) coronavirus infection on peripheral blood lymphocytes and their subsets. Int J Infect Dis 2005; 9: 323-30. [Crossref]
13. Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, et al. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Sci Rep 2016; 6: 25359. [Crossref]
14. Al-Tawfiq JA, Hinedi K, Abbasi S, Babiker M, Sunji A, Eltigani M. Hematologic, hepatic, and renal function changes in hospitalized patients with Middle East respiratory syndrome coronavirus. Int J Lab Hematol 2017; 39: 272-8. [Crossref]
15. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497-506. [Crossref]
16. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054-62. [Crossref]
17. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020; 46: 846-8. [Crossref]
18. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020; 323: 1061-9. [Crossref]
19. Zhang J, Wang X, Jia X, Li J, Hu K, Chen G, et al. Risk factors for disease severity, unimprovement, and mortality of COVID-19 patients in Wuhan, China. Clin Microbiol Infect 2020; 26: 767-72. [Crossref]

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Original Research

Comparison of Vacuum-assisted Closure and Conventional Dressing Treatment Modalities for Fournier's Gangrene

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ABSTRACT

Objective: The aims of this study were to evaluate the etiology and predisposing factors of patients with Fournier's gangrene (FG) and to compare the results and efficacy of vacuum-assisted closure (VAC) treatment with conventional dressings and debridement method.

Methods: The data of 52 patients diagnosed with FG and treated at our clinic between January 2013 and October 2018 were analyzed. Patients diagnosed with FG based on physical examination findings and anamnesis were analyzed. Patients with VAC applied (Group I) and not applied (Group II) were analyzed for demographics, etiology, wound culture results, predisposing factors, FG severity index, visual analog scale (VAS) for pain, number of debridements, requirement for analgesia, colostomy, length of hospital stay, and complications.

Results: Group I included 37 patients treated with conventional daily dressings, and Group II included 15 patients who were treated with VAC. No significant difference was determined between the groups with respect to etiology, microorganism type, or predisposing factors. Length of hospital stay was similar in both groups. Statistically significant differences were observed between the groups with respect to the number of debridements, VAS values, mean number of daily dressings, and use of analgesia ($P<0.001$).

Conclusion: VAC treatment does not decrease treatment duration, but less pain is felt during dressing changes as fewer dressings are used. Patient tolerance to treatment is also improved. It may be considered that the use of VAC treatment in wound care for patients with FG could increase patients' tolerance to treatment and quality of life.

Keywords: Fournier's gangrene, vacuum-assisted closure, debridement

INTRODUCTION

Fournier's gangrene (FG) was first defined by Jean Alfred Fournier in 1883 and continues to be known by his name (1). Fournier described this as a sudden-onset idiopathic disease in a series of 5 healthy young men, which develops in the penis and scrotum and leads to fulminating gangrene. Although Fournier defined the disease as idiopathic, there is current high incidence in men older than 50 years and, in the majority of cases, the etiological reason is known to be anorectal (30%–50%), urogenital (20%–40%), or skin (20%) infections originating from aerobic and anaerobic microorganisms (2, 3).

Early diagnosis and treatment of the disease, which is also known as necrotizing fasciitis, is of vital importance, with mortality rates varying between 7% and 75% (3). Aggressive surgical debridement must be applied to patients immediately and effective empirical parenteral antibiotic treatment must be started for all possible microorganisms (4–6). Repeated debridements are often applied to patients (7). After the first radical debridement, daily conventional dressings or vacuum-assisted closure (VAC) can be used for open wound treatment (4). However, the inability to tolerate daily dressings can create a need for analgesia or general anesthetic, which has a negative effect on quality of life for the patient.

The aims of this study were to evaluate the etiology and predisposing factors of patients with Fournier's gangrene and to compare the results and efficacy of VAC use with the conventional dressings and debridement method.

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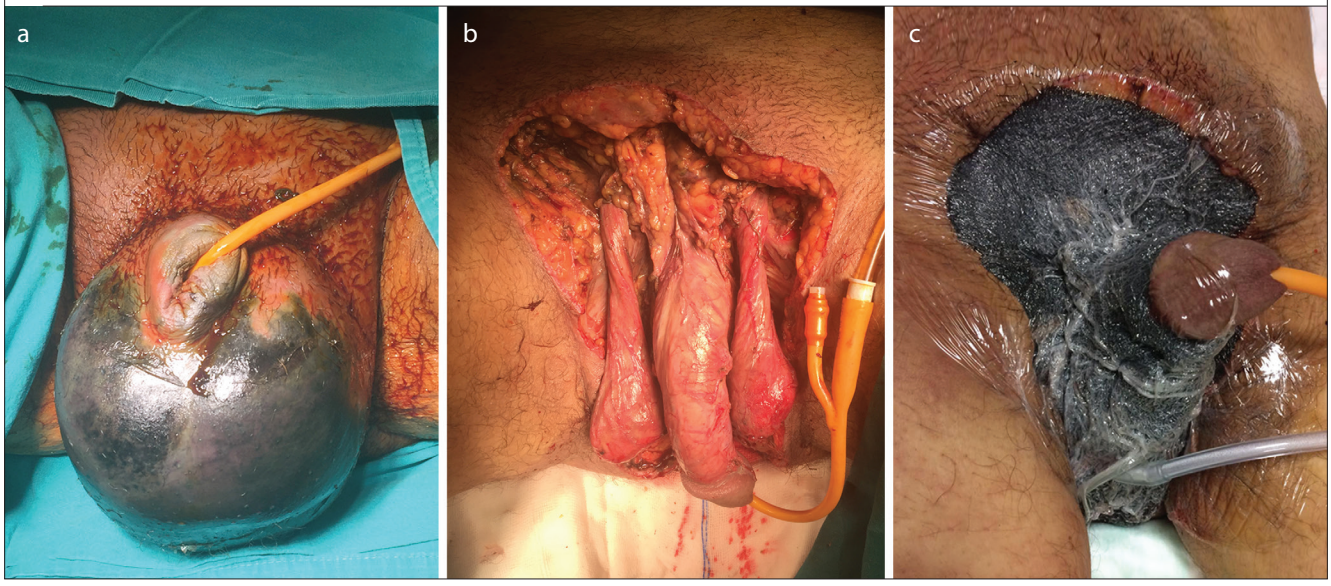
Figure 1. a-c. (a) The patient with Fournier's gangrene before treatment. (b) Same patient after the first surgical debridement procedure. (c) VAC application after surgical debridement.



METHODS

A retrospective examination was made of data for 52 patients diagnosed with Fournier's gangrene who were treated in our clinic between January 2013 and October 2018. Approval for the study was obtained from the local ethics committee of Health Sciences University, Adana City Training and Research Hospital (12.09.2018/281).

