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Letter to the Editor	500	No abstract	5	No tables	No media

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Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

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Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

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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Contents

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Review

Basic Problems in Serological Diagnosis of Cystic Echinococcosis

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ABSTRACT
Cystic echinococcosis (CE), which occurs in rural areas during most seasons, is an important public health problem in Turkey. Many challenges regarding both diagnosis and treatment of this disease have not yet been overcome, and despite significantly increasing patient care costs, surgical treatment remains the main option. Confirmation of diagnosis is usually performed by serological tests based on the detection of serum antibodies against crude parasitic extracts (hydatid fluid, HF); however, HF contains cross-reactive antigens that lead to false-positive results, indicating other parasitic and nonparasitic diseases. Moreover, certain patients are serologically negative for HF, despite suffering from CE, likely due to cyst stage, number, and size. The existing insensitive and nonspecific tests have been replaced with indirect hemagglutination test (IHAT), enzyme-linked immunosorbent assay (ELISA), and immunoblotting (IB) in recent years. As a result of the evident diagnostic problems, the World Health Organization/World Animal Health Organization recommendations were based on a sequential screening and confirmatory test model. The use of ELISA, IHAT, latex agglutination tests (LAT), immunofluorescence antibody test, and immunoelectrophoresis is recommended for primary screening. The accepted serological screening tests are IHAT, IFA, and ELISA in Turkey, with the Turkish Ministry of Health, Public Health Agency recommending that at least two serological screening tests are used to diagnose patients with CE, followed by confirmation using IB.In the present review, the laboratory tests used in the diagnosis of CE and their limitations and diagnostic algorithms are explained with reference to the current literature.
Keywords: Cystic echinococcosis, microbiology, serological diagnosis

INTRODUCTION

Cystic echinococcosis (CE), which occurs in rural areas during most seasons, is an important public health problem in Turkey. Although the disease does not discriminate among age and gender, its occurrence is greater in women aged 30-50 years who reside in rural areas and are in frequent contact with animals (1, 2).

Many challenges regarding both diagnosis and treatment of this disease have not yet been overcome, and despite significantly increasing patient care costs, surgical treatment remains the main option. Radiological imaging methods are generally used for the identification, evaluation, and screening of liver lesions (3, 4), with confirmation of the diagnosis, typically performed using serological tests (5).

A multidisciplinary team consisting of clinicians, radiologists, and microbiologists must work together for proper CE diagnosis. Clinician and laboratory cooperation is required for the differentiation of CE cysts from benign cysts, cavitary tuberculosis, mycoses, and benign and malignant neoplasms.

In the present review, the laboratory tests used in the diagnosis of CE and their limitations and diagnostic algorithms are explained with reference to the current literature, with a view to guiding clinicians with cases of CE.

CLINICAL AND RESEARCH CONSEQUENCES

Diagnosis

Detection of a cyst-like mass in an individual who works with livestock supports the diagnosis of CE in regions where *Echinococcus granulosus* is endemic. However, differential diagnosis from benign cysts, mycoses, cavitary tuberculosis, and benign or malign neoplasms must be made. Generally, a noninvasive confirmation of the diagnosis can be performed by the combined use of radiological imaging and immunological diagnostic techniques (5).

Radiological imaging methods, such as ultrasonography, computerized tomography, and magnetic resonance imaging, are frequently used for CE diagnosis. Radiological imaging is also used as a screening tool for the diagnosis of liver lesions, guided by a classification system for CE diagnosis defined by the World Health Organization (WHO) informal working group. Based on this classification, CE liver cysts are categorized as stages 1-5 (CE1-CE5), where stages CE1 and CE2 are considered as

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an active disease (3). Unilocular, oval, echo-free, double-walled cysts are classified as stage CE1. Generally, when the position of the patient is changed, a "snowflake effect" is observed due to free-floating protoscoleces. When daughter cysts are visible within mother cysts as a "wheel spoke" or "honeycomb" pattern, the cyst is classified as stage CE2. The laminate membrane on the inner surface of the cyst is dissolved as part of the degenerative process, described as the "water lily" sign, during stage CE3, which is the inactive staging transition phase. The cysts become inactive with a "ball of wool" appearance with heterogeneous internal structures during stage CE4. Finally, the cyst has a thick, arch-like, calcified wall that forms a conical ultrasonic shadow, and protoscoleces are no longer present during stage CE5, which is the inactive phase (3). These classification criteria enable the simple assessment and evaluation of cystic liver lesions in routine clinical settings (6).

Laboratory Diagnosis

Preanalytical considerations

There is a risk, although small, of this parasite infecting the laboratory personnel who handle tissue biopsies from positive patients or stool samples from experimental animals. Although most laboratory animals are commercially available, animal species used in certain investigations are not guaranteed to be pathogen free. The American College of Laboratory Animal Medicine defines animal species as primary and secondary. *Echinococcus* spp. has been reported to be at risk of transmission from dogs and sheep to humans; however, it is classified as low infectivity in biomedical research laboratories (7).

Biosafety level 2 (BSL2) laboratory applications are required to handle hydatid disease in a clinical laboratory. Great attention must be paid to the use of personal protective equipment and good hygiene practices, such as hand washing, to protect the laboratory personnel from infection with *Echinococcus* (7). A laboratory-based case of CE was reported in 2012 in a 37-year-old laboratory technician who had been working with positive CE specimens in a non-BSL2 laboratory for 20 years (8). Gloves should be used when coming into contact with stools or surfaces contaminated with fresh stool samples (9).

Microscopic approaches

The pathogen can be detected directly with microscopic examination of fluid obtained from fine-needle aspiration or liver biopsy samples. Hooks, protoscoleces, and laminate membrane fragments can be detected in smears prepared from sediments of centrifuged cyst fluid with microscopy. Ziehl-Neelsen staining can also be performed, giving a better contrast of hooklets (10). Cyst fluid microscopy may show infection and cystic vitality (11); however, certain cysts are sterile (acephalocysts) without the presence of germination capsules. Therefore, a negative microscopy result does not exclude CE infection, and the diagnosis should be confirmed by serological tests (5).

Immunological approaches

Serological methods for CE are based on the detection of serum antibodies against crude parasitic extracts (hydatid fluid, HF); however, HF contains cross-reactive antigens that lead to false-positive results, indicating other parasitic and nonparasitic diseases. In addition, HF produces nonspecific reactions in certain samples from healthy donors, and conversely, certain patients are serologically negative for HF, despite suffering from CE, likely due to cyst stage, number, and size. As a result of the pitfalls in detecting antibodies, alternative laboratory methods have been developed, such as the detection of circulating antigens, peripheral cytokines, and parasitic DNA (12).

E. granulosus Antigens

Native antigens

Hydatid fluid is the main source of antigens most commonly used in serological tests for the detection of antibodies in patients affected with CE. The most pertinent disadvantage of HF as an antigen source is that it cannot be produced in the laboratory but has to be collected from naturally infected animal and human cysts (12). Hence, HF composition varies greatly depending largely on the host, the stage of cystic development, and the parasitic genotype (13).

Hydatid fluid is a complex mixture of glycoproteins, lipoproteins, carbohydrates, and salts formed during parasite metabolism, with certain components including serum albumin and immunoglobulins being internalized from the host. The most well-defined and abundant immunogenic antigens in HF are antigen B (AgB) and antigen 5 (Ag5) (12). AgB is a highly immunogenic 120-160 kDa protein that acts as a protease inhibitor, eliciting a Th2 cell response in patients with progressive CE, which inhibits neutrophil recruitment and activation of T helper cells (14, 15). AgB-like antigens are also present in parasites of the *Taenia* genus, including *Taenia solium* and *Taenia saginata* (16). Ag5 is a 400 kDa thermolabile protein that is highly abundant in HF (14) and is thought to have important functions in the cyst development (12). Since Ag5 shows high homology with antigens in the *Taenia* species, it can cause cross-reaction when used in diagnostic tests (17). Semi-purified fractions enriched in AgB and/or Ag5 can be obtained from HF in different ways; however, this has not yet been standardized. HF and its fractions are heterogeneous since they are usually collected from infected animals, leading to false-positive and -negative test results when used as an antigen for the detection of antibodies (12). Recombinant antigens have been developed as an alternative due to the cross-reactivity of native antigens.

Recombinant antigens

The AgB isoforms, such as AgB1, AgB2, AgB3, and AgB4, are produced as recombinant proteins by different laboratories for use as antigens; however, recombinant AgB5 is yet to be successfully produced. The methods used to obtain recombinant antigens have not reached consensus among different laboratories, leading to different diagnostic performances (12). Synthetic peptides have been identified as alternatives to recombinant proteins, and their standardization is thought to be better since they are produced chemically following the amino acid sequence. Nevertheless, a single peptide cannot provide sufficient diagnostic sensitivity; thus, several peptide antigens can be combined to

increase sensitivity (18). Although Ag5 is produced as a recombinant protein, its various available versions and different immune reactivities render it less than ideal. Recently, other recombinant antigens from protoscolices, oncospheres, and adult worms have been described (12).

Serological diagnosis

In recent years, the existing insensitive and nonspecific tests have been replaced with indirect hemagglutination test (IHAT), enzyme-linked immunosorbent assay (ELISA), and immunoblotting (IB) (19, 20). CE elicits a strong antibody response in many patients with different isotypes (IgG, IgM, IgA, and IgE). Antibodies against oncosphere antigens first appear several weeks after infection, followed by those against the laminar layer, cystic fluid, and protoscolices (12). The most commonly used methods for CE diagnosis are the detection of specific IgG antibodies using an HF antigen in diagnostic tests, such as ELISA and IHAT, and in the confirmatory IB test.

Studies have reported that the sensitivity of IgG-ELISA varies from 63% to 100%. False-negative results with ELISA are due to various factors, such as early and inactive cyst stages, cyst number and size, cyst placement outside the liver, and parasitic genotype (21-23). Another problem with ELISA is false positivity. When HF is used as an antigen, it causes various false-positive results in healthy donors from different geographical regions (24). Cross-reactivity to HF can be observed in other parasitic (alveolar echinococcosis, cysticercosis, ascariasis, and amebiasis) and nonparasitic (malignant) diseases (23, 25, 26). Moreover, the HF anti-echinococcal antibody level cannot be used as an indication of successful treatment, since it can remain high for many years despite cyst removal. Therefore, when antibodies other than IgG were investigated, they were found to provide better results with respect to patient follow-up, although this is still a question of debate (27).

Antibody responses to HF have been found to be highly variable both qualitatively and quantitatively at different times during infection, both in different patients and in the same patient. This variability is due to cyst number, size, stage, and location, in addition to parasitic genotype and the applied treatment (12). Recombinant antigens have also been used in studies with more sensitive and specific diagnostic purposes; however, most of the current studies have been conducted in a small number of patients, often with unknown clinical variables. Variable susceptibility and specificity rates are common for all tested recombinant antigens (12).

There are various commercial kits available that are based on ELISA, IHAT, and immunochromatography (IC), containing crude or semi-purified HF fractions; however, the antigenic source is rarely specified. IHAT tests have reported a sensitivity of 34.9%-88% and a specificity of 44%-70% (28-31). Several commercial ELISA kits were tested and compared with in-house ELISA, determining variable false-negative and -positive results. IB has been found to be more sensitive than either ELISA or IHAT techniques (12). As a result of the evident diagnostic problems, there exist the WHO/World Animal Health Organization recommendations based on a sequential screening and confirmatory test model. The use of ELISA, IHAT, latex agglutination test, immunofluorescence antibody test, and immunoelectrophoresis is recommended for primary screening (32). Over the past few years, sensitive and easy-to-use tests, such as IC and the dot immunogold filtration assay, have been commercially developed. Compared with other tests, IC is more advantageous with respect to its various features, such as a short test duration, no requirement for specialist staff, and easy interpretation of results (12). In addition to being more economical than other techniques, IC does not require to be transported or stored under refrigeration. Higher sensitivity ratios have been determined for IC compared with the antigens used in other tests (33, 34). A flowchart adapted from the "Republic of Turkey Ministry of Health, Public Health Agency, Cystic Echinococcosis, Field Guide for Laboratory Diagnosis of Infectious Diseases" hown in Figure 1 (35).

Serological studies have alternatively been performed using noninvasive urine specimens, with similar sensitivity and higher specificity rates being detected by ELISA (36). Currently, there is a need for the production of easy-to-use tests containing few recombinant antigens, which can be used as both primary screening and secondary confirmation tests. To date, there is no ideal test for use in patient follow-up; accordingly, it is necessary to validate the standardized antigens identified from patients with detailed clinical traits from a large number of serum samples (12).

Antigen detection

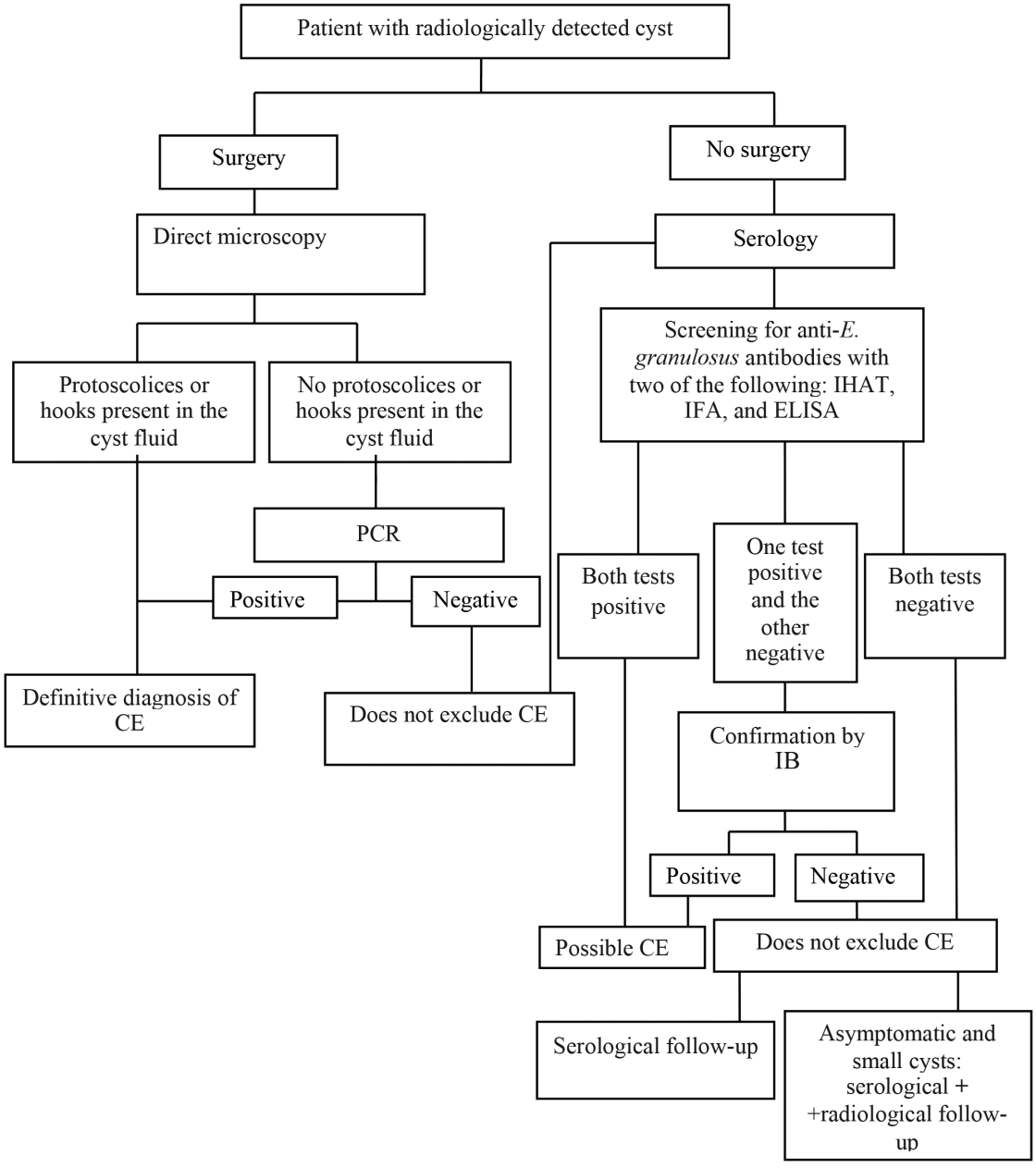
Antibody detection does not only show the presence of disease but also the exposure to an *Echinococcus* infection, and serum antibodies can often be detected for up to 10 years after removal of a hydatid cyst (24). An alternative for CE diagnosis is the detection of antigens in bodily fluids and serum, which may be more advantageous than the detection of antibodies in the early stages of infection and during patient follow-up, since circulating antigens initially decrease in successfully treated patients (12). Antigen detection may be useful in diagnosing antibody-negative patients; however, circulating antigens in patients with CE are often below the detection limits due to a low release or binding of released antigens to antibodies in the circulation (37). Diagnostic samples may need to be treated prior to testing to separate antigens from antigen-antibody complexes, and for this reason, antigen detection may be more time-consuming (12). The combined use of two tests that detect antigens and antibodies has been reported to increase sensitivity (38).