Patients diagnosed with Fournier's gangrene based on physical examination findings and anamnesis were analyzed. Diagnostic criteria for the physical examination were findings such as genital, perineal, and perianal sensitivity; induration; erythema; fluctuation; necrosis; and subcutaneous crepitation. Patients with simple abscess and inflammation of the urogenital and perianal region that had not progressed to the fascia were excluded from the study. Initially, all patients were treated with empirical broad-spectrum antibiotics (third generation cephalosporin, aminoglycoside, and metronidazole). According to the culture and antibiogram results, the antibiotics were switched if necessary. Informed consent was obtained from the patients, and they were admitted for surgery on the same day. Aggressive debridement was applied to all necrotic tissues until live and normal bleeding fascia was obtained. For patients with possible fecal contamination, a colostomy was performed. Tissues were irrigated with hydrogen peroxide and povidone iodine. Repeated debridements were applied to patients with infection and necrosis that persisted after the first debridement. Postoperatively, fluid replacement was administered to the patients, low-molecular weight heparin was started, and blood transfusion and nutritional support were administered if necessary.

Main Points:

- In the treatment of Fournier's gangrene, after the first radical debridement, daily traditional dressings or vacuum assisted closure (VAC) can be used for open wound treatment.
- In the current study, statistically significant differences were determined between the groups with respect to the number of debridements, VAS values, mean number of daily dressings, and use of analgesia.
- The number of repeated debridements was greater in the patients followed up with conventional dressings compared with those with VAC.
- The patients with VAC applied were found to have lower VAS values and require less analgesia.
- VAC treatment does not decrease treatment duration, but less pain is felt during dressing changes as fewer dressings are used. Patient tolerance to treatment is also improved.

For the patients who planned to have VAC, an Exsudex vacuum pump (Haromed bvba, Ghent, Belgium) was applied with 80 to 120 mmHg subatmospheric negative pressure within 24 to 48 hours after the acute phase. The device was set for 10 minutes of negative pressure, followed by 2 minutes of rest. The vacuum dressing was changed every 72 hours. Patients who did not use VAC were followed up with daily debridement and dressings changed twice a day (Figure 1).

The patients with VAC applied and not applied were analyzed with respect to demographic data, etiology, culture results, predisposing factors, Fournier's gangrene severity index (FGSI) score, visual analog scale (VAS) for pain, number of debridements, requirement for analgesia, colostomy, length of hospital stay, and complications. The FGSI score was defined by Laor et al. (8) as the score obtained from the 9 parameters of body temperature; pulse; respiratory count; and serum sodium, potassium, creatinine, bicarbonate, hematocrit, and white cell values, indicating the severity of the disease (Table 1).

Table 1. Fournier's gangrene severity index (FGSI)

Physiological Variable/Point Assignment	High Abnormal Value				Normal	Low Abnormal Value			
	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature (˚C)	>41	39–40.9	–	38.5–38.9	36–38.4	34–35.9	32–33.9	30–31.9	<29.9
Heart rate	>180	140–179	110–139	–	70–109	–	55–69	40–54	<39
Respiratory rate	>50	35–49	–	25–34	12–24	10–11	6–9	–	<5
Serum sodium (mmol/L)	>180	160–179	155–159	150–154	130–149	–	120–129	111–119	<110
Serum potassium (mmol/L)	>7	6–6.9	–	5.5–5.9	3.5–5.4	3–3.4	2.5–2.9	–	<2.5
Serum creatinine (mg/100 mL x2 for acute renal failure)	>3.5	2–3.4	1.5–1.9	–	0.6–1.4	–	<0.6	–	–
Hematocrite (%)	>60	–	50–59.9	46–49.9	30–45.9	–	20–29.9	–	<20
White blood count (total/mm3 x1000)	>40	–	20–39.9	15–19.9	3–14.9	–	1–2.9	–	<1
Serum bicarbonate (venous, mmol/L)	>52	41–51.9	–	32–40.9	22–31.9	–	18–21.9	15–17.9	<15

Table 2. Etiology, morbidity and predisposing factors for Fournier's gangrene

	Group I (Conventional) n (%)	Group II (VAC)	Total n (%)	P Value
Origin				
Urogenital[a]	26 (70.3)	10 (66.7)	36 (69.2)	0.523
Anorectal	8 (21.6)	4 (26.7)	12 (23.1)	0.726
Other	3 (8.1)	1 (6.7)	4 (7.7)	0.674
Mortality	4 (10.8)	2 (13.3)	6 (11.5)	0.565
Colostomy	2 (5.4)	1 (6.7)	3 (5.8)	0.648
Predisposing factors				
DM	22 (59.5)	10 (66.7)	32 (61.5)	0.628
Renal failure	4 (10.8)	2 (13.3)	6 (11.5)	0.565
Hepatic dysfunction	2 (5.4)	1 (6.7)	3 (5.8)	0.648
Malignancy	1 (2.7)	1 (6.7)	2 (3.8)	0.498
Alcoholism	1 (2.7)	1 (6.7)	2 (3.8)	0.498

DM, diabetes mellitus.
[a]Urogenital includes the urethra, prostate, urinary bladder, and genitalia.

Statistical Analysis

Data obtained in the study were analyzed statistically using Statistical Package for Social Sciences software, version 20.0 (IBM SPSS Corp.; Armonk, NY, USA). Conformity of quantitative variables to normal distribution was assessed using the Kolmogorov-Smirnov test and the Shapiro Wilk test. The Mann Whitney U test was applied to quantitative variables, and the Chi-square test and Fisher's Exact test to categorical-nominal variables. A value of P less than 0.05 was accepted as statistically significant.

RESULTS

Patients diagnosed with FG to whom surgical debridement was applied were separated into 2 groups according to the open wound treatment. Group I included 37 patients treated with conventional daily dressings, and Group II included 15 patients with VAC applied. All of the patients were men with a mean age of 57.1±10.7 years in Group I and 58.6±11.1 years in Group II (P=0.785). No significant difference was determined between the groups with respect to etiology, microorganism type, or predisposing factors. In Group I, 22 (59.5%) patients had diabetes, 1 (2.7%) had alcoholism, and 4 (10.8%) had renal failure. In Group II, 10 (66.7%) patients had diabetes, 1 (6.7%) had alcoholism, and 2 (13.3%) had renal failure (Table 2).

The mean FGSI score was calculated as 4.6±3.3 in Group I and 3.9±3.3 in Group II (P=0.433). A total of 4 (10.8%) patients in Group I and 2 (13.3%) in Group II died (FGSI scores 10–12). In the first group, 3 patients with diabetes mellitus (DM) and 1 patient with hepatic dysfunction died as a result of sepsis. In the second group, 1 patient with DM and 1 patient with DM and kidney failure died as a result of sepsis. The general mortality rate of 6 patients was found to be 11.5%. Colostomy was opened in 2 patients in Group I and in 1 patient in Group II (P=0.648). The responsible microorganism was identified in 42 (80.8%) patients. The bacteriological results are shown in Table 3.