Levels of cytokines and peripheral blood mononuclear cells (PBMCs) in seronegative patients have also been investigated as diagnostic markers of the disease. Highly proliferative PBMC responses have been reported in certain patients with low antibody titers. Of note, the use of PBMC levels during the follow-up of treated patients may be impractical, since these levels remain high for a long period following treatment (39). Although a relationship between cytokine levels and CE has been established in certain clinics, further investigation is required (12).

Molecular diagnosis

DNA-based molecular tests for the presence of *Echinococcus* should measure true infection status with high sensitivity and

Figure 1. Flowchart for the laboratory diagnosis of patients with CE in Turkey. Adapted from the Republic of Turkey Ministry of Health, Public Health Agency, Cystic Echinococcosis, Field Guide for Laboratory Diagnosis of Infectious Diseases (35)



specificity, and be safe, and be cost-effective for the laboratory personnel. With the emergence of molecular and biochemical approaches for the detection of parasites, different methods have been developed to identify *Echinococcus* strains (12). Such studies, mainly based on polymerase chain reaction (PCR) approaches, have identified species, genotypes, and haplotypes of *E. granulosus*. PCR is the preferred method for parasite identification, molecular epidemiological studies, and confirmatory purposes (40, 41). Real-time PCR (qPCR) offers many advantages over conventional PCR in detecting parasitic infections due to the increased sensitivity and specificity, reduced reaction time, and quantitative detection of the amount of DNA in the sample (41, 42). However, DNA detection by PCR-based methods cannot assess parasitic viability or exclude the presence of a PCR-negative disease (43).

CONCLUSION

Despite the standardization and cross-reactivity challenges, a combination of radiological imaging and screening with confirmatory serological tests is the preferred choice for the diagnosis of CE. The specificities and sensitivities of hydatid serological tests differ depending on the antigen used. An ideal test should

be highly sensitive and highly specific; however, it is challenging to develop novel serological tests that are better than HF-based tests. PCR should be used for seronegative patients with radiologically suspected CE cases.

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Review

Is *Blastocystis* spp. Friendly?: A Current View of the Intestinal Microbiota

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ABSTRACT

The intestinal microbiota has become the center of attention, not only in microbiology but also in all fields of medicine. There has been an intense activity in studies that investigate the composition and function of the intestinal microbiota. The imbalance in the diversity of bacteria that constitute microbiota has been defined as "dysbiosis" and associated with various diseases. *Blastocystis* spp. is a eukaryotic protist and the most prevalent protozoan of the human gastrointestinal system. The frequency of observed colonization of *Blastocystis* spp. in asymptomatic cases has made its association with diseases controversial. It was found in some studies that there is a positive correlation between *Blastocystis* and the bacterial diversity of the intestinal microbiota. This implies that the parasite may play a role in intestinal homeostasis. Human and animal studies on this subject play an important role in understanding this relationship.

Keywords: Bacterial diversity, *Blastocystis* spp., dysbiosis, intestinal microbiota

INTRODUCTION

The gastrointestinal tract is the host of "the inner microbial world" that includes thousands of different species of microorganisms. It is part of a system in which, separate from our standard knowledge, many important events related to human health occur. Fecal microbiota consists of 93% bacteria, 5.8% virus, 0.8% archaea, and 0.5% eukaryotes. Meta-taxonomic analyses reveal that humans have 63-84 bacterial phyla, and it is estimated that nearly 15 phyla are localized in the gastrointestinal tract (1). This group consists of approximately 1014 microorganisms/g stool and weighs up to 2 kg. Of the colonic microbiota, 90% is composed of two dominant phyla called *Firmicutes* and *Bacteroides*. These individually bear high variability at the species level. Although the intestinal microbiota contains a low number of phyla, high diversity is exhibited with respect to species. Despite the significant differences between individuals in the adult fecal microbiota, a stable state is achieved in the individual after a certain period (2). Changes in the microbial composition are referred to as dysbiosis. These are associated with inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), colorectal cancer, metabolic syndrome, rheumatic diseases, allergy and atopic diseases, heart diseases, and psychiatric disorders (3).

Diet during early childhood, continuing the same eating habits for a protracted period, antibiotic use, the genetic structure of an individual, and sanitation affect the variability of human fecal microbiota (4).

Blastocystis spp. is a unicellular, anaerobic, and eukaryotic microorganism that is present in the gastrointestinal tract of humans and many animal species. It is classified as being in the stramenopile phylum and is the only member of this phylum that is present in human intestines. Carrying *Blastocystis* spp. is very common globally, and its prevalence has been reported to be 22%-56% in European countries and 37%-100% in Asian and African countries. The genetic diversity of *Blastocystis* spp. is very high and includes 17 subtypes (STs). Among these subtypes, ST1-9 and ST12 are isolated from humans, whereas ST3 is the one most frequently detected. ST4 in particular, which is the second most frequently identified subtype in Europe, is rarely seen in South America, Africa, and Asia (5).

Blastocystis has been associated with diarrhea, abdominal pain, and vomiting, while its role in diseases has not yet been completely explained. Studies that investigate symptomatology with subtypes were not able to precisely define pathogenic and non-pathogenic subtypes. The detection of long-term colonization in asymptomatic cases highlights the fact that one has to consider whether this agent is a member of the intestinal environment or not (6-8). It is necessary to investigate the relation-

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ship between *Blastocystis* and intestinal microbiota to determine this. Intestinal microbiota studies gained speed, especially after 2010, with the development of next-generation sequencing techniques that have made it possible to conduct metagenomic analyses. However, the number of studies in this field that focus on *Blastocystis* is very limited (9-15).

In this review, studies that investigate the relationship between *Blastocystis* and intestinal microbiota have been reviewed and summarized, and opinions regarding whether *Blastocystis* can be a "biomarker" for healthy intestines or not have been evaluated.

Clinical and Research Consequences

The presence of high bacterial diversity in the intestinal microbiota is considered an indicator of health. The coexistence of *Blastocystis* spp. and high bacterial diversity has drawn attention to the possibility that this protist microorganism can be a biomarker of gastrointestinal system health (10).

In a meta-analysis that evaluated studies investigating the prevalence of *Blastocystis* in cases of IBS, the relative risk was reported to be 2.34 in cases with IBS that exhibit *Blastocystis* colonization. This is in comparison with cases without IBS (9). In the same study, the aim was to evaluate the relationship between the presence of *Blastocystis* and intestinal microbiota with respect to IBS pathophysiology. In this study, which employs the quantitative polymerase chain reaction method conducted in France, the prevalence of *Blastocystis* was found to be 23.2% in patients with IBS and 16.1% in controls, and the most frequently detected subtype was ST4. A significant decrease was found in *Bifidobacterium* spp. in constipated male patients with IBS infected with *Blastocystis*. There was a decrease in the amount of *Faecalibacterium prausnitzii*, which has anti-inflammatory properties, in male control patients who did not have gastrointestinal complaints who had *Blastocystis*. It was found that *Bacteroides* spp. was higher, whereas *Bifidobacterium* spp., *Desulfovibrio* spp., *Clostridium leptum*, and *F. prausnitzii* were lower in constipated patients with IBS who did not have *Blastocystis* than in the control group. *F. prausnitzii* and *Bifidobacterium* spp. are known as being protective bacteria. This is due to their anti-inflammatory, anti-carcinogenic, and immunostimulant effects. The amount of these bacteria was found to have decreased in *Blastocystis* carriers. This implies that this parasite might be associated with inflammatory events. An inverse correlation between *Blastocystis* colonization and *Bacteroides* spp. has been found. This is in addition to the other studies that will be discussed subsequently. Investigators asserted the hypothesis that *Blastocystis* and dysbiosis of the intestinal microbiota might be associated in the pathophysiology of constipation-predominant IBS (9).

In the first study, which investigates the relationship between *Blastocystis* and intestinal microbiota with metagenomic analysis, the prevalence of *Blastocystis* was found to be 20.3% in healthy individuals and 14.9% in patients with ulcerative colitis. Patients with Crohn's disease did not have *Blastocystis*. *Blastocystis* positivity was less frequently seen than *Ruminococcus* and *Prevotella* enterotypes in cases involving *Bacteroides*-predominant enterotype. This reveals a positive correlation of *Blastocystis* colonization with bacterial species diversity. The fact that patients with Crohn's disease had decreased bacterial diversity, in addition to no *Blastocystis* colonization, makes it possible to explain this hypothesis. Investigators have shown that *Blastocystis* colonization was less frequently seen in patients with dysbiosis, as in cases of Crohn's disease and ulcerative colitis. There is no information on whether *Blastocystis* selects a specific microbiota directly or indirectly. Interestingly, the same study revealed that among healthy individuals, the rate of *Blastocystis* positivity was found to be 30.9% in lean individuals and 11.1% in obese individuals (p=0.008). In this study, conducted in Denmark, lean individuals had high bacterial species diversity in their microbiota, whereas obese individuals had lower diversity. Another remarkable result was the positive correlation between bacterial species diversity in the intestinal microbiota and the relationship between *Blastocystis* and leanness (10).

Differences in the intestinal microbiota were observed between healthy controls and patients with IBS with diarrhea in a metagenomic study conducted in Australia, which compared fecal microbiota in patients with IBS with and without *Blastocystis*. It was also found that *Blastocystis* carriage had no effect on fecal microbiota (12). It is known that patients with IBS have a higher *Firmicutes*/*Bacteroides* ratio and lower fecal bacterial diversity (16). Moreover, it is thought that *Blastocystis* leads to IBS symptoms by affecting the intestinal microbiota (12).

Audebert et al. (13) investigated the intestinal microbiota of patients with and without *Blastocystis* colonization in their study conducted in France using metagenomic analysis. The study found that *Blastocystis* colonization showed a positive correlation with bacterial diversity, and that there was a higher incidence of *Clostridia* class and Ruminococcaceae and Prevotellaceae families in cases who had *Blastocystis* colonization than in those without *Blastocystis* colonization. There were more cases of Enterobacteriaceae family in patients without *Blastocystis* colonization. In addition, there was more abundance of *Faecalibacterium* and *Roseburia* genera that contain butyrate-producing bacteria in cases with *Blastocystis* colonization. Butyrate is an important metabolite for human colon health since it is the main energy source for colonic epithelial cells, possesses anti-inflammatory properties, and is able to regulate gene expression, apoptosis, and enterocyte differentiation. Data obtained from this study indicate that parasite colonization is associated with a healthy colon environment, rather than the association of intestinal dysbiosis that is linked to *Blastocystis* with infectious and inflammatory diseases of the bowel.

The first study that addressed the relationship between *Blastocystis* and intestinal microbiota in Turkey investigated the stool samples of patients with cirrhosis using metagenomic analysis (14). It was found that the prevalence of *Blastocystis* colonization (0%) is lower in cases with hepatic encephalopathy (HEP) than in cases who did not develop HEP (38.1%) (p=0.006). It is known that intestinal dysbiosis plays a role in the development of HEP in patients with cirrhosis. Decreased intestinal motility, gastric acid, and pancreatobiliary secretions, as well as portal hypertension, affect the microbiota composition in

patients with cirrhosis (17). Yildiz et al. (14) found a tendency for the negative correlation between *Blastocystis* colonization and bacterial diversity, although it was not statistically significant. In addition, they found a negative correlation between *Bacteroidetes* phylum and *Blastocystis* colonization, as seen in the previous studies (9-11, 14, 15).

Forsell et al. (15) investigated the effect of travel on *Blastocystis* carriage and its relationship with the intestinal microbiota in Swedish travelers. They found that traveling did not have any effect on *Blastocystis* carriage. There was no significant difference between the groups with and without *Blastocystis* colonization, with respect to fecal microbiota composition. Interestingly, an increased amount of *Sporolactobacillus* and *Candidatus Carsonella* was detected with *Blastocystis* colonization. In addition, a negative correlation with *Bacteroides* enterotype and increased bacterial diversity at the genus level was detected with *Blastocystis* carriage. *Sporolactobacillus* species produce lactic acid from the sugars contained in vegetables that are consumed as part of the diet. Therefore, investigators are of the opinion that *Blastocystis* colonization can be associated with a healthy microbiota and a diet that contains vegetables.

Studies involving helminths have also shown a positive correlation between helminths and increased bacterial diversity. It was found that *Trichuris trichiura* treatment is effective in the restoration of intestinal dysbiosis and the regulation of mucosal barrier functions in macaque monkeys with chronic diarrhea (18). It was seen that helminth colonization is associated with increased bacterial species diversity in Malaysian individuals infected and not infected with helminths (19).