The length of hospital stay was similar in both groups (Group I: 20.3±11.1 d; Group II: 23.5±17.0 d) (P=0.754). Statistically significant differences were determined between the groups with respect to the number of debridements, VAS values, mean number of daily dressings, and use of analgesia. The mean number of daily dressings was 1.7±0.3 in Group I and 0.3±0.1 in Group II

Table 3. Bacteriological results

	Group I (Conventional) n (%)	Group II (VAC) n (%)	Total n (%)
Escherichia coli	10 (27.0)	6 (40.0)	16 (30.8)
Staphylococcus aureus	5 (13.5)	2 (13.3)	7 (13.5)
Proteus vulgaris	3 (8.1)	1 (6.7)	4 (7.7)
Enterococcus faecium	3 (8.1)	1 (6.7)	4 (7.7)
Acinetobacter baumannii	1 (2.7)	2 (13.3)	3 (5.8)
Klebsiella pneumoniae	3 (8.1)	–	3 (5.8)
Pseudomonas aeruginosa	2 (5.4)	–	2 (3.8)
Enterobacter cloacae	1 (2.7)	–	1 (1.9)
Staphylococcus haemolyticus	–	1 (6.7)	1 (1.9)
Streptococcus anginosus	–	1 (6.7)	1 (1.9)
No bacterial growth	9 (24.3)	1 (6.7)	10 (19.2)

Table 4. Characteristics of patients

	Group I (Conventional) n=37, Mean±SD	Group II (VAC) n=15, Mean±SD	P Value
Age	57.1±10.7	58.6±11.1	0.785
FGSI	4.6±3.3	3.9±3.3	0.433
LOS	20.3±11.1	23.5±17.0	0.754
Number of daily dressings (mean)	1.7±0.3	0.3±0.1	<0.001
VAS	6.9±1.4	4.9±1.5	<0.001
Number of debridements	2.5±1.0	1.9±0.7	0.034
Number of daily analgesics (mean)	2.3±0.4	1.7±0.3	<0.001

FGSI, Fournier's gangrene severity index; LOS, length of stay; SD, standard deviation; VAS, visual analog scale for pain.

(P<0.001). The number of debridements was 2.5±1.0 in Group I and 1.9±0.7 in Group II (P=0.034). VAS values were determined as a mean of 6.9 ±1.4 in Group I and 4.9±1.5 in Group II (P<0.001). The mean daily analgesia requirement was 2.3±0.4 in Group I and 1.7±0.3 in Group II (P<0.001) (Table 4).

DISCUSSION

Urogenital, colorectal, or cutaneous polymicrobial infections of aerobic and anaerobic microorganisms and local trauma are often encountered in FG etiology (3). Although perianal and rectal infections have often been reported in the literature (9), for the majority of patients in the current study, urogenital, perineal, and scrotal infections were involved in the etiology. This was attributed to FG developing secondary to perianal and rectal infections being referred more often to the General Surgery Clinic and that the Urology Clinic was consulted in cases of FG involving the penoscrotal region.

In a review of 1726 cases by Eke, it was emphasized that FG was observed 10-fold more in adult men (3), although pediatric (10, 11) and female (12) cases have been reported in the literature. Yucel et al. (13) reported that the increased incidence of FG in elderly patients was associated with a weak immune response secondary to chronic diseases, increased incidence of impaired circulation, and more vascular pathologies being observed at advanced ages. There is a high likelihood of diabetes and obesity in patients with FG (2). Predisposing factors for FG include diabetes, obesity, cancer, alcoholism, advanced age, poor hygiene, malnutrition, trauma, renal failure, liver disease, and other immune-suppressing conditions (2, 9, 14, 15). DM is the most frequently encountered predisposing factor (3, 9). Diabetes and alcohol consumption are known to impair the immune system, and diabetes also causes distal arterial disease. In the current study, the rate of DM was found to be 59.5% in Group I and 66.7% in Group II.

Colostomy is recommended in patients with FG with perianal sphincteric, anorectal involvement (16). In the current study, colostomy was performed in only 3 patients. The reason for this lower rate according to the previous reports in the literature is believed to be the fact that patients with FG at our clinic were generally those in whom infection had developed from the scrotum and perineal region.

The causes of death of patients with FG include severe sepsis (8), coagulopathy (17), acute renal failure (18), diabetic ketoacidosis (17), or multiple organ failure (19). When Laor et al. (8) used a cut-off value for FGSI score of 9, they reported that the probability of mortality in patients with a score greater than 9 was 75% and the survival probability for those with a score of 9 or less was 78%. In the current study, no significant difference was found between patients with conventional dressings and those with VAC applied with respect to mortality rates. The FGSI scores of the patients with mortality were greater than 9. The general mortality rate was determined to be 11.5%, which is with the findings of the current literature.

The application of negative pressure to extensive tissue defects formed after aggressive debridement aims to facilitate wound healing through the elimination of bacterial contamination, infected material, and exudates; the reduction of edema; and the acceleration of granulation tissue formation (20, 21). Assenza et al. (22). reported that VAC treatment reduced the hospitalization time of patients and allowed early reconstructive surgery. Cuccia et al. (23) also stated that VAC treatment shortened the length of stay in hospital. In a prospective evaluation of the efficacy of VAC treatment in 35 patients diagnosed with FG, Czymek et al. (9). reported that VAC treatment was not superior to conventional dressings with respect to length of hospital stay and clinical

results but that the treatment was clinically effective and successful for extensive wounds. In the current study, no significant difference was determined between the 2 groups with respect to length of stay in hospital.

Open wound care is a problematic process often requiring lengthy hospitalization. Conventional dressings generally require more than 1 intervention within 24 hours, which can be a painful procedure and vexatious for both the patient and the clinician. For wound care with VAC, the dressing is changed once every 48–72 hours (24, 25), and patients experience less pain compared with care with conventional dressings (25). In addition, patients can be mobilized with a VAC device. Reducing the number of dressing changes and mobilization increases patient comfort. An important potential advantage of the use of a VAC device in the genitourinary region is that the drape creates a barrier against fecal contamination (24). Previous studies in the literature reported that VAS scores and the requirement for daily analgesia are significantly higher in patients treated with conventional dressings compared with those treated with VAC (25, 26). Similarly, in the current study, the patients with VAC applied were found to have lower VAS values and require less analgesia.

In a review of 1641 patients with FG, it was reported that surgery was applied a mean of 2.2 ± 1.6 times and debridement a mean of 1.5 ± 1.0 times (2). Cuccia et al. stated that there were no major complications with VAC treatment. It was safe, it reduced the number of debridements, and it increased patient comfort (23). In the current study, the number of repeated debridements was greater in the patients followed up with conventional dressings compared with those with VAC.

In a study by Öztürk et al. (26) the time from first debridement to wound closure was found to be similar in both groups, but wound healing in the VAC group occurred with fewer interventions than in the conventional dressings group, which resulted in a significant increase in patient comfort. Because the VAC treatment system is portable, patients are not bed-bound, and even if only partially, mobility can be maintained, allowing independent bathroom use, unlike patients with conventional dressings. In addition, there is no bad odor originating from the wound or bed-wetting related to the dressing. There is also less restriction on oral intake owing to the lower need for analgesia and sedation. Therefore, the use of VAC was observed to have a significant positive impact on quality of life of the patient compared with conventional treatment (26). The same authors reported that, in addition to VAC treatment being more comfortable for the patient, it is also preferred by clinicians. As the number of interventions required is reduced, the clinician spends less time on treatment, pain-related complaints are reduced, and there is a lower requirement for analgesia. Consequently, the use of VAC for wound care of patients with FG is easier for both the patient and the clinician.

No cost comparisons were made in the current study but there are previous studies that showed the cost of VAC treatment was similar to or slightly less than conventional treatment (26, 27). As there are more dressings and debridements in the conventional method, it is believed that a similar result would also be obtained in the current study.