CONCLUSION

Many recent studies have detected an increased fecal bacterial diversity in individuals who have *Blastocystis* colonization. This situation implies that this protist may be a beneficial component for intestinal homeostasis. Lukes et al. asserted that the use of protists, such as *Blastocystis*, may be beneficial in helminth treatment due to its potential of stimulating the immune system, especially in cases with allergy and IBD (20). Once this hypothesis is confirmed with future studies, commensalism and even mutualistic relationships between *Blastocystis* and individuals will need to be reshaped, at least under certain conditions.

Moreover, there are a few studies that investigate the relationships between *Blastocystis* and intestinal microbiota, and these studies have not yet provided conclusive results regarding the cause-effect relationships. Is microbiota with dysbiosis not suitable for *Blastocystis* colonization, or does *Blastocystis* affect the structuring of microbiota composition by affecting intestinal homeostasis? Answers to these questions will be found by performing long-term prospective metagenomic studies conducted on humans containing case-control groups (13). On the other hand, animal models colonized by *Blastocystis* are urgently needed to understand the functional effect of *Blastocystis* on the bacterial microbiota.

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Original Research

Investigation of the Relationship of Vitamin D, Parathyroid Hormone, Calcium Serum Level, and Insulin Resistance among Obese Individuals

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ABSTRACT

Objective: Several studies have shown that 25-hydroxyvitamin D, parathyroid hormone (PTH), and serum calcium (Ca) may play functional roles with insulin resistance.

Methods: This case–control study included 60 obese individuals with impaired fasting glucose who were compared with 60 non-obese individuals with normal fasting glucose who visited the endocrinology and metabolism department. Fasting plasma glucose, fasting insulin, triglyceride, PTH, and serum 25-hydroxyvitamin D (25(OH)D) were measured from all subjects in the morning after approximately 8 h of fasting using methods with respect to the standard operating procedures. Homeostatic model assessment of insulin resistance (HOMA-IR) was used for determining insulin resistance. The statistical analysis was attained by SPSS.

Results: A lack of association was found between serum 25(OH)D and insulin resistance (HOMA-IR) before and after adjusting PTH, Ca, and various variables either in all participants or after exclusion of participants with HOMA-IR ≤2.5. On the other hand, PTH showed a significant inverse correlation with fasting insulin (p=0.022) and HOMA-IR (p=0.023) after adjusting 25(OH)D, serum Ca, and various variables and exclusion of participants with HOMA-IR ≤2.5.

Conclusion: In the present study, we did not find a relationship between insulin resistance and vitamin D levels. Further studies are needed for clarifying the relationship between insulin resistance and vitamin D levels.

Keywords: Calcium, insulin resistance, obesity, parathormone, vitamin D

INTRODUCTION

25-Hydroxyvitamin D 25(OH)D and parathyroid hormone (PTH) are important physiological regulators of extracellular calcium (Ca) metabolism. The effects of intestinal Ca absorption are elevated by vitamin D (1, 2). In vitro and in vivo studies showed that vitamin D status may play a functional role in glucose homeostasis (3). Associations between low vitamin D and the risk of type 2 diabetes were indicated by many studies (4, 5). On the other hand, there are many studies which support the opposite. 25(OH)D level, insulin resistance, and diabetes in non-Hispanic Whites and Mexican Americans have a common inverse association, but not in non-Hispanic Blacks as observed in a study conducted by the Third National Health and Nutrition Examination Survey (6). A study of Chinese individuals showed that serum 25(OH)D level was inversely related with insulin resistance (7, 8), whereas in a Canadian Cree study, these parameters were found to be not connected with insulin resistance or beta cell function. However, these studies did not present the data on serum Ca, nor did they adjust PTH levels for Ca concentrations as we have done in our study. The objective of the present study was to investigate the relationship of 25(OH)D, PTH, and Ca serum levels with IR among obese individuals with impaired fasting glucose (IFG) compared with non-obese individuals with normal fasting glucose.

METHODS

Subjects

This study was conducted on 60 obese adult individuals with IFG with a body mass index (BMI) ≥30 kg/m^2 as diagnosed by physicians. The mean age of the patients was 47.7 (20-60) years. Patients who had serious disease, pregnant women, or patients who take any medication drugs were excluded from the study. The results of these 60 case study individuals were compared

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with 60 healthy individuals with normal fasting glucose with a BMI <30 kg/m^2 as the control group. The mean age of the healthy individuals was 40.3 (20-60) years. Patients' tobacco use (response set smoker or nonsmoker) and typical alcohol consumption (response set drinking or nondrinker) data were gathered.

Study samples were collected from outpatients attending to Gaziantep University endocrinology and metabolic diseases clinic from October 2016 to January 2017. All participants were informed about the study procedure through a written consent form before participation. The study complied with the Declaration of Helsinki, and the research protocol was approved by Gaziantep University School of Medicine Research Ethics Committee (Decision date: 08.15.2016, Decision no: 2016/238).

A BMI ≥30 kg/m^2 was defined as general obesity (9), whereas the explanation of IFG is according to the current American Diabetes Association guidelines. The explanation of IFG is a serum glucose level of 100-125 mg/dL, and homeostatic model assessment of insulin resistance (HOMA-IR) ≤2.5 was agreed as the natural range for insulin resistance.

After overnight fasting, blood samples were obtained from the subjects by venipuncture in the morning and centrifuged immediately, and serum samples were analyzed directly without storage. In the central laboratory of Gaziantep University, a serum concentration of total Ca and triglyceride (TG) was measured by enzymatic methods, whereas fasting plasma glucose (FPG) was measured by the hexokinase method by an autoanalyzer. Fasting plasma insulin and PTH levels were assayed by the chemiluminescent immunoassay technique by an autoanalyzer.

Insulin resistance was estimated by using HOMA-IR. HOMA-IR was calculated and estimated fasting plasma insulin value (mIU/mL) and the FPG value (mg/dL), given in the same amount and homeostatic model assessment-beta cell function (HOMA2-B%) and homeostatic model assessment-sensitivity (HOMA2-S%) (10).

Statistical Analysis

Anderson-Darling test was done to assess if continuous variables follow normal distribution. If continuous variables followed normal distribution, then mean and standard deviation would be used. However, if they did not follow normal distribution, then median and interquartile range (25%-75%) were used. Discrete variables were used to present the data, chi-square test was used to analyze the discrete variable, and Fisher's exact test was used to analyze the distribution between the two groups (used instead of chi-square for 2×2 table, if total sample <20 and if two or more with expected frequency <5). Two samples t-test was used to analyze the differences in means between the two groups (if both follow normal distribution with no significant outlier). Mann-Whitney U test was used to analyze the differences in the median of the two groups (if they do not follow normal distribution).

Linear regression analysis was performed to assess the relationship between different variables. If one or both of them followed normal distribution, then Pearson regression was used, but if both did not follow normal distribution, then Spearman correlation was used. Scatter plot was used to present the regression analysis, r (correlation coefficient or standardized beta is a representative of magnitude and direction of the relationship), r<0.25 weak, 0.25-0.5 mild, 0.5-0.75 moderate, and >0.75 strong correlation. A negative sign indicates inverse relationship, whereas a positive sign represents direct relationship.

Multiple linear regression analysis was applied by using dummy variables (sex, alcoholic, and smoking) after adjustment of possible confounders (age, sex, BMI, smoking, alcoholic, TG, 25(OH)D, and serum Ca), and the results were shown using partial correlation coefficient (which represents the correlation coefficient after adjustment).

Statistical Package for the Social Sciences (SPSS) 20.0.0 version (SPSS IBM Corp.; Armonk, NY, USA) Minitab 17.1.0, MedCalc 14.8.1, and GraphPad Prism 7.0 software packages were used for statistical analysis. A p value <0.05 was considered to be significant.

RESULTS

In Table 1, regarding the characteristics of the subjects, obese individuals were compared with those with IFG, and the results of the control non-obese subjects were compared with normal fasting glucose subjects with respect to demographic and biochemical parameters.

Table 2 shows the simple regression analysis between serum 25(OH)D versus demographic and biochemical parameters. Significant and inverse correlation between 25(OH)D, BMI, and PTH was found, whereas no correlation with other demographic and biochemical parameters was reported. However, among the control group subjects, there was no correlation between 25(OH)D and various variables (demographic and biochemical parameters) as shown in Table 2.

Table 3 shows the simple regression analysis between serum PTH versus demographic and biochemical parameters. Only serum PTH showed a significant correlation with 25(OH)D (p=0.032, inverse correlation) and with HOMA2-B% (p=0.049). In the control group, there was no correlation between serum PTH and various variables (demographic and biochemical parameters) as shown in Table 3.

Table 4 shows the simple regression analysis between serum Ca level versus demographic and biochemical parameters. Only Ca showed a significant correlation with HOMA2-S% (p=0.008, inverse correlation) in the control group, whereas no correlation was found between serum Ca and various variables (demographic and biochemical parameters) in the case group.

In Table 5, multiple regression analysis was performed on serum 25(OH)D versus IR-related parameters adjusted for PTH, as well as Ca, age, sex, BMI, smoking, and alcohol consumption. No significant correlation was demonstrated between 25(OH)D and IR-related parameters after adjusting these variables in the case

Table 1. Baseline characteristics of the subjects

Variables	Case N=60	Control N=60	p
Age (years)	47.7[a]±9.5[b]	40.3[a]±14.6[b]	0.001 [Sig.]
Sex			0.449
Female	40[c] (66.7%)[d]	36[c] (60.0%)[d]	
Male	20[c] (33.3%)[d]	24[c] (40.0%)[d]	
Smoking			0.461
Nonsmoker	36[c] (60.0%)[d]	32[c] (53.3%)[d]	
Smoker	24[c] (40.0%)[d]	28[c] (46.7%)[d]	
Alcoholic			0.685
No consumption	44[c] (73.3%)[d]	42[c] (70.0%)[d]	
Consumption	16[c] (26.7%)[d]	18[c] (30.0%)[d]	
Height (cm)	161[a]±8[b]	169[a]±8[b]	<0.001 [Sig.]
Weight (kg)	90[a]±11[b]	73[a]±6[b]	<0.001 [Sig.]
BMI (kg/cm^2)	34.3[a]±4.5[b]	25.3[a]±2.5[b]	<0.001 [Sig.]
FPG (mg/dL)	105.1[a]±3.7[b]	89.4[a]±7.0[b]	<0.001 [Sig.]
F.I (mIU/mL)	10.5[e] (6.2–15.5)[f]	8.5e (5.0–14.0)[f]	0.204
25(OH)D (ng/mL)	20.0[a]±9.8[b]	18.9[a]±9.9[b]	0.536
PTH (pg/mL)	44.6[e] (34.0–59.1)[f]	44.9[e] (40.0–60.0)[f]	0.502
S.Ca (mg/dL)	10.0[a]±0.5[b]	9.7[a]±0.5[b]	0.009 [Sig.]
TG (mg/dL)	134.4[a]±9.4[b]	106.5[a]±20.4[b]	<0.001 [Sig.]
HOMA-IR	2.78[e] (1.58–3.92)[f]	1.88[e] (1.14–2.84)[f]	0.015 [Sig.]
HOMA2-B%	83.4[e] (58.1–108.9)[f]	108.5[e] (66.6–131.3)[f]	0.006 [Sig.]
HOMA2-S%	73.0[e] (51.2–120.4)[f]	91.5[e] (55.0–152.5)[f]	0.152

[a]Mean; [b]standard deviation; [c]number; [d]percentage; [e]median; [f]interquartile range
BMI: body mass index; FPG: fasting plasma glucose; F.I: fasting insulin; 25(OH)D: 25-hydroyvitamin D; PTH: parathyroid hormone; S.Ca: serum calcium; TG: triglyceride; HOMA-IR: homeostatic model assessment—insulin resistance; HOMA2-B%: homeostatic model assessment—beta cell function; HOMA2-S%: homeostatic model assessment-sensitivity; Sig.: significant

and control groups. When subjects with HOMA-IR ≤2.5 were excluded and data were reanalyzed, 25(OH)D results were also not significantly correlated with IR-related parameters in either the case or the control group as shown in Table 5.

DISCUSSION

In the present study, serum 25(OH)D had no relationship with insulin resistance (HOMA-IR) occasionally adjusting for PTH, Ca, and various variables either in all participants or after excluding participants with HOMA-IR ≤2.5. There is a modest and inverse association in the middle of serum 25(OH)D and IR, and multiple adjustment was widely reported by studies. Recently, the con-

Table 2. Correlation between 25(OH)D and various variables

	Control		Case	
Variables	r	p	r	p
Age (year)	−0.96	0.467	0.131	0.317
Weight (kg)	0.080	0.544	−0.106	0.421
Height (cm)	−0.047	0.720	0.236	0.069
BMI (kg/m^2)	0.199	0.127	−0.286	0.027 [Sig.]
PTH (pg/mL)	−0.216	0.098	−0.278	0.032 [Sig.]
S.Ca (mg/dL)	0.051	0.700	−0.002	0.991
FPG (mg/dL)	0.078	0.551	−0.015	0.911
F.I (mIU/mL)	−0.118	0.370	0.131	0.319
TG (mg/dL)	−0.014	0.917	−0.094	0.473
HOMA-IR	−0.122	0.393	0.134	0.306
HOMA2-B%	−0.101	0.441	0.081	0.540

BMI: body mass index; FPG: fasting plasma glucose; F.I: fasting insulin; PTH: parathyroid hormone; S.Ca: serum calcium; TG: triglyceride; HOMA-IR: homeostatic model assessment—insulin resistance; HOMA2-B%: homeostatic model assessment—beta cell function; Sig.: significant

Table 3. Correlation between PTH and various variables

	Control		Case	
Variables	r	p	r	p
Age (years)	0.192	0.143	0.022	0.869
Weight (kg)	0.227	0.081	0.180	0.168
Height (cm)	0.101	0.445	−0.009	0.945
BMI (kg/m^2)	0.111	0.398	0.189	0.149
25(OH)D (ng/mL)	−0.216	0.098	−0.278	0.032 [Sig.]
S.Ca (mg/dL)	−0.068	0.603	−0.137	0.298
FPG (mg/dL)	0.036	0.785	0.077	0.561
F.I (mIU/mL)	−0.049	0.709	−0.239	0.065
TG (mg/dL)	−0.122	0.355	−0.069	0.602
HOMA-IR	−0.037	0.779	−0.236	0.071
HOMA-B%	−0.141	0.282	−0.255	0.049 [Sig.]
HOMA-S%	0.247	0.057	0.060	0.647

BMI: body mass index; FPG: fasting plasma glucose; F.I: fasting insulin; S.Ca: serum calcium; TG: triglyceride; HOMA-IR: homeostatic model assessment—insulin resistance; HOMA-B%: homeostatic model assessment—beta cell function; HOMA-S%: homeostatic model assessment-sensitivity; 25(OH)D: 25-hydroyvitamin D; Sig.: significant

centration of 25(OH)D was approximately the same as indicated by the Framingham Offspring Cohort Study (mean=15 ng/mL) and many Chinese studies (n=3262) (20). The Framingham Cohort Study showed that the participants in the highest 25(OH)D tertile had a 12.7% lower HOMA-IR score (p-trend <0.001) than those in the lowest tertile category (7). In other words, other studies showed clearly that 25(OH)D had a relationship with HOMA-IR after adjusting for age, sex, current smoking, BMI, and WC (p<0.001). Meanwhile, a highly important regression coefficient for 25(OH)D as a predictor of HOMA-IR (p<0.01) followed after settlement for multivariables, having and containing BMI, in a study conducted on a Chinese population (8).