Limitations of this study can be considered as not being prospective or randomized in design. However, the number of patients is believed to be sufficient when the low incidence of FG is taken into consideration.

CONCLUSION

The use of VAC in wound care after aggressive debridement in patients with FG is more comfortable for both the patient and the clinician. Although VAC treatment does not shorten the treatment process, less pain is felt during dressing changes as fewer dressings are used and patient tolerance to treatment is increased. This reduces the need for analgesia and reduces the number of repeated debridements with anesthesia. Quality of life is improved for the patients, as they can be easily mobilized and use the bathroom independently. Therefore, it is considered that the use of VAC treatment in the wound care of patients with FG will increase over time.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Health Sciences University, Adana City Training and Research Hospital (12.09.2018/281).

Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

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REFERENCES

1. Fournier JA. Jean-Alfred Fournier 1832-1914. Gangrène foudroyante de la verge (overwhelming gangrene). Sem Med 1883. Dis Colon Rectum 1988; 31: 984-8. [Crossref]
2. Sorensen MD, Krieger JN. Fournier's Gangrene: Epidemiology and Outcomes in the General US Population. Urol Int 2016; 97: 249-59. [Crossref]
3. Eke N. Fournier's gangrene: a review of 1726 cases. Br J Surg 2000; 87: 718-28. [Crossref]
4. Chennamsetty A, Khourdaji I, Burks F, Killinger KA. Contemporary diagnosis and management of Fournier's gangrene. Ther Adv Urol 2015; 7: 203-15. [Crossref]
5. Hong KS, Yi HJ, Lee R-A, Kim KH, Chung SS. Prognostic factors and treatment outcomes for patients with Fournier's gangrene: a retrospective study. Int Wound J 2017; 14: 1352-8. [Crossref]
6. Wagner S, Greco F, Hoda MR, Kawan F, Heynemann H, Fornara P. Is intensive multimodality therapy the best treatment for fournier gangrene? Evaluation of clinical outcome and survival rate of 41 patients. Surg Infect (Larchmt) 2011; 12: 379-83. [Crossref]

7. Misiakos EP, Bagias G, Papadopoulos I, Danias N, Patapis P, Machairas N, et al. Early Diagnosis and Surgical Treatment for Necrotizing Fasciitis: A Multicenter Study. Front Surg 2017; 4: 5. [Crossref]
8. Laor E, Palmer LS, Tolia BM, Reid RE, Winter HI. Outcome prediction in patients with Fournier's gangrene. J Urol 1995; 154: 89-92. [Crossref]
9. Czymek R, Schmidt A, Eckmann C, Bouchard R, Wulff B, Laubert T, et al. Fournier's gangrene: vacuum-assisted closure versus conventional dressings. Am J Surg 2009; 197: 168-76. [Crossref]
10. Ward L, Eisenson D, Fils J-L. Fournier's gangrene of the penis in a 12-year-old patient secondary to phimosis. R I Med J (2013) 2016; 99: 45-6.
11. Zgraj O, Paran S, O'Sullivan M, Quinn F. Neonatal scrotal wall necrotizing fasciitis (Fournier gangrene): a case report. J Med Case Rep 2011; 5: 576. [Crossref]
12. Hahn HM, Jeong KS, Park DH, Park MC, Lee IJ. Analysis of prognostic factors affecting poor outcomes in 41 cases of Fournier gangrene. Ann Surg Treat Res 2018; 95: 324-32. [Crossref]
13. Yücel M, Özpek A, Başak F, Kılıç A, Ünal E, Yüksekdağ S, et al. Fournier's gangrene: A retrospective analysis of 25 patients. Ulus Travma Acil Cerrahi Derg 2017; 23: 400-4. [Crossref]
14. Norton KS, Johnson LW, Perry T, Perry KH, Sehon JK, Zibari GB. Management of Fournier's gangrene: an eleven year retrospective analysis of early recognition, diagnosis, and treatment. Am Surg 2002; 68: 709-13.
15. Singh A, Ahmed K, Aydin A, Khan MS, Dasgupta P. Fournier's gangrene. A clinical review. Arch Ital Urol Androl 2016; 88: 157-64. [Crossref]
16. Li Y-D, Zhu W-F, Qiao J-J, Lin J-J. Enterostomy can decrease the mortality of patients with Fournier gangrene. World J Gastroenterol 2014; 20: 7950-4. [Crossref]
17. Slater DN, Smith GT, Mundy K. Diabetes mellitus with ketoacidosis presenting as Fournier's gangrene. J R Soc Med 1982; 75: 530-2.
18. Lamb RC, Juler GL. Fournier's gangrene of the scrotum. A poorly defined syndrome or a misnomer? Arch Surg 1983; 118: 38-40. [Crossref]
19. Scott SD, Dawes RF, Tate JJ, Royle GT, Karran SJ. The practical management of Fournier's gangrene. Ann R Coll Surg Engl 1988; 70: 16-20.
20. Ozkan OF, Koksal N, Altinli E, Celik A, Uzun MA, Cıkman O, et al. Fournier's gangrene current approaches. Int Wound J 2016; 13: 713-6. [Crossref]
21. Fabian TS, Kaufman HJ, Lett ED, Thomas JB, Rawl DK, Lewis PL, et al. The evaluation of subatmospheric pressure and hyperbaric oxygen in ischemic full-thickness wound healing. Am Surg 2000; 66: 1136-43.
22. Assenza M, Cozza V, Sacco E, Clementi I, Tarantino B, Passafiume F, et al. VAC (Vacuum Assisted Closure) treatment in Fournier's gangrene: personal experience and literature review. Clin Ter 2011; 162: e1-5.
23. Cuccia G, Mucciardi G, Morgia G, Stagno d'Alcontres F, Gali A, Cotrufo S, et al. Vacuum-assisted closure for the treatment of Fournier's gangrene. Urol Int 2009; 82: 426-31. [Crossref]
24. Silberstein J, Grabowski J, Parsons JK. Use of a Vacuum-Assisted Device for Fournier's Gangrene: A New Paradigm. Rev Urol 2008; 10: 76-80.
25. Yanaral F, Balci C, Ozgor F, Simsek A, Onuk O, Aydin M, et al. Comparison of conventional dressings and vacuum-assisted closure in the wound therapy of Fournier's gangrene. Arch Ital Urol Androl. 2017; 89: 208-11. [Crossref]
26. Ozturk E, Ozguc H, Yilmazlar T. The use of vacuum assisted closure therapy in the management of Fournier's gangrene. Am J Surg 2009; 197: 660-665. [Crossref]
27. Driver VR, Eckert KA, Carter MJ, French MA. Cost-effectiveness of negative pressure wound therapy in patients with many comorbidities and severe wounds of various etiology. Wound Repair Regen 2016; 24: 1041-58. [Crossref]

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Original Research

The Effect of Music and Massage on the Pain Scales and Vital Signs of ICU Patients with Hemodialysis Catheter

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ABSTRACT

Objective: Uncontrolled pain with any ICU patient may affect the course of medical applications negatively. Insertion of a hemodialysis (HD) catheter is a painful process. The main aim of this study is to evaluate the effect of music and massage on the pain level during hemodialysis catheterization.