Table 4. Correlation between calcium and various variables

	Control		Case	
Variables	**r**	**p**	**r**	**p**
Age (years)	−0.214	0.100	0.123	0.349
Weight (kg)	0.072	0.585	0.056	0.673
Height (cm)	0.029	0.826	0.043	0.742
BMI (kg/m^2)	0.042	0.749	0.028	0.831
PTH (pg/mL)	−0.068	0.603	−0.137	0.298
25(OH)D (ng/mL)	0.051	0.700	−0.002	0.991
FPG (mg/dL)	0.348	0.006	−0.154	0.240
F.I (mIU/mL)	0.177	0.175	0.051	0.696
TG (mg/dL)	0.150	0.254	0.029	0.823
HOMA-IR	0.181	0.167	0.031	0.813
HOMA-B%	0.152	0.248	0.143	0.275
HOMA-S%	−0.342	0.008 [Sig.]	−0.113	0.390

BMI: body mass index; FPG: fasting plasma glucose; F.I: fasting insulin; TG: triglyceride; HOMA-IR: homeostatic model assessment—insulin resistance; HOMA-B%: homeostatic model assessment—beta cell function; HOMA-S%: homeostatic model assessment-sensitivity; 25(OH)D: 25-hydroyvitamin D; Sig.: significant

In this report, the reason behind the lack of an association between 25(OH)D and HOMA-IR is not clear and needs to be debated.

Various possibilities in vitamin D metabolism in our homogenous, ethnically distinct population in comparison with other cohorts previously recorded in the literature have explained the clear loss of influence of 25(OH)D on IR (HOMA-IR) in this study because 1,25-dihydroxyvitamin D3 mediates biological effects by vitamin D receptor binding including facilitating the biosynthetic capacity of B-cells.

A vital role in glucose metabolism has been detected to be attained by the PTH by bringing about insulin resistance (9, 10). The correlations of essential inverse were clear between PTH with fasting insulin (p=0.022) and HOMA-IR (p=0.023) after adjustment of 25(OH)D, serum Ca, and various variables and exclusion of participants in addition to HOMA-IR ≤2.5. The relationship of PTH to insulin resistance stems from data that patients with primary hyperparathyroidism were clear to be at a serious risk of death from coronary artery illness by 1.71-1.85 times which has to be into one's consideration as an additional evidence (11). This result shows that primary hyperparathyroidism has a relationship with insulin resistance (or decreased insulin sensitivity), as the concept between coronary artery illness and insulin resistance is well based (12). Therefore, our study is the same with these reports which depict the relationship between plasma PTH and insulin resistance.

It is common that mechanisms that clearly show the vital role of Ca in IR are also not fully elucidated. Increasing intracellular Ca concentration can affect the movement of glucose mediated by insulin and insulin secretion as it has been proposed by some studies (13, 14).

This study has some limitations. First, its small sample size may have an effect on the association. Second, only subjects who visited Gaziantep University Hospital were investigated, so we cannot generalize these study results to other adult populations. Third, our samples were collected in the winter season (from October 2016 to January 2017), where sunlight is another essential source of plasma 25(OH)D. We did not have an exact measurement of sunlight exposure. We did not provide help for IR

Table 5. Partial correlation coefficients between 25(OH)D and IR-related parameters adjusted for age, sex, BMI, smoking, alcoholic, PTH, and serum calcium

	Control				Case			
	All		HOMA-IR >2.5		All		HOMA-IR >2.5	
Variables	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
FPG (mg/dL)	0.007	0.958	0.007	0.981	−0.029	0.838	−0.047	0.820
F.I (mIU/mL)	−0.211	0.129	−0.360	0.226	−0.005	0.973	0.087	0.674
HOMA-IR	−0.206	0.138	−0.328	0.274	0.001	0.992	0.613	0.104

FPG: fasting plasma glucose; F.I: fasting insulin; HOMA-IR: homeostatic model assessment—insulin resistance; BMI: body mass index; PTH: parathyroid hormone

by hyperinsulinemic euglycemic clamp, a gold standard for the measurement of IR. However, HOMA-IR is highly correlated with the clamp technique.

CONCLUSION

We did not find a relationship between insulin resistance and vitamin D levels. Further studies are needed for clarifying the relationship between insulin resistance and vitamin D levels.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University of School of Medicine (Decision date: 08.15.2016, Decision no: 2016/238).

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

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Original Research

Residual Stress Levels on the Cortical Section of Vertebral Bone Tissue

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ABSTRACT

Objective: Residual stress can cause deformation and cracks in the bone tissue. The aim of our study is to measure the residual stress level and distribution in the cortical bone of the extremities of vertebrates.

Methods: Residual stress levels in the bone tissue of 12 sheeps aged 2 years were measured by observing the cortical parts of 6 different (C1, C3, Th1, Th 13, L1, and L6) vertebral bones by means of the X-ray diffraction method. This method is recognized as the one that can measure residual stress in the bone tissue most accurately. By means of special methods, the cortical part of vertebral bone was separated from its trabecular part. The bone tissue was left to stand for a long time to dry completely. Measurements were performed on completely dried tissues using an X-ray diffraction apparatus. The residual stress values obtained from all the subject groups were compared statistically.

Results: It was found that the residual stress level was the highest in C3 and that it showed a statistically significant change as compared to the levels in C7, Th1, and Th13. Although the level in C3 was high as compared to the levels in L1 and L6, it was not statistically significant.

Conclusion: The residual stress level in the C3 vertebral cortical section was significantly higher than other parts and was interpreted as such by us, i.e., anatomically, it is one of the vertebrae that keep the head upright and is the vertebra carrying the maximum load in all natural processes.

Keywords: Deformation, nano-cracks, residual stress, vertebral bone

INTRODUCTION

Residual stress is defined as the stress that exists in bone tissue without any external force (1, 2). Residual stress is also seen in living soft tissues, such as blood vessels (3), but primarily the existence of residual stress was has been shown in bone tissue (4). There are many physical methods used to measure residual stress, which include hole drilling, deep hole creation, sectioning method, Contour method, X-ray diffraction, ultrasonography, and the Barkhausen noise method (5). Of these methods, the X-ray diffraction method is considered to be the technique that provides the most extensive results, especially in bone tissue. Residual stress of bone tissue from the femur of cattle was measured by means of the X-ray diffraction method (6).

Cortical bone has a regular composite structure that is shaped with hydroxyapatite (HAP) minerals and collagen matrix (7-12). When the bone tissue is deformed, the distance between the lattice planes of HAP crystals shows the deformation changes in the bone tissue (10). The stress undertaken by HAP crystals can be measured by assessing the deformation in interplanar spacings and by comparing it with a reference (7, 11). Bone tissue regenerates continuously through new osteon structuring (13). New tissues develop under in-vivo loads (14, 15).

Due to non-uniform structures in the bone tissue, residual stress could be formed between changed (old and new) areas without any external force.

The purpose of this study was to measure residual stress levels in the cortical portions of the vertebral bone tissue of 12 2-year-old sheep without applying any external force, assess whether there was any difference between the stress in the cervical, thoracic, and lumbar regions, and thus find which the region with a higher probability of deformation as compared to the others. For this purpose, the first and last vertebrae of the cervical, thoracic, and lumbar regions were selected.

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METHODS

Separation of the Cortical Section

A total of 12 cervical vertebrae (III, IV, V, VI, and VII), 13 thoracic vertebrae, and 6 lumbar vertebrae were dissected from the sheeps' vertebral column and freed from the surrounding soft tissues. This dissected vertebrae were fixed to a table using a clamp system and bone blocks were obtained by resecting 2 mm-thick pieces from the obtained samples from the top and bottom surfaces of the corpus vertebrae using a Stryker bone cutter. All the procedures were performed by the same operator under aseptic conditions with the use of an apron, gloves, and operation glasses. The bone tissue was set out for a long time to dry it completely. Measurements were performed on completely dried tissues using the X-ray diffraction tool. Ethics committee approval is not required since this study was performed on the slaughtered sheep vertebral bone.

Residual Stress Analysis

The known methods to measure residual stress are: X-ray diffraction method, Neutron diffraction method, Barkhausen noise method, and ultrasonic method. Among these, the most reliable measurement method is accepted as the X-ray diffraction method (Methods of Measuring Residual Stresses in Components N. S. Rossinia, M. Dassistia, K. Y. Benyounisb and A. G. Olabi). For this reason, we measured all our samples using this method (5).

The residual stress value can be determined by measuring the σ and 2θ diffraction angle. The Bragg law, which is the basic method for X-ray diffraction, is given by the following equation:

$2d\sin\theta = n\lambda$

This equation establishes a relationship between the θ Bragg angle and the distance between the planes at the knowing (hkl) plane using characteristic X-rays with a monochromatic wavelength.

$\nu\theta$The values of E, ν, and θ in the formula are obtained with some operations.

E is the Young's modulus (MPa), ν is the Poisson rate, and θ is the diffraction angle in a non-stress situation.

The ψ angle between the sample surface and the lattice plane changed normally during the stress measurement, as shown in Figure 1. Detector scans to measure the intensity of X-ray were refracted by the sample.

To obtain the stress value, a graphic of the 2θ (horizontal part) and $\sin^2\psi$ (vertical part) graphic was drawn using various ψ angles. The slope of this graph is multiplied with stress constant K, which is determined by the type of material. Following this, the stress value is obtained.

Figure 1. Cortical section of Vertebral bone of 2-year-old sheep

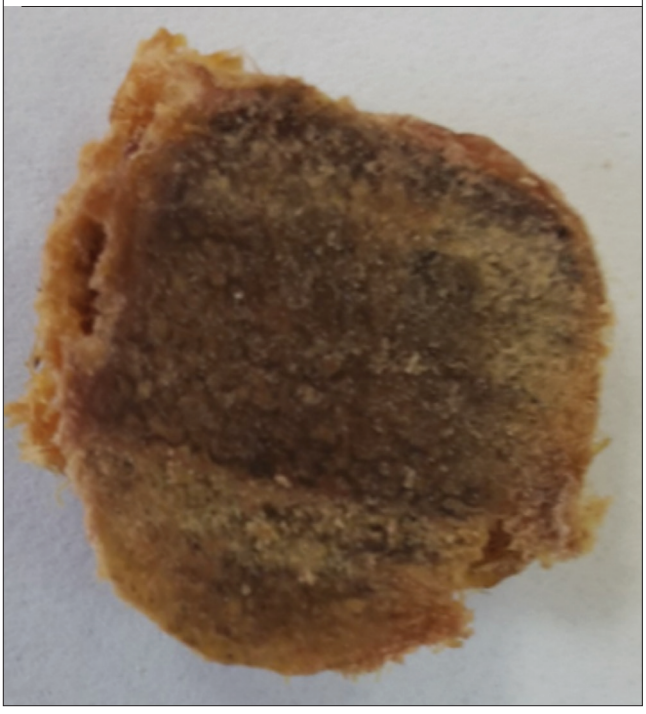
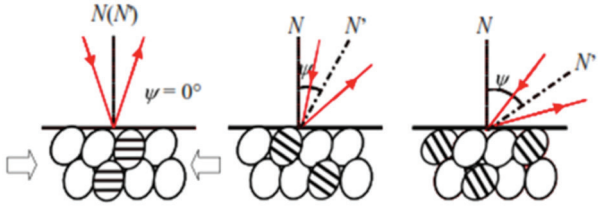


Figure 2. Residual stress measurement by X-ray refraction method



The measurements of the samples in this study were performed from up part. The measurement of residual stress by the X-ray diffraction (XRD) method is shown in Figure 2. XRD spectra were recorded by using a Rigaku X-ray diffractometer (RINT 2000 series, model D/max 2000, Rigaku Co., Japan) with a multipurpose attachment. A characteristic CuKα (l.5406 nm) X-ray source, a tube with a voltage of 40 kV, and a tube current at 40 mA was used. The incidence angle was fixed at 1° during scanning. The XRD analyzes were carried out using the symmetrical Bragg-Brentano configuration (θ-2θ) with parallel beam geometry. The diffraction profiles were measured between 10°-120°, which includes the diffraction angles of the (2 1 1), (1 1 2), and (3 0 0) lattice planes of hydroxyl apatite (HAP) crystals. The XRD peaks were found to have a hexagonal crystal structure with a P63/m (176) space group (JCPDS 98-000-0050).

The study defined 2θ as the angle at the peak position of the profile, the peak position was determined as the midpoint of the full width at half maximum intensity of the profile (FWHM method), 2θ was measured at the various ψ conditions accord-

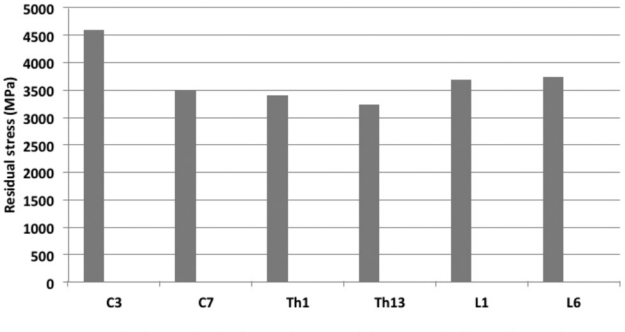
Table 1. Mean and standard deviation of samples

	N	Mean	Standard deviation	Standard error
C3	12	4602.41	1345.33	388.36
C7	12	3320.00	595.45	171.89
Th1	12	3411.66	653.24	188.57
Th13	12	3236.58	959.19	276.89
L1	12	3688.00	1058.25	305.49
L6	12	3738.75	479.66	138.46

Table 2. Statistical results of samples of C7, Th1, Th13, L1, L6 sections are compared to C3

I group	J group	p
C3	C7	0.030
C3	Th1	0.032
C3	Th13	0.008
C3	L1	0.253
C3	L6	0.356

Figure 3. Residual stress levels of cortical section of vertebral bone tissue



ing to the sample situation, and $q(2\theta)/q(\sin^2\psi)$ was calculated by the linear least-squares method. To determine residual stress, a residual stress calculation software was used. The samples were smoothed and the unit cell parameters of the sample obtained from the XRD pattern (a=9.364, b=9.364, c=6.881Å, alpha=90°, beta=90°, and gamma=120°) were used for residual stress calculation (Rigaku, Jade 7 software). Based on previous literature, the values of the Young's modulus and Poison rate for this sample were taken as 70 GPa and 0.3, respectively (16).

Statistical Analysis

Residual stress levels that were measured in the cortical bones C3, C7, Th1, Th13, L1 and L6 were statistically compared with each other using ANOVA oneway and Benforanni statistical methods in the Statistical Package for the Social Sciences (SPSS IBM Corp.; Armonk, NY, USA) 20 version package software (Table 1).

RESULTS

Residual stress level measurements of the cortical part of 12 sheep vertebrae were made by using the X-ray diffraction method. Residual stress levels that were measured in the cortical bones C3, C7, Th1, Th13, L1, and L6 were statistically compared with each other using the one-way analysis of variance and Benforanni statistical methods available in the SPSS version 20 software (Table 1). It was found that residual stress levels were the highest in the cortical bone of the C3 vertebra. The residual stress level of C3 showed statistically significant changes as compared to C7, L1, and L6 (C3-C7 $p<0.030$; C3-L1 $p<0.032$; C3-L6 $p< 0.008$). Results are shown in Figure 3. When compared with C3, there was a difference in the levels of Th1 and Th13 but this difference was not statistically significant (Table 2).

DISCUSSION

The value of residual stress depends on the stress constant Kx, which is calculated with the elasticity modulus Ex and the coefficient $k^*_{x'}$ which in turn, depend on HAP crystal structures, collagen fibers, and ultimately, tissue stress (10). However, the X-ray diffraction method directly measures the distribution of residual stress without using the elasticity modulus.

Yamado et al. (1) compared residual stress and osteon population density and observed a higher proportion of residual stress in areas with a high osteon population density. These results were found compatible with some past results (17, 18). However, the authors suggested that this might change depending on whether the anterior or posterior positions were sampled. This is because mineral crystal orientation could change in these regions and it does not have a direct relationship with the orientation and organization of collagen fibers (19). Yamado et al. (1) suggested that non-uniform structures of tissues are derived from osteon formation and the internal organization of these entities down to the nanostructural levels may be explained by the spatial differences in the patterns of residual stress.

Adachi et al. (20) measured residual stress in the vertebral bone by the cutting method and suggested that the bone tissue develops a stress condition to eventually become more uniform.

Residual stress might be important for mechanical strength of the tissue. Bone tissue can distribute the applied force and energy among the cells to counter daily mechanical stresses, but it may fail to distribute the same level of force after certain levels of stress, leading to the occurrence of residual stress in the tissue. This residual stress causes hair cracks in the tissue, which may easily lead to bone fragility.

Residual stress could also manifest differently in anterior and posterior positions (6). It has been shown that residual stress in the anterior position is higher than the stress seen in the posterior position in the femurs of cattle. For this reason, residual stress could be associated with energy distribution or composite distribution of the stress environment (6).

More generally, residual stress could be associated with the formation of osteon structures and/or their collagen/lamellar/crystalline organization. Some studies have also revealed a relationship between residual stress and osteon population density. However, this relationship was not found to be particularly strong.

More complex studies are needed in order to completely explain the residual stress-fragility relationship. For example, it will be necessary to examine the non-uniform distributions and collagen fiber orientation (CFO) in collagen attachments, the relationship between osteon types and/or CFO heterogeneity and residual stress, distribution of HAP crystal structures, and their orientation.

Further, residual stress may show differences in bones of different species and not only in anterior and posterior bones, since the vertebrae and other parts of the skeleton are exposed to different loads in human beings and other animals. For instance, since the cervical, thoracic, and lumbar vertebrae are loaded differently in four-footed animals, the residual stress may occur at different levels between such animals and even between different animals in the same species. For this reason, it is important to assess whether or not the residual stress levels show differences depending on different loads in cortical sections of C3, C7, Th1, Th13, L1, and L6 in adult sheep bone.

In our study, the residual stress level of C3 showed a statistically significant change as compared to C7, Th1, and Th13. A statistically insignificant difference was observed between L1 and L6, which may be due to the fact that C3 is the vertebra that carries the maximum load to support the cranium in a sheep's skeleton. Undoubtedly, this load is present at different levels in other vertebrae because all of them were found to exhibit residual stress. However, it was seen most clearly in C3 due to its anatomical structure and its maximum load-bearing position.

CONCLUSION

With the existing methods and under in-vivo conditions, it is impossible to measure whether such a difference occurs in human beings at the present time. This is because the vertebral bone would need to be isolated from the body for all these methods.

Hydroxyapatite crystal structures, lattice planes and their deformations, osteon structures, osteon population density, and deformations can provide some information about residual stresses in the bone tissue.

Ethics Committee Approval: Ethics committee approval is not required since this study was performed on the slaughtered sheep vertebral bone.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - C.S.; Design - C.S., A.İ.U.; Supervision - C.S.; Resources - C.S.; Materials - C.S., A.İ.U.; Data Collection and/or Processing - C.S., A.İ.U.; Analysis and/or Interpretation - Ş.Y., A.İ.U.; Literature Search - C.S.; Writing Manuscript - C.S., A.İ.U.

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

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Original Research

Comparison of Pulmonary Artery Catheter and Central Venous Catheter for Early Goal Directed Targeted Therapy in Sepsis and Septic Shock

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ABSTRACT

Objective: The aim of the present study was to compare the effect of the pulmonary artery catheterization (PAC) method and the central venous catheterization (CVC) method on hemodynamic and inflammatory parameters in early goal-directed therapy (EGDT).

Methods: This was a randomized prospective study. Patients with sepsis and septic shock within 12 h of diagnosis were included in the study. Each group received strict protocolized resuscitation for 72 h.

Results: The mean age of the patients was 63.4±14.5 years. The study included 15 (52%) male and 14 (48%) female patients. The length of stay in the hospital and the duration of mechanical ventilation were similar between the two groups. The length of stay in the intensive care unit was shorter in the CVC group (p=0.025). High mobility group box 1 levels were lower at 72 h in the CVC group (p=0.026). In the early resuscitation period, it was found that in the CVC-directed therapy group, the urine output and the mean arterial blood pressure were higher, but vasoconstrictor need was lower (p<0.05).

Conclusion: In the early resuscitation period, CVC-directed therapy is more effective, and rapid correction of hemodynamic parameters results in shorter intensive care unit stay. PAC is not superior to CVC-guided therapy in the late period.

Keywords: Early goal directed therapy, sepsis, septic shock

INTRODUCTION

Sepsis is a common disease with a high mortality rate (1). Fundamentally, the treatment consists of the removal of the trigger and the prevention of the tissue hypoperfusion and organ dysfunction. Several minutes after endotoxin release, cytokines are released, and these play an important role in pathogenesis (2). Mortality is significantly decreased by fluid resuscitation and early antibiotic induction which are the major steps in early goal-directed therapy (EGDT) (3).

Fluid resuscitation in EGDT is based on the central venous pressure (CVP) and central venous oxygen saturation ($ScVO_2$) measurements. In the early hemodynamic stage, vital signs, CVP (4), and urinary output (5) cannot detect global tissue hypoxia. A more definitive method to show the balance between systemic oxygen delivery and oxygen demand is the manipulation of preload, afterload, and contractility (6). Pulmonary artery (PA) catheter can be used to measure stroke volume, cardiac output (CO), mixed venous oxygen saturation (SvO_2), and intracardiac pressures to guide diagnosis and treatment (7).

The aim of the present study was to evaluate PA effectiveness in EGDT in a protocol-based study. For this reason, we used two different EGDT protocols based on two different catheterization methods in sepsis and septic shock to compare their effectiveness on hemodynamic goals and inflammatory parameters. The Protocolized Care for Early Septic Shock (PROCESS), Australasian Resuscitation in Sepsis Evaluation, and Protocolized Management in Sepsis trials show that EGDT has no mortality benefit compared with usual care (8-11). Our study was planned before these trials.

METHODS

This prospective randomized study was conducted in Erciyes University School of Medicine, Medical Intensive Care Unit (MICU) be-

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tween August 2010 and January 2012. Our study was approved by the ethics committee of Erciyes University School of Medicine (date: 08/07/2010; ethics committee decision no: 2010/61).

All patients or their families were informed about the study. For conscious patients, information was directly given to the patients, and for unconscious patients, families were informed. Written informed consent was obtained from the patients or their legally authorized representatives.

Inclusion Criteria

- Patients with severe sepsis and septic shock as defined according to the Surviving Sepsis Guidelines (12)
- Patients who were aged >18 years.

Exclusion Criteria

- Chronic liver disease
- Chronic renal failure
- Renal replacement therapy
- Pregnant
- Expected to die within 48 h
- Referred from another health care institution while they were being hospitalized there
- Patients who were aged <18 years
- Severe sepsis or septic shock diagnosis delayed more than 12 h
- Patients with chronic liver disease and chronic renal failure since they can affect lactate level.

The primary end points in our study were mortality and hemodynamic goals. A total of 29 patients were enrolled in the study. Baseline demographics, admission Glasgow Coma Scale, Acute Physiology Age Chronic Health Evaluation (APACHE II) score, daily Sepsis-Related Organ Failure Assessment score, arterial blood gas, arterial lactate levels, hourly urine output, vasopressor dose, length of hospital stay, length of intensive care unit (ICU) stay, duration of mechanical ventilation (MV), and 28-day mortality rate were recorded. Clinical data were collected at baseline; at 3, 6, 12, and 24 h; and on days 2 and 3. In addition, blood high mobility group box 1 (HMGB1) levels were measured at 20 and 72 h. Dopamine and noradrenaline were the initial choices as vasoconstrictor agents. Adrenaline was used in addition to dopamine or noradrenaline. During catheterization and measurements, midazolam (Dormicum®) and/or vecuronium (Norcuron®) were used for sedation and neuromuscular blockade, respectively.

Patients were randomly divided into the pulmonary artery catheterization (PAC) group (13, 14) and central venous catheterization (CVC) group. PA was used in the PAC group, whereas a CV catheter was used in the CVC group.

The patients were enrolled in the study within 12 h of severe sepsis or septic shock diagnosis. In each group, their own protocol was applied for at least 72 h.

PAC Group

In the PAC group, PA was inserted, and the protocol published by Pinsky and Vincent was used (15). For CO measurements, the thermodilution technique and a Vigilance CEDV (Edwards Lifesciences Corp., Irvine, CA, USA) device were used (15).

CVC Group

The protocol published by Rivers et al. in 2001 was used in the CVC group (13). To measure $ScVO_2$, blood extracted from the central catheter was immediately analyzed by a Siemens Rapidlab 1265, blood gas analysis device.

Statistical Analysis

The analysis was conducted using R 3.0.2 (www.r-project.org). Shapiro-Wilk test, histogram, and q-q plots were used to assess the normality of data. Levene's test was used to test variance heterogeneity. To compare the differences between the groups, a two-sided independent samples t test and Mann-Whitney U test were performed. In addition, one-way repeated measures analysis of variance and Friedman tests were used for time comparisons. A P value <5% was considered as statistically significant.

RESULTS

The mean age of the patients enrolled in the study was 63.4±14.5 years. A total of 29 patients were recruited into the study. There were 15 (52%) male and 14 (48%) female patients in the study (Table 1).

Table 1. Baseline demographic and clinical characteristics

Variables	CVC (n=15)	PAC (n=14)	p
Age (year) (±SD)	62±12	64±16	0.690
Male, n (%)	7 (46.7)	8 (57.1)	0.424
Underlying disease			
DM, n (%)	4 (26)	3 (21)	0.742
COPD, n (%)	1 (6.7)	4 (28)	0.119
CRF, n (%)	1 (6.7)	1 (7.1)	0.960
Malignancy, n (%)	3 (20)	1 (7.1)	0.316
CHF, n (%)	2 (13)	5 (35.7)	0.159
PVD, n (%)	2 (13)	2 (14.3)	0.941
SAH, n (%)	2 (13)	2 (14.3)	0.941
CVD, n (%)	2 (13)	1 (7.1)	0.584
pH (±SD)	7.34±0.1	7.30±0.1	0.278
Lactate (mmol/L) (±SD)	3.78±2.5	3.41±1.6	0.641
SOFA score day 1 (±SD)	7±3	9±2	0.141
SOFA score day 3 (±SD)	6±3	8±3	0.244
No. of organ failures	2 (13.3%)	4 (28.6%)	0.442

CVC: central venous catheterization; PAC: pulmonary artery catheterization; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; CRF: chronic renal failure; CHF: chronic heart failure; PVD: peripheral vascular disease; SAH: systemic artery hypertension; CVD: cerebrovascular disease

Table 2. Effects of early goal–directed therapy protocols on hemodynamic parameters in the early phase of treatment

Variables	CVC (n=15)	PAC (n=14)	p
Median fluid amount (mL/h) (min/max)			
1 h	150.0 (50.0–600.0)	112.0 (50.0–1000.0)	0.228
2 h	100.0 (50.0–200.0)	100.0 (50.0–500.0)	0.854
3 h	100.0 (50.0–500.0)	100.0 (50.0–1000.0)	0.323
4 h	100.0 (50.0–200.0)	100.0 (50.0–260.0)	0.081
5 h	100.0 (50.0–200.0)	100.0 (50.0–260.0)	0.056
6 h	100.0 (50.0–200.0)	112.5 (50.0–260.0)	0.094
Median urine (mL/h) (min/max)			
1 h	0.00 (0.00–50.00)	0.00 (0.00–486)	0.782
2 h	0.00 (0.00–50.00)	5.00 (0.00–150.0)	0.706
3 h	40.00 (0.00–100.0)	10.00 (0.00–150.0)	0.116
4 h	20.00 (0.00–100.0)	10.00 (0.00–1 00.0)	0.138
5 h	50.00 (0.00–200.0)	5.00 (0.00–75.0)	0.028*
6 h	50.00 (0.00–250.0)	0.00 (0.00–100.0)	0.005
Vasoconstrictor need (µg/kg/min) (min–max)			
3 h	0.00 (0.00–0.50)	2.65 (0.00–20.0)	0.001
6 h	0.00 (0.00–0.25)	2.75 (0.00–20.0)	0.001
Cumulative fluid (mL/h) (min/max)			
Day 1	1395.0 (0.00–6729.0)	2972.0 (1278.0–9469.0)	0.023
Day 2	2167.0 (0.00–8965.0)	3285.0 (800.0–8004.0)	0.097
Day 3	2145.0 (0.00–5760.0)	2365.0 (0.00–5000.0)	0.678
Lactate (mmol/L) (±SD)			
3 h	2.85±2.46	3.29±1.70	0.579
6 h	2.54±1.62	3.20±1.66	0.292
MAP			
1 h	78.6±19.2	76.2±14.9	0.722
2 h	72.13±14.92	74.4±11.1	0.645
3 h	76.06±11.6	72.7±16.4	0.529
4 h	76.0±11.5	69.1±8.88	0.083
5 h	76.9±10.1	69.7±6.90	0.037
6 h	77.2±9.5	71.2±10.0	0.109
CVP (mmHg)			
3 h	8.66±4.04		
6 h	9.93±3.63		
PCWP (mmHg)			
3 h		15.50±4.25	
6 h		16.78±4.37	
$SCVO_2$ (±SD)			
3 h	67.0000±8.23		
6 h	67.4286±7.27		
SVO_2 (±SD)			
3 h		73.214±8.1	
6 h		74.571±7.9	

CVC: central venous catheterization; PAC: pulmonary artery catheterization; CVP: central venous pressure; PCWP: pulmonary capillary wedge pressure; $SCVO_2$: central venous oxygen saturation; SVO_2: mixed venous oxygen saturation

*<0.05

In the early phase (within 6 h), the patients in the PAC group had significantly higher vasoconstrictor needs (p=0.014) at 3 and 6 h (Table 2). The mean arterial pressure (MAP) (P<0.05) and urine output (p<0.05) were lower at only 5 h in the PAC group. The other clinical parameters were similar between both groups in the early phase. The length of stay in the hospital, the duration of MV, and 28-day mortality were similar between the two groups. In the CVC group, the length of stay in the ICU was shorter (p=0.025). In the CVC group, 24 h after the resuscitation period, lactate levels were lower. We did not observe any PAC-related complications. HMGB1 levels were lower at 72 h in the CVC group (p=0.026).