Methods: This randomized controlled prospective trial was conducted on 220 patients who were hospitalized between January 2020 and July 2020 due to emergence of HD. Fifty-eight patients were listened to music with headphones while another 58 were hand massaged and another 56 were listened to music during hand massaging, and 50 patients were monitored without any extra applications.

Results: The average APACHE-2 (Acute Physiology and Chronic Health Evaluation) score of the patients was 19.57±8.98. The respiratory rate and heart rate of Music&massage (Mm) group was lower than the starting (p=0.027, p=0.043). There was a decrease in BPS (Behavioral Pain Scale) of intubated Mm and music group (p=0.001, p= 0000). Considering non-intubated patients, Mm, only music and only massage groups showed a significant decline in terms of WONG (Wong-Baker faces pain rating scale) (p=0.000, p=0.001, p=0.000). The Mm group showed that patients were more sedatized in terms of RASS (Richmond Agitation-Sedatation Scale) (p=0.046).

Conclusion: Use of music and massage during HD catheterization in ICU has contributed to improved vital signs and less pain along with more sedatized patients.

Keywords: Complementary medicine, hemodialysis, intensive care unit, massage, music, pain

INTRODUCTION

Pain is one of the main stress factors for intensive care unit (ICU) patients. Endotracheal intubation, chest tube insertion, hemodialysis (HD) catheterization, deep respiration and coughing exercises, endotracheal aspiration, wound care, changing bed position, being bedridden for a long period, and other situations known to cause pain (1). Approximately 33% of ICU patients experience pain in bed, whereas almost half of them feel pain during healthcare processes (2, 3). Severe pain negatively affects hemodynamic stability and immunity (4). Therefore, studies have been conducted to determine whether complementary medicine would help pain management in the ICU. The Pain, Agitation/Sedation, Delirium, Immobility, and Sleep Disruption in Adult Patients in the ICU (PADIS) guideline suggests the use of music and massage, two complementary medicine methods, to ICU patients to decrease pain and anxiety (5). Music arouses both physiological and psychological responses in the listener (6). Listening to music affects the right lobe of the brain and triggers morphine and endorphin secretion while lowering adrenaline level, heart rate, and blood pressure (7). The use of massage to treat patients is a traditional method (8). Studies have shown that massage therapy increases lymphedema, endorphin level, and body temperature while decreasing anxiety, depression, blood pressure, and heart rate along with sleep regulation (9). Applying massage therapy in the ICU is simple and has no negative physiological effect (9). According to PADIS guideline, only a limited number of studies on the use of music and massage therapies are found in the literature (5). To the best of our knowledge, no study investigated the contribution of using music and massage therapies to pain management in third-line ICU in Turkey. The present study was conducted to meet the need for investigating the effect of using music and massage therapies on pain in the literature.

METHODS

This is a randomized controlled prospective study conducted between January and July 2020 in the 26-bed third-stage gen-

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eral ICU and 12-bed anesthesia ICU of the Training and Research Hospital. The study included 220 HD patients who were divided into four groups, and each patient was catheterized for HD. Power analysis was performed before the study. Musical and hand massage therapies were each applied to 58 patients, respectively. Music and received hand massage therapies were simultaneously applied to 56 patients. Furthermore, 50 patients were monitored without any extra treatment. Four types of methods were written on 220 pieces of papers. Subsequently, they were put in envelopes, and these envelopes were put into a box. We were blinded to the contents of the papers. In case of HD process, the patient's nurse picked one of the envelopes, and the method written was applied for the catheterization of the patient. The type of music was chosen by the patient if s/he was conscious; otherwise, the family members chose the type of music. Massages were performed by physiotherapists on duty in routine and in the ICU. Choosing the hand massage depends on factors, such as easy access to the hand of patient that has no venous or peripheral intravenous access. After checking the dermal integrity, hand massage was performed for 5 min on the right palm, and 5 min on the back of the hand. Subsequently, the same process was applied on the left hand.

HD catheterization was performed by the same specialist in the ICU unit. The vital signals of the patient were followed on the monitor and recorded. The pain scales were recorded by patient's nurse. The mean duration of catheter insertion was 35 min.

Sedation levels were evaluated using Richmond Agitation–Sedation Scale (RASS). The patients were administered paracetamol as analgesic, and propofol as sedative. Many scales are used for conscious and cooperative patients, with WONG as the easiest and most reliable (10). Ventilator-supported patients were evaluated using the Behavioral Pain Scale (BPS), a reliable scale composed of three subscales: upper extremity movements, facial expressions, and ventilation cooperation. These three subscales have four subscales (12 scales). A score of five indicates that the patient is in pain (11, 12). WONG and BPS were used for conscious and unconscious patients, respectively.

Inclusion Criteria

- ✓ Patients' age ≥18 years
- ✓ Patients without language or communication problems
- ✓ Patients expected to be in the ICU for ≥72 h
- ✓ Patients who agreed to participate
- ✓ Patients in need of urgent HD catheterization.

Main Points:

- Uncontrolled pain with any ICU patient may affect the course of medical applications negatively.
- Complementary use of music and massage on HD catheterized patients in ICU has contributed to improved vital signs and less pain along with more sedation.
- The study suggests adding music and hand massage therapy to nursing applications.

Exclusion Criteria

- ✓ Patients with hearing problems
- ✓ Patients with thrombus, hematoma, ecchymosis, phlebitis in the massage area, and those receiving anticoagulant infusion
- ✓ Patients hospitalized in the ICU for the second time during the study
- ✓ Patients already catheterized for HD
- ✓ Patients with thrombosis, local infections, and burns in the catheter area
- ✓ Patients with anatomic anomalies
- ✓ Patients with history of allergy to lidocaine, chlorhexidine, and povidone–iodine
- ✓ Patients (if the patient is unconscious the patient's relative) who refused to participate in the study.

Catheter Insertion

Femoral vein cannulation was performed using the Seldinger method (13). After placing the patient in the supine position, the leg was mildly abducted and externally rotated. Catheterization was performed on the side of the catheter. Femoral artery pulsation was performed 2–3 cm below the inguinal ligament, and the catheter was inserted through 1–1.5 cm medial of the area in 45° to the femoral vein. A guide wire was inserted (Figure 1). HD catheter was inserted through the guide wire. Each lumen of the catheter was checked for bleeding. In case of bleeding, the lumens were rinsed, and the catheter was fixed (Figure 2).

Ethical Considerations

Gaziantep University ethics Council approved this study (date: December, 25, 2019; reg. number: 2019/474). Written consents were obtained from conscious patients or from legal guardians of unconscious patients. Before the study, all participants and their relatives were briefed about catheterization and the complementary medicine methods in the study. They were also informed that they have the right to waive any time, and refusal of participation will not affect their treatment.

Statistical Analysis

Data were analyzed using frequency, percentage analysis, and complementary statistics. Moreover, Chi-squared analysis and T-test were used for categorical and continuous variables, re-

Figure 1. Central catheter insertion

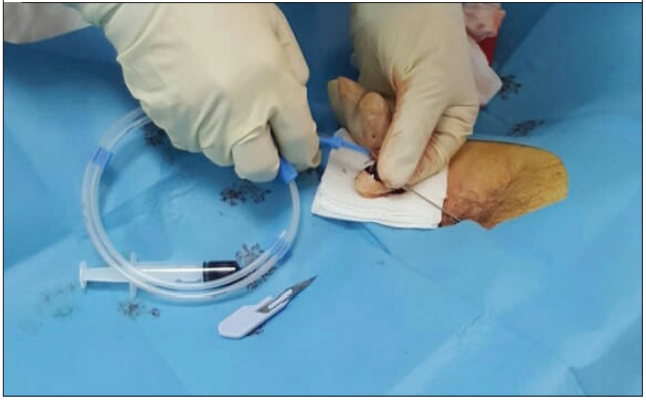


Table 1. Demographic data

Variable	Total (n=222)
Age, mean (SD)	58.32 (18.67)
Sex, n (%)	
Male	148 (66.66)
Female	74 (33.33)
Marital status, n (%)	
Married	165 (74.32)
Single	57 (25.67)
Education, n (%)	
Primary school	72 (32.43)
High school	138 (62.16)
University	12 (5.40)
GCS mean (SD)	11.13 (4.01)
APACHE–II mean (SD)	19.57 (8.98)
Intubation, n (%)	
Intubated	69 (31.08)
Non–intubated	153 (68.91)
Analgesic, n (%)	
Yes	115 (51.80)
No	107 (48.19)
Sedation, n (%)	
Yes	32 (14.41)
No	190 (85.58)
RASS, mean (SD)	−1.46 (1.93)

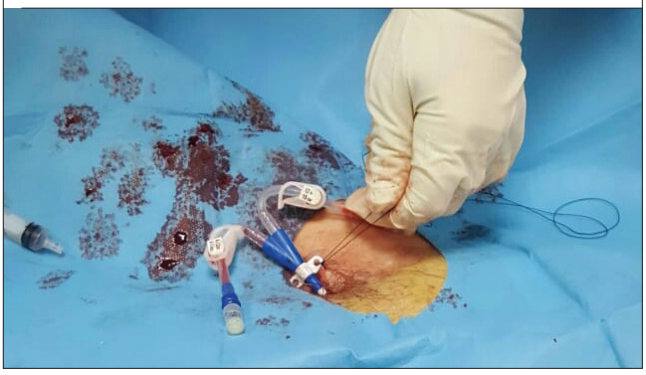
GCS, Glasgow Coma Scale; APACHE, Acute Physiology and Chronic Health Evaluation; RASS, Richmond Agitation–Sedation Scale.

spectively. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). Significance was determined for $p<0.05$.

RESULTS

The study included 220 patients, of which 148 (66.66%) were men, and 165 (74.32%) were married. The educational level of 72 (32.43%), 138 (62.16%), and 12 (5.40%) participants was primary school, high school, and university, respectively. Moreover, 32 (14.41%) patients were intubated, and 115 (51.80%) were administered analgesics, and 32 (14.41%) were administered sedatives. The mean Glasgow coma scale was 11.13±4.01. The mean Acute Physiology and Chronic Health Evaluation 2 (APACHE-2) score was 19.57±8.98. Meanwhile, the RASS scale was −1.46±1.93 (Table 1). The demographical features of the subgroups were not significantly different (Table 2). A statistically insignificant decrease was found in the systolic and diastolic BP after 90 min of music and massage (Mm) therapy group (respectively, p=0.056, p=0.073). The respiratory and heart rates of the Mm group was lower than baseline, which was statistically significant (respectively, p=0.027, p=0.043). A decline was observed in the BPS score of intubated patients in the only music and Mm groups (respectively, p=0.001, p=0000). A significant decline in the WONG scale score of non-intubated patients in the only music, only massage, and Mm groups (respectively, p=0.000, p=0.001, p=0.000). Despite no medical intervention received, the RASS score of Mm patients indicated more sedation (p=0.046) (Table 3).

Figure 2. In case of bleeding, lumens were rinsed and the catheter was fixed



DISCUSSION

The study showed a significant improvement in pain scores and vital parameters in Mm group patients. This group had lower respiratory and heart rates than baseline. According to RASS, the combined effect of music and massage improved sedation. The BPS score of intubated patients and WONG scale score of non-intubated patients were significantly lower. Music has long been used as a complementary medicine method. The literature suggests that the effective period of music treatment is between 25 and 90 min (14). Therefore, for the current study, the use of music was limited to 30 min. Indeed, there was no exact consensus for the type of music used (6). It is well known that personal tastes play a role in choosing the right kind of music (15). Jasemi et al. (16) stated that making cancer patients listen to their favorite music significantly lowers the pain level. Regional characteristics, cultural and ethnic features along with personal tastes, and education have an effect on responses to the music people listen. There may be various responses to the same type of music. It may be correct that we have chosen the type of music in accordance with the personal tastes of our patients, and it revealed improvements in the treatment. Considering the pain scales of intubated and non-intubated patients, both values were lower and statistically significant (17). Music therapy has a physiological effect on lowering respiratory rate, arterial blood pressure, heart rate, and stress hormone responses (18). Chan et al. have made 66 patients, who were C-clamped after percutaneous coronary intervention, listen to music for 45 min, and the control group did not listen to any music. The heart rate, respiratory rate, and oxygen saturation levels of the music group were

Table 2. Demographic features of each group

Variable	Music (n=58)	Massage (n=58)	Music/massage (n=56)	Control (n=50)	p
Age, mean (SD)	57.88 (19.49)	58.47 (20.51)	56.47 (19.61)	59.37 (20.38)	0.324
Sex, n (%)					
Male	38 (65.52)	39 (67.24)	38 (67.85)	33 (66.00)	0.597
Female	20 (34.48)	19 (32.75)	18 (32.14)	17 (34.00)	0.590
Marital status, n (%)					
Married	43 (74.14)	43 (74.13)	42 (75.00)	37 (74.00)	0.475
Single	15 (25.86)	15 (25.86)	14 (25.00)	13 (26.00)	0.231
Education, n (%)					
Primary school	18 (31.03)	19 (32.75)	19 (33.92)	16 (32.00)	0.747
High school	37 (63.79)	35 (62.06)	34 (62.50)	32 (64.00)	0.591
University	3 (5.17)	4 (5.17)	3 (5.35)	2 (4.00)	0.440
GCS mean (SD)	11.05 (4.55)	11.53 (3.94)	11.40 (3.86)	11.00 (6.83)	0.367
APACHE-II mean (SD)	19.86 (9.94)	20.98 (8.89)	20.37 (7.92)	19.31 (8.02)	0.437
Intubation, n (%)					
Intubated	19 (32.76)	18 (31.03)	17 (30.35)	15 (30.00)	0.349
Non-intubated	39 (67.24)	40 (68.69)	39 (69.64)	35 (70.00)	0.124
Analgesic, n (%)					
Yes	31 (53.45)	30 (51.72)	29 (51.78)	25 (50)	0.766
No	27 (46.55)	28 (48.27)	27 (48.21)	25 (50)	0.584
Sedation, n (%)					
Yes	8 (13.79)	9 (15.51)	8 (14.28)	7 (14.00)	0.742
No	50 (86.21)	49 (84.48)	48 (85.71)	43 (86)	0.691
RASS, mean (SD)	−1.34 (1.78)	−1.54 (1.23)	−1.61 (1.12)	−1.29 (1.98)	0.657