Table 3. Effects of early goal–directed therapy protocols on hemodynamic parameters in the late phase of treatment

Variables	CVC (n=15)	PAC (n=14)	p
Median urine (mL/day) (min/max)			
Day 2	1030 (50–6500)	2055 (200–3600)	0.158
Day 3	1330 (240–4900)	1972 (400–8400)	0.051
Median fluid amount (mL/day) (min/max)			
Day 2	4430 (1752–10,096)	4368 (300–9550)	0.880
Day 3	4300 (1791–6907)	4005 (1500–8110)	0.938
Noradrenaline dose (µg/kg/min) (min/max)	0.000 (0.0–0.1)	0.000 (0.0–1.5)	0.172
Dopamine dose (µg/kg/min) (min/max)	0.000 (0.0–20)	0.000 (0.0–20)	0.533
MAP (mmHg)			
12 h	84.6±15.7	68.7±11.2	0.005
24 h	92.8±16.5	66.6±15.6	0.000
Lactate (mmol/L) (±SD)			
12 h	1.80 (1.20–2.10)	2.52 (1.7–3.0)	0.102
24 h	1.6 (1.08–1.72)	2.64 (1.5–3.0)	0.029*
HMGB1, 20 h (ng/mL)	4.59±5.2	5.4±2.8	0.594
HMGB1, 72 h (ng/mL)	1.44±1.1	2.6±1.6	0.026*
Duration of MV (day) (min–max)	4 (1–65)	6 (1–24)	0.554
Length of stay in the ICU (day) (min–max)	5 (4–65)	14 (4–45)	0.025*
Length of stay in the hospital (day) (min–max)	6 (4–87)	19 (4–135)	0.058
Mortality, n (%)	8 (53%)	6 (43%)	0.424

CVC: central venous catheterization; PAC: pulmonary artery catheterization; MV: mechanical ventilation; MAP: mean arterial pressure; ICU: intensive care unit
*: $p<0.05$

Table 4. Culture isolates

	CVC	PAC	p
C. albicans, n (%)	3 (20)	1 (7)	0.316
A. baumannii, n (%)	3 (20)	6 (43)	0.184
S. maltophilia, n (%)	0 (0)	1 (7)	0.292
E. coli, n (%)	2 (13)	5 (35)	0.159
C. pneumoniae, n (%)	1 (6)	0 (0)	0.326

CVC: central venous catheterization; PAC: pulmonary artery catheterization

The patients were followed up for 72 h in the late phase; there was no difference with respect to daily urine amount, amount of fluid, and vasoconstrictor needs between the two groups (Table 3). Nine (60%) patients in the CVC group and 9 (64%) patients in the PAC group had positive culture results (Table 4).

DISCUSSION

In this single-center randomized study, we found that there was no difference between the treatment methods using PAC-directed therapy and CVC-directed therapy. In the CVC group, the length of stay in the ICU was shorter. Vasoconstrictor requirements were lower in the CVC group in the initial resuscitation period. Lactate levels were lower 24 h after the resuscitation period. The study protocol proposed by Pinsky and Vincent (15) was used in the PAC group, and the protocol proposed by Rivers et al. (13) was used in the CVC group. Our study was conducted in 2010; for this reason, the 2008 international sepsis guidelines were used as basis. The 2012 and 2016 international sepsis guidelines also have similar suggestion for hemodynamic goals with 2008 (11). The most important thing in management is early fluid resuscitation and infection control.

There are multiple studies about PA use in critically ill patients in specific and mixed patient populations with both positive and negative results (14, 16).

Although we had a very small patient group, our study can answer important questions since we used a specific homogenous

patient group. In 2001, Rivers et al. (13) reported that 28-day mortality decreases by 16% in the EGDT group. The major advantage of PA-directed therapies is that they can provide information about hemodynamic data which cannot be detected by clinical signs and CVC (17).

If PA data are interpreted correctly, they can detect intravascular volume in hypotensive patients, differentiate shock type, and monitor tissue oxygenation with SvO_2 levels. Despite all these beneficial effects, PA is expensive, experienced staff are needed for catheterization, and most physicians believe that usage can increase mortality. Therefore, these negative studies resulted in a significant decrease in PA use in critically ill patients (18). The first major study published in 1996 by Connors et al. (18) called the Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments (SUPPORT) study showed increased 30-day mortality, length of ICU stay, and cost with PA use. In the SUPPORT study, the PAC group patients had a higher APACHE II score, lower MAP, and lower serum albumin levels than the other group, which may suggest that further studies need to be conducted (18). Other studies which criticize PA use concluded that PA use did not provide any beneficial effects. However, in all these studies, no particular protocols were used for PA use (16, 19-21).

In our study, the CVC group had a shorter ICU stay, meaning that effective early resuscitation results in more ICU-free days.

In the PROCESS study, there was no difference between the EGDT group and the usual care group with respect to early resuscitation and 60-day mortality rates. The study seriously questioned whether CVC-directed therapy is an indispensable method for sepsis and septic shock treatment (9). This landmark study is published in 2014. The results of the study indicate that awareness of sepsis is increased until this year and can be managed with fewer devices.

We did not observe any PA-related complications in our study, probably because of having only a small number of patients. The same person collected all of the data, the physician initiative was not taken into account, protocols were strictly applied to each group, the patient group was not changed, and the protocol was implemented until the end of the study.

Baseline positive end-expiratory pressure and PaO_2/FiO_2 values were different between the two groups. Although the envelope method was used, patients in the PAC group had more severe respiratory failure than those in the CVC group. This difference can be due to the small patient number. However, similar APACHE II scores in both groups show that there was no bias in selection of the patient. In the early stage, urine output and MAP were lower, and vasoconstrictor need was higher in the PAC group, indicating that early hemodynamic goals were not reached.

In contrast to the acute phase, in the late phase, there were no significant differences with respect to hemodynamic parameters, MV day, or 28-day mortality between the two treatment methods. This means that PAC-directed therapy is not superior to CVC-directed therapy. The length of stay in the ICU was shorter in the CVC-directed therapy group, meaning that effective early resuscitation results in more ICU-free days.

Since the study was conducted in a medical ICU, most of the patients had comorbidities. In the CVC group, the major co-morbidity was diabetes mellitus (26%). Patients with diabetes mellitus have a higher risk of infection (22). In the PAC group, the major co-morbidity was congestive heart failure, which also has infection risk.

Gram negative infections were associated with an increased risk of mortality in several studies (22). *Acinetobacter baumannii* was isolated in 13 (20%) patients in the CVC group, whereas it was 6 (43%) patients in the PAC group. We thought that the increased ratio of *A. baumannii* in the PAC group was due to longer ICU stay, but it had no effect on mortality rate.

Although there are many studies comparing the efficiency of the PAC and CVC methods, until now, no study has measured cytokine levels as well. Cytokines, such as tumor necrosis factor alpha, interleukin (IL)-1, IL-6, and HMGB1, have been shown to play an important role in organ dysfunction and cardiovascular disorders in sepsis and septic shock (23). No single biomarker showed sensitivity and specificity >90% for the diagnosis of sepsis or the prediction of outcome (24).

High mobility group box 1 is a late mediator released from macrophages 20 h after activation and remains at the plateau level for 72 h, so it can be detected within 20-72 h at the beginning of sepsis (24). Since it can be detected in serum for a long time, we preferred HMGB1 as a cytokine in our study. HMGB1 levels were low in both groups at 20 h, but at 72 h, the level was significantly lower in the CVC group, which may mean that the inflammatory process was less activated with CVC-directed therapy because of the faster recovery of hemodynamics in this group.

The study was conducted on a very small group of patients and in a single center. Catheters were replaced by only two physicians who had PAC insertion training. All those reasons affect the patient number and study result.

Since the study was conducted in a medical ICU, the results cannot be generalized to other ICUs, such as surgical ICU and mix ICU.

We did not accept all patients. We excluded moribund patients since the protocol should be applied for 72 h.

CONCLUSION

In the early resuscitation period, CVC-directed therapy is more effective on hemodynamic parameters. In the late period, PAC is not superior to CVC-guided therapy. PA is expensive, insertion is a complex process, and it needs special training. For all those reasons, we do not recommend PAC use for hemodynamic monitoring in sepsis and septic shock in medical ICU patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University (date: 08/07/2010; decision no: 2010/61).

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

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Original Research

Squamous Cell Carcinoma of the Lower Lip; Is Prophylactic Neck Dissection Required and Evaluating Predictive Factors

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ABSTRACT

Objective: This research introduces a series of 87 cases of squamous cell carcinoma of the lower lip. We aim to evaluate the efficacy of prophylactic neck dissection and assess determinative factors for local recurrence and regional metastasis in lower lip carcinomas.

Methods: Medical records of 87 consecutive patients who were diagnosed with squamous cell carcinoma (SCC) of the lower lip were retrospectively analyzed for specific parameters at the Ear Nose Throat (ENT) and Plastic & Reconstructive Surgery clinic at the Dr. Ersin Arslan Training and Research Hospital and the ENT clinic at the Gaziantep University School of Medicine between 2011 and 2017. Patients who had been previously operated upon in other centers were excluded from the study. Tumor excision with safe margins confirmed by frozen sections and supraomohyoid neck dissection was performed on all the patients involved in this study. The minimum follow-up was 12 months for all patients. The median follow-up was 23 months (ranging from 12 to 72 months).

Results: The data analysis indicated that local recurrence was significantly related to the tumor size, depth, and proximity of the tumor to surgical margins. Local recurrence was detected in 8 (9.1%) of 87 patients. Time from onset of disease to the diagnosis, size of the tumor, and proximity of the tumor to the lip commissure play an important role in the spread regional metastasis. Overall the regional metastasis rate was found to be 22.9% (20 of the 87 patients). The occult metastasis rate was found to be 9.1% (8 of the 87 patients). The overall survival rate of patients with lower lip carcinoma, who underwent a supraomohyoid neck dissection and excision of the tumor was 96.5% (84 of the 87 patients).

Conclusion: Although lower lip cancers generally have a good prognosis after a proper surgical intervention, it can be life-threatening in case of neck metastasis or local recurrence. Certain factors, such as tumor size, location, and time interval between onset of disease and diagnosis are influencing the prognosis. Prophylactic neck dissection should be performed when the risk of cervical lymph node metastases is high in patients, especially to cases in which the tumor is close to the commissure. Early detection, prophylactic neck dissection, and follow-up with frequent intervals are essential for a good prognosis.

Keywords: Carcinoma, lip reconstruction, lower lip scc, prophylactic neck dissection

INTRODUCTION

Lip cancer is a frequent malignant disease of the oral cavity and is responsible for approximately 30% of malignant tumors of the oral cavity (1, 2). SCC is the most common type of cancer of the lip and rare tumors include adenocarcinomas and melanomas (3, 4). The lower lip is the most frequently involved part and accounts for more than 90% of lip cancer cases. SCC of the lower lip has a favorable prognosis because a lesion of the lower lip can be seen easily and diagnosed early. Nevertheless, mortality due to lower lip SCC may still occur (5). Frequently, lower lip involvement is thought to be more closely related to increased exposure to solar radiation as compared to the upper lip. However, the predisposing factors of lip cancer are not only limited by solar radiation, but also include tobacco, immunodeficiency, chronic ulcerations, and genetic predisposition (5, 6). Surgery consisting of full-thickness resection of the skin, mucosa, and underlying muscle tissues to ensure a safe surgical margin is the only option for the definitive treatment of lower lip SCC. Although various reconstruction techniques to be performed after safe excision of the tumor have been defined, the reconstruction of the defect is still a challenging issue, especially in large tumors (7). In addition to surgical intervention of the primary lesion site, prophylactic neck dissection should be performed even on clinically N0 neck tumors, to completely cure the occult metastasis to regional lymph nodes, if it is present. Although there is a consensus that therapeutic neck dissection should be performed in patients

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with cervical nodal involvement, the indications of prophylactic neck dissection still remain unclear and controversial. The most significant factor that directly affects the survival of a patient with lower lip SCC is the nodal involvement. Even if the tumor is in the T1 stage, if it is located at the lip commissure, the possibility of cervical metastasis should be taken into consideration due to the rich lymphatic network of the lip commissure.

METHODS

Eighty-seven consecutive patients who were diagnosed with lower lip SCC, underwent surgical excision of the tumor, and either had therapeutic or prophylactic neck dissection done as a primary treatment, were retrospectively analyzed. Verbal informed consent was obtained from all the patients prior to the start of the study. Patients who were previously treated at other medical centers and were subsequently referred to our clinic were not included in the study. Medical records were scanned for particular parameters including age, gender, solar radiation exposure, tobacco usage, tumor size, location of tumor, time from onset of disease to diagnosis, clinical and radiological lymph node involvement, and control examination reports. Local ethical committee approval was taken from Gaziantep University (263/2018).

Statistical Analysis

Analysis of the data was performed using the Statistical Package for Social Sciences software version 20.0 (SPSS IBM Corp.; Armonk, NY, USA) and a p-value of <0.05 was considered statistically significant. Descriptive statistics were used to analyze the data (minimum, maximum, mean, and standard deviation). To test the relationship between categorical variables, we used the x^2 test.