GCS, Glasgow Coma Scale; APACHE, Acute Physiology and Chronic Health Evaluation; RASS, Richmond Agitation-Sedation Scale.

statistically significantly decreased compared with that of the control group (19). Besides perioperative applications, music therapy has been proven beneficial for many procedures, such as bronchoscopy, pleural drainage, and central venous catheter insertion (20). Catheter insertion is one of the most painful procedures applied in the ICU. Many HD patients are admitted to medical ICUs, and they need urgent HD treatment. Pain management may be difficult during this non-elective procedure. Using painkillers at high doses may be harmful for patients with organ dysfunctions. Complementary medical methods, such as music and massage therapies, are easy to apply and inexpensive compared with their positive effects. Zengin et al. have made one part of 100 cancer patients listen to music during port catheter insertion. Following the procedure, serum cortisol, ACTH levels, and blood pressure of the two groups were compared. The hormone level, respiratory rate, blood pressure, and pain scores of the music group were lower than those of the control group (21). The present study did not focus on cortisol or ACTH levels due to loss of diurnal rhythm in the ICU. In addition, the cortisol levels of HD patients are expected to be above normal levels because some patients will have multiorgan dysfunction, respiratory failure, or hypotensive shock. ICUs are departments where vital signs are the most important, and many painful procedures are performed. Thus, many studies suggest the application of music therapies in nursing (6). Music therapy is widely used in cardiology departments. Twenty-three studies on 1461 patients have shown that music therapy lowers the blood pressure and heart and respiratory rates (22). Besides conscious patients, unconscious patients also benefit from music therapy, especially in the ICU. Ajri et al. (23) stated that music therapy decreases the pain score of unconscious patients. Fariba et al. (24) showed that when unconscious and ventilator-supported patients listen to music for three consecutive days, pain control is easily achieved at the end of the third day. Music therapy was

Table 3. Vital signs and pain scales

Variables (SD, min-max)	Before catheterization 30 dk	Catheterization 30 dk	Catheterization 30-60 dk	p
Systolic blood pressure (mmHg)				
Normal	128.55±19.65	127.88±17.89	126.95±17.35	0.409
Music	126.64±24.29	128.00±23.15	124.72±25.52	0.249
Massage	125.93±22.31	124.88±21.62	123.03±22.00	0.327
Music and massage	130.53±23.79	128.43±21.59	127.33±21.87	0.056
Diastolic blood pressure (mmHg)				
Normal	74.91±14.43	75.71±13.57	75.64±13.82	0.737
Music	75.43±18.34	74.52±17.67	74.36±18.1	0.590
Massage	71.33±14.19	71.31±11.95	71.66±13.19	0.914
Music and massage	73.83±14.72	71.62±15.21	71.34±13.88	0.073
Respiratory rate (cycles/min)				
Normal	19.59±4.63	19.64±4.43	19.31±4.57	0.708
Music	19.47±5.09	19.72±5.12	19.84±4.46	0.735
Massage	20.10±5.48	20.17±5.25	19.79±5.28	0.735
Music and massage	20.33±5.11	19.55±4.87	18.98±5.01	0.027
Heart rate (bpm)				
Normal	94.28±18.19	94.95±18.24	93.02±17.08	0.155
Music	95.12±3.13	95.12±3.59	95.00±3.52	0.828
Massage	94.45±2.86	94.55±2.87	94.74±2.67	0.341
Music and massage	95.60±16.11	94.26±15.69	93.71±16.45	0.043
Oxygen saturation (%)				
Normal	94.16±3.21	94.50±3.26	94.52±3.25	0.235
Music	94.72±2.73	94.71±2.44	94.91±2.62	0.584
Massage	94.52±15.60	93.76±14.63	93.05±15.28	0.239
Music and massage	95.38±2.63	95.48±3.10	95.43±2.95	0.916
RASS (median)				
Normal	−1.31 (1.88)	−1.29 (1.78)	−1.28 (1.85)	0.923
Music	−1.32 (1.71)	−1.30 (1.77)	−1.31 (1.74)	0.874
Massage	−1.47 (1.23)	−1.49 (1.24)	−1.42 (1.19)	0.432
Music and massage	−1.41 (1.46)	−1.52 (1.39)	−1.66 (1.92)	0.046
BPS				
Normal	3 (1.00-3.75)	3 (1.00-3.75)	3 (1.00-3.75)	0.097
Music	3 (1.25-3.75	2 (1.00-3.75)	1 (1.00-2.75)	0.001
Massage	3 (1.00-3.00)	3 (1.00-3.00)	3 (1.00-3.00)	0.223
Music and massage	3 (2.000-5.000)	3 (1.000-5.000)	3 (0-5.000)	0.000
WONG scale				
Normal	1 (0-2.00)	1 (0-2.00)	1 (0-2.00)	0.368
Music	2 (2.00-2.00)	2 (1.00-2.00)	1 (1.00-2.00)	0.000
Massage	2 (1.000-2.000)	2 (0-2.000)	1 (0-2.000)	0.001
Music and massage	2 (2.00-3.00)	2 (1.00-2.00)	2 (1.00-2.00)	0.000

RASS, Richmond Agitation-Sedatation Scale; BPS, Behavioral Pain Scale; WONG, Wong-Baker faces pain rating scale.

shown to decrease anxiety and the need for sedatives in mechanical ventilator-supported patients (25). The present study did not include the data of sedative need as music and massage therapies were applied only during catheter insertions. Local anesthesia was applied on the catheter insertion area, and this process continued without any extra sedation if the patient received routine sedation. The relationship between consecutive application of music or massage therapy for several days and the need for sedation may be revealed through future studies. The PADIS guideline suggests the use of music and massage therapies in ICUs. Five randomized controlled studies stated that the pain scores were significantly reduced when massage therapy was applied for 30 min. These studies utilized hand, foot, and body massages. These were included as suggestions suggestion in the guideline because massages reduce the need for opioids. However, studies on this subject are lacking (5). Massage and music therapy have long been used since ancient times (8). Hajbaghery et al. (26) stated that anxiety, diastolic blood pressure, and heart and respiratory rates were significantly reduced after applying full-body massage therapy for 60 min on ICU patients compared with the control group. Meanwhile, Vibhu et al. conducted a study on 104 cardiac surgery patients who were made to listen to music postoperatively on the first day. The patients received a mild massage therapy before room transfer. The massage group reported a significant decrease in pain compared with the control group (27). The present study utilized massage and music therapies on different days. Thus, there is no evidence on whether they have a combined effect. Music and massage therapies were applied on the same type of patients in this study. Having four different groups, having different personnel for insertion and records, and having no idea about the method applied on each patient make the study valuable. In addition, application of the combined therapy in one of the groups and possible determination of the combined effect of therapies make it more valuable. Hand massage therapy is important in terms of accessibility. It can be applied without risking the sterility of the patient during catheterization. Similar to the present study, Boitor et al. (28) stated that hand massage therapy improved the vital signs and reduced the pain score. To the best of our knowledge, this is the first study investigating the contribution of using music and massage therapies to pain management in a third-line ICU in Turkey. However, the study has some limitations.