RESULTS

The study consists of a total of 87 patients, 70 (80.4%) males and 17 (19.6%) females, ranging from 34-87 years of age with the average age being 64 years. The overall male-to-female ratio was 4 to 1. Males were found to have a higher risk for lower lip cancer as compared to females. The distribution of patients according to age is displayed in Table 1. Lower lip cancer was more frequently detected between the ages of 61-70 years (27 of 87, i.e., 31%). Analysis of the medical records revealed that 62 patients (71.2%) were affected by solar radiation exposure as the predisposing factor (farmers, open area workers, rural citizens) and 72 patients (82.7%) smoked more than 20 cigarettes per day. In this study,

Table 1. Patients characteristics and tumor stage

	Characteristics	(n)	%
Age	<40	3	3.46
	41–50	14	16.09
	51–60	13	14.94
	61–70	27	31.03
	71–80	13	14.94
	>80	17	19.54
Gender	Male	70	80.4
	Female	17	19.6
Tobacco	Smokers	72	82.75
	Non–smokers	15	17.25
T stage	T1	29	33.33
	T2	38	43.67
	T3	17	19.54
	T4	3	3.46

Figure 1. Distribution of tumors location and lymph node involvement ratio

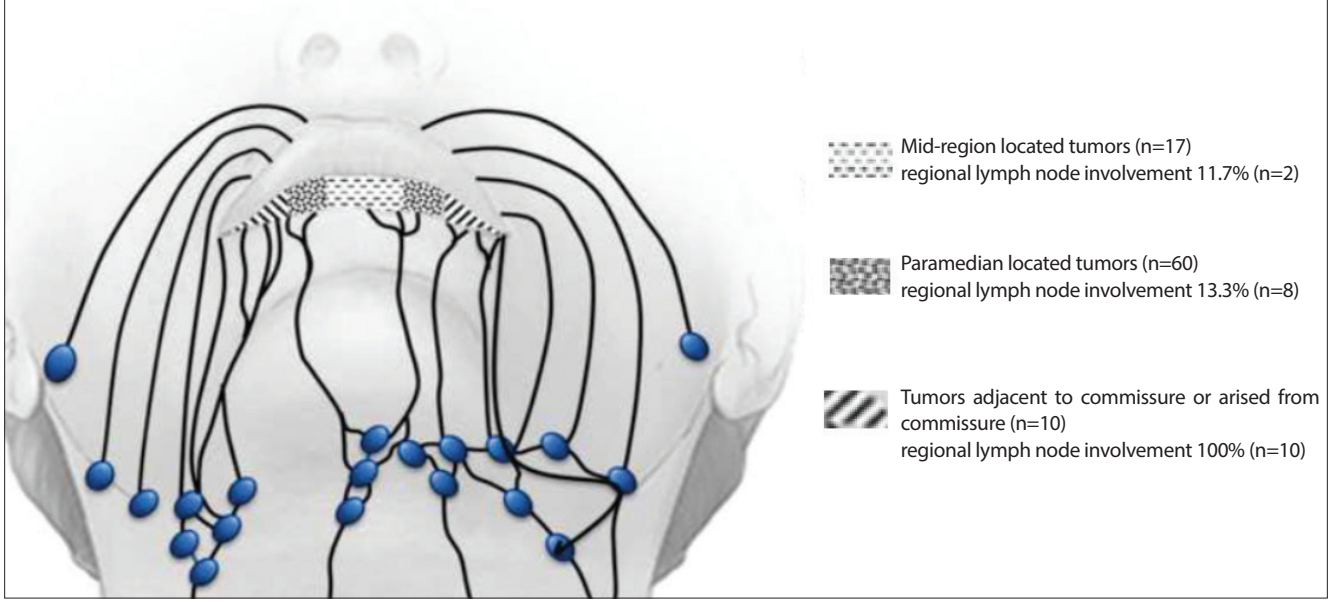


Table 2. Correlation between parameters and recurrence and metastasis

Parameters	Recurrence	Metastasis
Age	NS	NS
Gender	NS	NS
Size	p<0.001	p=0.021
Onset time of disease	NS	p<0.001
The proximity of tumor to commissure	NS	p<0.001
Surgical margin positivity	p=0.017	p<0.001
Thickness (depth)	p=0.02	p<0.001
Tobacco	p<0.001	NS

NS: stands for not significant

the locations of the tumors were classified in 3 groups: median, paramedian, and adjacent to the commissure. The distributions of the localization of tumor were n=17 (19.6%), n=60 (68.9%) and n=10 (11.5%) respectively (Figure 1). The analysis of cases with neck metastasis showed that there is a significant relationship between lymph node involvement and tumor location (Figure 1). After surgical intervention, metastases to the regional cervical lymph nodes were detected in all of the 10 patients whose tumor had originated from the commissure or close to the commissure. The distribution of the TNM stage is presented in Table 1. There were 29 patients (33.3%) in stage T1, 38 patients (43.7%) in T2, and 17 patients (19.5%) in stage T3. In 3 patients (3.5%), the tumor was in stage T4. Lymph node involvement, as confirmed by fine needle aspiration biopsy, was detected in 12 (13.8%) patients by ultrasonography and physical examination pre-operatively. Histologic evidence revealed that the depth of tumor ranged from 2-10 mm, with the average depth in patients with regional lymph node metastasis being 6 mm (minimum=3 mm, maximum=10 mm). Pathological examination of neck dissection specimen revealed the fact that regional lymph node metastasis was present in 20 (22.9%) patients actually. In 8 patients, there was no detectable clinical lymph node involvement pre-operatively, but after pathological examination, regional lymph node metastasis was found, therefore, the occult metastasis rate was 9.1%. No distant metastasis was identified and all patients were classified as stage M0. We identified a significant relationship between the tumor size at diagnosis and the regional lymph node involvement. Neck dissection, therapeutic in 12 patients and prophylactic in 75 patients, was performed on all patients. Considering the location of the tumor, either unilateral or bilateral neck dissection were performed. We performed unilateral neck dissection in 81 patients with tumors located at the commissure or close to the commissure. In cases where the tumor was located at the midline of the lip and had spread to the contralateral side or had invaded both sides, we performed bilateral neck dissection. Patients having cervical metastases with extracapsular extension and soft tissue invasion were referred to post-operative radiotherapy. Our follow-up policy stated that patients were to report for examination each month for the first 3 months, then every 3 months for the 1st year, and finally, 2 times per year following the 1st year.

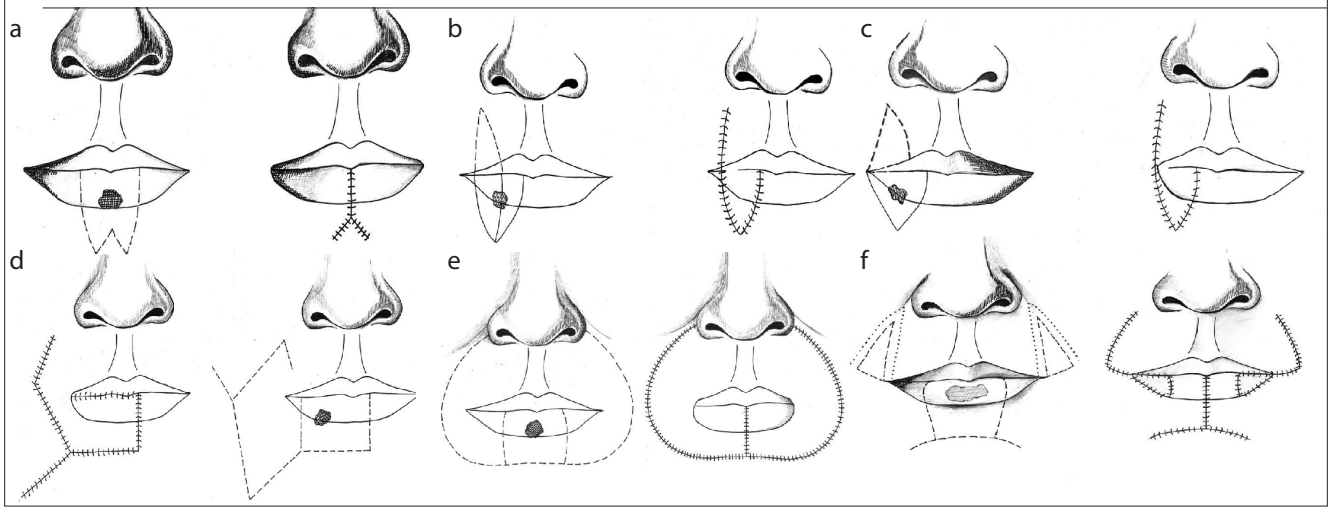
Statistical analyses revealed that parameters such as size and depth of the tumor, time from onset of disease to diagnosis, surgical margin status, and tumor location had a significant effect on the regional recurrence of the tumor and the cervical lymph node involvement (Table 2). Time from the onset of disease to the diagnosis is an another important predictive factor influencing the regional lymph node involvement, which we observed in 20 patients with lymph node metastasis, where the average time from the onset of the disease to the diagnosis was 32 months (ranging from 18-48 months). In 67 patients without lymph node metastases, the average time from the onset of disease to the diagnosis was 6 months (ranging from 3-18 months). For this reason, disregarding the chronic lesions at the lower lip may lead to neck metastasis if they are malignant in nature.

A recurrent case was defined as the growth of tumor at the same location after completion of primary treatment. Local recurrence occurred in 8 (9.1%) patients after a mean follow-up duration of 16 months (ranging from 8-24 months). All the recurrent cases were treated by surgical re-excision, after which surgical intervention adjuvant radiotherapy was advised and these patients were followed-up at more frequent intervals. Various surgical techniques were identified for the reconstruction of lip after tumor excision. For 36 patients (41.3%) with defects spanning less than one-third length of the lower lip, we preferred a U, V, or W- shaped excision and primary suturing for reconstruction. The remaining 51 (58.7%) patients' defects were reconstructed using the Gilles Fan flap, Estlander flap, Abbe flap, Bernard flap, and Karapandzic flap techniques according to the tumor location and defect size (Figure 2). The median follow-up time was 23 months (minimum 12 months, maximum 72 months). Unfortunately, during our follow-up period, 3 patients died, primarily due to lower lip cancer. All 3 patients were living in rural areas, therefore, they neglected to report regular check-ups and disregarded local recurrence of disease and regional lymph node involvement. The overall survival rate of patients with lower lip carcinoma who underwent neck dissection and excision of tumor was 96.5% (84 of the 87 patients).

DISCUSSION

Lip cancer is the common form of malignant oral cavity neoplasms and the frequently involved site in most cases was the lower lip. Moreover, in some studies, it has been advocated that lip cancer is the second most frequent skin cancer in the head and neck region (1, 2). Frequent involvement of the lower lip is attributed to excessive exposure to sunlight, since the lower lip receives considerably more direct sunlight than the upper lip. Solar radiation and smoking are well-known risk factors for the development of lip cancer (3). The gender distribution in our study revealed a considerable tendency toward men, which is consistent with the literature (4). Various treatment options have been defined for the treatment of SCC of the lower lip,

Figure 2. a-f. Lower lip reconstruction techniques. (a) Wedge resection and primary closure for small tumors of the lower lower lip. (b) Abbe flap for laterally located tumors of the lower lower lip. (c) Estlander flap for tumors Involving oral commissure. (d) Gilles Fan flap for midline located tumors of the lower lower lip. (e) Karapandzic flap for midline located large tumors of the lower lower lip. (f) Bernard-Burrow flap for midline located large tumors of the lower lower lip



including surgery, radiotherapy, chemotherapy, and some combinations of these. Among these options, we preferred surgery that consisted of tumor excision, therapeutic or prophylactic neck dissection, and reconstruction of defects in all patients. We aimed for complete removal of the tumor with a safe surgical margin, and consequently, the surgical treatment involved excision of full-thickness skin, mucosa, and underlying muscle. Adequate resection of the tumor and maintaining a safe surgical margin are contentious issues, since there is no consensus on them. A study advocated that a 3 mm distance seems to be adequate for maintaining a safe surgical margin after excision of early SCC (T 1/T 2) of the lower lip, when a frozen section test was performed for the margins. If a frozen section is not available, at least a 6 mm distant excision to the tumor is recommended (5). Another study has suggested that excision of the tumor should be done at least 6 mm away from the healthy tissue to ensure the maintenance of a safe surgical margin in the surgical treatment of primary squamous cell carcinomas (6). In another study, it was emphasized that the excision should be performed at least 10 mm away from the tumor tissue in order to provide a safe surgical margin (7). In our study, we obtained frozen section for all the cases and resected normal tissue with the tumor to obtain safe surgical margins ranging from 6-10 mm according to the tumor size, depth, and location. We performed additional excisions as long as the frozen test was positive for disease. Nevertheless, even though additional excisions were performed according to frozen section reports, the histologic examination revealed the presence of tumors that were more than 2 mm closer to surgical margins in 9 patients. In various studies, the rate of regional lymph node metastases was reported between 3% to 29% (1, 4). In this study, the lymph node involvement was detected in 12 patients (13.8%) and the initial diagnosis that was made by ultrasonography and physical examination was also confirmed by fine needle aspiration biopsy. After surgery, the pathological examination of the dissected neck specimen revealed that regional lymph node metastasis was present in 20 patients (22.9%). Therefore, in 8 (9.1%) patients with a clinically N0 neck, occult lymph node metastasis was present at diagnosis. Eight patients with N0 stage with occult metastasis were in T1 (n=1) and T2 (n=7) stages. Neck metastases were detected and treated at an early stage via prophylactic neck dissection in 8 of 87 patients (9.1%). A value of 9.1% is neither too high nor too low as an occult metastasis rate. Authors have reported a poor prognosis for cases that demonstrate nodal involvement during the follow-up period, and for this reason, the surgical approach in clinically N0 patients remains a controversial issue (8). Some other authors, who have adopted the "wait-and-see" policy for N0 necks, advocated that if nodal involvement occurs during the follow-up period, the therapeutic neck dissection combined with radiotherapy should be applied (1). Some studies have supported the use of aggressive surgery, who claimed that a prophylactic neck dissection should be performed in all clinical N0 cases (9-11). Recently, according to some authors, lymphatic mapping and sentinel lymph node biopsy were recommended for deciding whether neck dissection should be performed or not (12). Furthermore, considering that regional metastasis directly affected the patients' survival, it will be a reasonable approach to perform prophylactic neck dissection on patients with lower lip cancer. Based on our experience, the prognosis is poor despite salvage surgery and radiotherapy if there is cervical lymph node metastasis when no prophylactic neck dissection is performed.

Therefore, our surgical approach to lower lip SCC includes excision of the tumor with safe surgical margins confirmed by frozen sections and performing a prophylactic or therapeutic neck dissection regarding the N stage of the neck. The

size, stage, depth, and high histologic grade are found to be related with higher regional lymph node metastasis rates in certain studies (12, 13). On the contrary, some studies claim that the tumor size does not correlate closely with lymph node involvement (14, 15). In our study, we stated that particular factors such as size and depth of tumor, time from onset of disease to diagnosis, surgical margin status, and lesion location has a significant effect on recurrence and regional lymph node involvement (Table 2). We could not find any significant relationship between tobacco usage and regional lymph node metastasis, however, all the recurrent cases were found to be smoking more than one packet cigarette per day. As shown in Figure 1, we found an increase in the rate of regional lymph node metastasis in cases where the tumor was located close to the commissure.