Limitations

Even if the study was conducted in two different ICUs, they were in the same medical center. Therefore, the findings cannot be generalized. The study may be conducted on a wider patient group in multiple centers because the anxiety of the patient may be due to his/her acute HD need, and it may improve with dialysis process. It is speculated that patients may not respond to our tests because most of them were in respiratory distress. Furthermore, it speculated that the anxiety levels of patients may not be evaluated correctly because some of the patients were intubated, and some of them were sedated. The conscious subgroup should be evaluated for anxiety levels, but the number of patients was not sufficient. Anxiety can be assessed in larger patient subgroups.

CONCLUSION

Complementary use of music and massage on HD catheterized patients in the ICU has contributed to improved vital signs and less pain along with more sedation. The study suggests adding music and hand massage therapy to nursing applications.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University (date: December, 25, 2019; reg. number: 2019/474).

Informed Consent: Written consents were obtained from conscious patients or from legal guardians of unconscious patients.

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REFERENCES

1. Ayasrah SM, O'Neill TM, Abdalrahim MS, Sutary MM, Kharabsheh MS. Pain assessment and management in critically ill intubated patients in Jordan: a prospective study. Int J Health Sci 2014; 8: 287-98. [Crossref]
2. Gelinas C. Management of pain in cardiac surgery ICU patients: Have we improved over time? Intensive Crit Care Nurs 2007; 23: 298-303. [Crossref]
3. Chanques G, Sebbane M, Barbotte E, Viel E, Eledjam J, Jaber S. A prospective study of pain at rest: Incidence and characteristics of an unrecognized symptom in surgical and trauma versus medical intensive care unit patients. Anesthesiology 2007; 107: 858-60. [Crossref]
4. Skrobik Y, Ahern S, Leblanc M, Marquis F, Awissi DK, Kavanagh BP. Protocolized intensive care unit management of analgesia, sedation, and delirium improves analgesia and subsyndromal delirium rates. Anesth Analg 2010; 111: 451-63. [Crossref]
5. John WD, Yoanna S, Céline G, Dale MN, Arjen JCS, Pratik PP, et al. Clinical Practice Guidelines for the Prevention and Management of Pain, Agitation/Sedation, Delirium, Immobility, and Sleep Disruption in Adult Patients in the ICU. Crit Care Med 2018; 46: e825-73.
6. Chlan L, Tracy MF. Music therapy in critical care: indications and guidelines for intervention. Crit Care Nurse 1999; 19: 35-41. [Crossref]
7. Nichols TW. Music as Medicine: The Science of How Music Can Help Induce Sleep, Relieves Anxiety and Pain in Patients. J Biomusic Eng 2015; 3: 1. [Crossref]
8. Cutshall SM, Wentworth LJ, Engen D, Sundt TM, Kelly RF, Bauer BA. Effect of massage therapy on pain, anxiety, and tension in cardiac surgical patients: a pilot stud. Complement Ther Clin Pract 2010; 16: 92-5. [Crossref]
9. Bauer BA, Cutshall SM, Wentworth LJ, Deborah EP, Messnerc C, Woode K. Effect of massage therapy on pain, anxiety, and tension after cardiac surgery: a randomized study. Complement Ther Clin Pract 2010; 16: 70-5. [Crossref]
10. Wong D, Whayley L. Clinical handbook of pediatric nursing; ed2; p373 St Louis C.V. Mosby Company; 1986.
11. Alderson SM, McKechnie SR. Unregocnised, undertreated, pain in ICU: causes, effects, and how to do better. Open Journal of Nursing 2013; 3: 108-13. [Crossref]

12. Rahu MA, Grap MJ, Ferguson P, Joseph P, Sherman S, Elswick RK. Validity and sensivity of 6 pain scales in critically ill, intubated adults. Am J Crit Care 2015; 24: 514-24. [Crossref]
13. Trerotola SO. Hemodialysis Catheter Placement and Management. Radiology 2000; 215: 651-8. [Crossref]
14. Almerud S, Petersson K. Music therapy-a complementary treatment for mechanically ventilated intensive care patients. Intensive Crit Care Nurs 2003; 19: 21-30. [Crossref]
15. Wong HLC, Nahas VLI, Molassiotis A. Effects of music therapy on anxiety in ventilator-dependent patients. Heart Lung 2001; 30: 376-87. [Crossref]
16. Jasemi M, Eghtedar S, Aghakhani N, Khodabandeh F, Sayadi L, Kheirkhahi N. Music therapy reduces the intensity of pain among patients with cancer. Thrita 2013; 2: 76-9. [Crossref]
17. Lee OKA, Chung YFL, Chan MF, Chan WM. Music and its effect on the physiological responses and anxiety levels of patients receiving mechanical ventilation: a pilot study. J Clin Nurs 2005; 14: 609-20. [Crossref]
18. Bradt J, Dileo C, Denise G. Music interventions for mechanically ventilated patients. Cochrane Database Syst Rev 2014; 12: CD006902. [Crossref]
19. Chan M. Effects of music on patients undergoing a c-clamp procedure after percutaneous coronary interventions: a randomized controlled trial. Heart Lung 2007; 36: 431-9. [Crossref]
20. Hole J, Hirsch M, Ball E, Meads C. Music as an aid for postoperative recovery in adults: a systematic review and meta-analysis. Lancet 2015; 386: 1659-71. [Crossref]
21. Zengin S, Kabul S, Al B, Sarcan E, Doğan M, Yildirim C. Effects of music therapy on pain and anxiety in patients undergoing port catheter placement procedure. Complement Ther Med 2013; 21: 689-96. [Crossref]
22. Bradt J, Dileo C, Noah P. Music for stress and anxiety reduction in coronary heart disease patients. Cochrane Database Syst Rev 2013; 12: CD006577. [Crossref]
23. Ajri M, Zaherimogadam M, Ajri Khameslou E, Farokhnezhad P. The effect of preferred music on physiological and behavioral parameters of pain in unconscious patients admitted to the Intensive care unit. Advances in Nursing & Midwifery 2015; 24: 1-8.
24. Fariba Y, Ali N, Mohammad NT, Sara S. Effect of music on pain intensity among patients with loss of consciousness in an intensive care unit. Medical - Surgical Nursing Journal 2016; 4: 35-40.
25. Chlan LL, Weinert CR, Heiderscheit A, Mary FT, Debra JS, Guttormson JL, et al. Effects of patient-directed music intervention on anxiety and sedative exposure in critically ill patients receiving mechanical ventilatory support: a randomized clinical trial. JAMA 2013; 309: 2335-44. [Crossref]
26. Hajbaghery M, Abasi A, Rajabi-Beheshtabad R. Whole body massage for reducing anxiety and stabilizing vital signs of patients in cardiac care unit. Med J Islam Repub Iran 2014; 28: 47.
27. Vibhu RK, Linda FC, Susan JH, Sue S, Barbara K. Complementary Alternative Medical Therapies for Heart Surgery Patients: Feasibility, Safety, and Impact. Ann Thorac Surg 2006; 81: 201- 5. [Crossref]
28. Boitor M, Martorella G, Arbour C, Midbaud C, Gelinas C. Evaluation of the preliminary effectiveness of hand massage therapy on postoperative pain of adults in the intensive care unit after cardiac surgery: a pilot randomized controlled trial. Pain Manag Nurs 2015; 16: 354-66. [Crossref]