We preferred reconstruction techniques for lip defects after surgery, depending on tumor size, location, and excision width. According to a recent study, combined surgical approaches including Karapandzic, Abbe, Estlander, and Stein flaps may offer excellent aesthetic and functional outcomes in subtotal and total lower lip defects and should be noted as a reliable reconstructive treatment of choice in patients having more than 70% of lower lip defects (16).

Aesthetic and functional expectations should be considered while removing the cancerous tissue, however, the risk of local recurrence and metastasis should not be ignored. Frequent and regular follow-up after surgical treatment is essential for decreasing mortality and morbidity.

Lastly, electrochemotherapy (ECT) is an innovative therapeutic modality based on the application of electrical pulses to the lesion site that increases the permeability of cell membranes to raise the uptake of chemotherapeutic agents, thus enhancing their cytotoxic effects. Additionally, ECT offers a therapeutic treatment option for aged and frail patients who, due to their delicate health condition, are either ill-suited for or refuse surgical interventions (17).

CONCLUSION

We have reported a retrospective study consisting of 87 patients who were treated by excision of tumor and neck dissection in all cases. The tumor size, depth, time from onset of disease to diagnosis, and the location are found to be associated with the recurrences and regional lymph node involvement. According to our experiences and the results of our study, we strongly recommend that prophylactic neck dissection should be performed, not only for accurate clinical staging, but also for treating occult metastases if present. Therefore, our clinical approach is to perform prophylactic neck dissection for lower lip cancer, even if the patient is clinically 0 nodal and involvement-free. Early diagnosis and immediate surgical intervention contribute to decreased morbidity and mortality. Therefore, dentists and general practitioners must remain vigilant about persistent lesions, and suspected patients should be referred to ENT specialists without losing time.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (263/2018).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.G., S.U.; Design - S.G.; Supervision - S.G., S.U.; Data Collection and/or Processing - S.G., S.U.; Literature Search - S.G.; Writing Manuscript - S.G., S.U.; Critical Review - S.G.

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Original Research

Radioprotective Effect of Caffeic Acid Phenethyl Ester on the Brain Tissue in Rats Who Underwent Total-Head Irradiation

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ABSTRACT

Objective: In this study, we evaluated whether caffeic acid phenethyl ester (CAPE) has a radioprotective effect on the damage in the rat brain tissue induced by gamma radiation, considering that it may inhibit the ionizing radiation damage.

Methods: A total of 36 Sprague–Dawley rats were divided into four groups to test the radioprotective effect of CAPE administered by intraperitoneal injection. An appropriate control group was also studied. On day 11, the brain tissue of all rats was removed and homogenized in phosphate buffer, and the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), paraoxonase (PON), arylesterase (ARE), ceruloplasmin (CER), lipid hydroperoxide (LOOH), and total-SH parameters were measured to determine if CAPE had a protective effect.

Results: The ARE and PON activity and the total-SH level were statistically increased compared to the IR group, whereas the LOOH, TOS, and OSI levels were significantly decreased.

Conclusion: The data obtained in the study suggest that the CAPE administration prior to irradiation may prevent the irradiation brain damage.

Keywords: Antioxidant enzymes, brain, caffeic acid phenethyl ester, irradiation, oxidative stress

INTRODUCTION

Brain tumors are recognized to be among the most malign tumors. According to the World Health Organization's (WHO) report, being the fourth-degree diffuse-type tumors, they have a high rate of mortality (1, 2). However, current treatment approaches are very limited, consisting of radical surgery, radiotherapy, and chemotherapy. Tumoral gliomas can diffuse throughout the brain by infiltrating into the lymphatic drainage system. Being effective in the brain, radiotherapy is preferred for diffuse tumors such gliomas. Nevertheless, the selective permeability of the blood–brain barrier prevents chemotherapeutic agents from substantially accessing tumoral cells that are diffuse throughout the brain (3, 4).

Radiotherapy, as one of the most frequent treatment methods, can be used in all types of cancer. Radiotherapy is preferred in 2 out of 3 patients who apply to clinics. In addition, the radiotherapy dose required to establish control over cancer cells is higher than usual, and that is why when applied, it causes damage in normal tissues (5). As the degree of damage in normal tissues caused by radiotherapy changes depending on the tissue radio-sensitiveness, it was shown in studies that there is a risk up to 8 times higher when the whole-body irradiation is used. When radiotherapy is applied to tumors located within the head and neck, brain, or eye, it can be observed that there are destructive effects on other nearby tissues, which depend on radiotherapy. It is known that reactive oxygen species (ROS) are accumulated and that DNA fragments are generated which cause these destructive effects following radiotherapy. Leading to the endoplasmic reticulum and mitochondrial membrane damage, accumulated ROS increase the damage to surrounding tissues, which depends on radiotherapy. It is pointed out in the literature that ROS and reactive nitrogen species (RNS) play a role in the pathogenesis of many diseases (6-9). Furthermore, it has been

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demonstrated in many studies that free radicals formed due to radiation infest other tissues and organs through systemic circulation (10, 11). In addition to the known antioxidant properties of caffeic acid phenethyl ester (CAPE), data on the radiation-protective ability of this agent are limited (6, 12). We hypothesized that CAPE, which antioxidant effects have been proven in many studies, could protect the brain tissue from radiation-induced oxidative damage. For this reason, we measured the oxidative biomarkers, total oxidant status (TOS), oxidative stress index (OSI), lipid hydroperoxide (LOOH), and antioxidant biomarkers, total antioxidant status (TAS), paraoxonase (PON), arylesterase (ARE), ceruloplasmin (CER), and total-SH in the brain tissue of rats with or without gamma radiation exposure to total cranium with a single dose of 5 Gray (Gy).

METHODS

This study is conducted using 36 male Wistar Albino rats weighing 200±20 gr. The rats were divided into four groups as the control (n=8), sham control (n=8), irradiation (IR) (n=10), and IR+CAPE group (n=10). Prior to total cranium irradiation, all rats except the sham control group were anesthetized with 80 mg/kg ketamine hydrochloride (Pfizer İlaç, İstanbul, Turkey) and placed on a tray in the prone position. The rats in the IR and the IR+CAPE groups received irradiation via 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, with the total cranium gamma irradiation being a single dose of 5 Gy, while the rats in the control and sham control groups received sham irradiation. Ten days after irradiation, all animals were killed by decapitation, and their brain tissues were removed and homogenized within a phosphate buffer (a single volume of the tissue sample and nine volumes of phosphate buffer as cold as ice). In the aftermath of this homogenization procedure, the obtained supernatant was put in five eppendorf tubes and kept at −80°C until biochemical assessment for protection from deformations. This study spectrophotometrically analyzed biochemical parameters like TAS, TOS, OSI, LOOH, PON, ARE, CER, and total SH, which have been chosen for assessing CAPE's antioxidant effectiveness.

The information regarding the groups can be found as following: Group 1: Sham control group (SCG): No drug application and/or surgical intervention was conducted in this control group.

Group 2: Control group (CG): As this group is the positive control of the fourth group, rats in this group were injected intraperitoneally (IP) with 0.25 ml dimethyl sulphoxide (DMSO) for 10 days.

Group 3: IR group: The head areas of the rats from this group were applied a single dose of 5 Gy on the 1st day. Thirty minutes prior to and throughout 10 days following this application, rats were given physiological saline solution IP.

Group 4: IR+CAPE group: The rats within this group were given a single dose of 5 Gy on the 1st day. Thirty minutes prior to and throughout 10 days following this application, subjects were applied IP 10 µmol/kg/day CAPE, which was thawed in DMSO. Rats in all groups were fed with regular food and water. At the end of the experiment, animals were decapitated being, which was identical to the protocol carried out in the first group, and advanced biochemical analyses were made following the removal of the brain tissue. CAPE was dissolved in DSMO immediately before the application. The final concentration of DMSO was 0.1%.

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 (2017/2).

Measurement of Antioxidant Parameters

Measurement of total antioxidant status

The method of measuring the TAS levels was as follows: This molecule gets decolorized as all antioxidant molecules reduce the ABTS cationic radical. The degree of decolorization is proportional to the total concentration of antioxidant molecules (13). During this procedure, Trolox, that is a water-soluble analogue of Vitamin E, was used as a calibrator. Results were presented as mmol Trolox equivalent/gr protein.

Measurement of Total SH

The level of total-SH groups in samples was measured according to Ellman's method (14). Results were presented as mmol/gr protein.

Measurement of Paraoxonase Enzyme Activity

Attached to paraoxonase HDL-cholesterol, lipophilic is a hydrophobic antioxidant enzyme. This enzyme's activity was measured by using a kit of Real Assay. In short, in this method, the PON enzyme hydrolyses the paraoxon substrate (O, O-diethyl-O-pnitrophenylphosphate) by reacting with it. Colored p-nitrophenol is a product of this hydrolyzing procedure. Monitored in the kinetic mode at 412 nm, this product's absorbance is expressed as U/g protein (15).

Measurement of Arylesterase Activity

The ARE activity e is measured with the Real Assay commercial kit. In this test, the enzyme contained by the sample that is to be measured triggers an enzymatic reaction with phenylasetat substrate, and hence creates phenol. The obtained phenol is measured colorimetrically, and the activity is thus determined (16). Results are presented as U/g protein.

Measurement of Ceruloplasmin Level

The ceruloplasmin level is determined according to the method suggested by Erel (17). Being a colorimetric method, it measures enzymatic oxidation of the ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Results are presented as U/gr protein.

Measurement of Oxidant Parameters

Measurement of lipid hydroperoxide level

The level of lipid hydroperoxide was measured by using the modified FOX2 assay method (18). In this method, ferrous ions that are in the reaction medium are oxidized to ferric ions by lipid hydroperoxides. The generated ferric ion chromogens form a complex molecule with xylenol orange and ferric ion and the absorbance of this formed colored molecule is measured at 560

Table 1. TAS, TOS, OSI, LOOH, PON, ARE, SER, and Total SH values

	TAS	TOS	OSI	LOOH	PON	ARE	CER	Total SH
Sham control group	0.36±0.078	25.33±2.76[e]	7.92±2.47[a]	1.31±0.076[b]	0.571±0.098[b]	10.30±0.468	56.91±3.32	0.0669±0.003
Control group	0.36±.078	28.16±3.69[e]	7.42±2.43[a]	1.27±0.043[c]	0.921±0.285[e]	10.59±0.697[a]	57.28±3.99	0.0666±0.002
IR group	0.32±0.103	35.16±3.84	11.97±3.54	1.46±0.093	0.351±0.060	9.48±0.745	53.37±7.48	0.0606±0.008
IR+CAPE group	0.38±0.043	27.39±2.35[e]	7.24±1.14[a]	1.17±0.169[e]	0.551±0.061[d]	10.68±0.657[a]	58.61±5.77	0.0710±0.005[a]

[a]: p<0.05; [b]: p<0.01; [c]: p<0.001; [d]: p<0.005; [e]: p<0.0001 vs. IR group

TAS: mmol Trolox equivalent/ gr protein; TOS: mmol/gr protein; OSI: arbitrary unit; LOOH: μmol/gr protein; PON: U/g protein; ARE: U/g protein; CER: U/ gr protein; Total SH: mmol/gr protein

nm in the endpoint mode. It is t-butyl hydroperoxide standard, which is freshly prepared as a calibrator used for this measurement. Results are presented as μmol/gr protein.

Measurement of total oxidant status level

While measuring TOS levels of samples, an assessment was made on the color change due to the oxidization of ferrous ion to ferric ions by oxidant molecules that samples contain. This is a method of colorimetric TOS measurement, which has been previously acknowledged within the scholarly literature (19). Results were expressed as μmol H_2O_2 equivalent/gr protein.

Calculation of Oxidative Stress Index

First, TOS and TAS units were calculated as μmol for the OSI calculation of samples. Then, OSI was calculated according to OSI (AU)=[(TOS μmol/L)/(TAS μmol/L)]x100 formula.

Measurement of Protein

It was the Bradford method that has been used during the protein designation conducted within this study (20). For standard curve, 25–300 μg series solutions that contain cattle serum albumin were prepared. Taken 0.1 mL from the prepared solution was added to 5 mL Coomassie Blue reactive dye. Five minutes after the mixture was made, its absorbance was measured at 595 nm. Calculations were made in accordance with the standard curve.

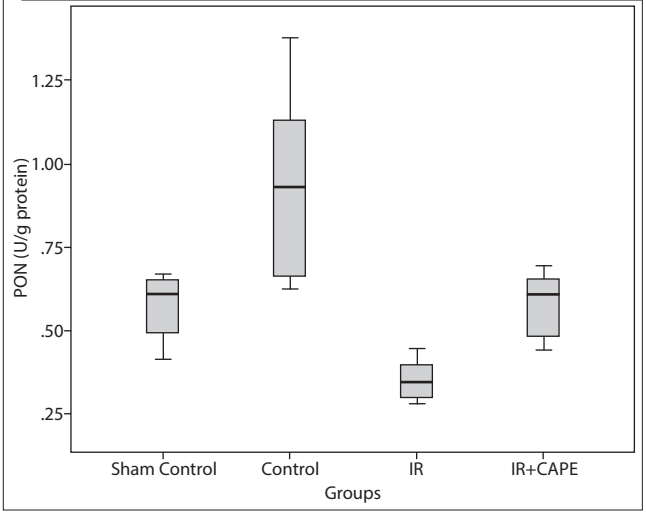
Statistical Analysis

The Kolmogorox–Smirnov test was used to check compliance with normal distribution. For comparing three independent group variables that have a normal distribution, analysis of variance and LSD multi-comparison tests were used. The interrelations between variables were tested using the Pearson correlation analysis. The frequency, percentage, and average of standard deviation values were given as introductory statistics. For statistical analyses, the SPSS for Windows version 22 (IBM Corp.; Armonk, NY, USA) package program was used, and a p-value ≤0.05 was accepted as statistically significant.

RESULTS

The TAS, TOS, OSI, LOOH, SER, total SH values, PON, and ARE activities of four groups that have been taken into account are summarized in Table 1 and Figures 1–6. When groups were assessed in terms of TAS levels, no significant change was observed in relation to control groups (p>0.05). However, when assessed in terms of TOS levels, there were statistically significant changes. A significant increase was observed when the TOS level of Group 3 (IR group) was compared with other groups (p<0.0001). It was detected that there were statistical differences in terms of OSI levels. The OSI level in Group 3 was found to be significantly increased in relation to Groups 1, 2, and 4 (p<0.05). Furthermore, there was no statistically significant difference observed between Groups 1, 2, and 4 (p>0.05).

Figure 1. Demonstration of the PON level distribution among groups by Box PLOT graph



It was observed that there were statistically significant differences when groups were assessed in terms of LOOH levels. It was noted that there was a significant increase in the LOOH level of Group 3 in comparison to Groups 1, 2, and 4 (p<0.01, p<0.001, p<0.0001, respectively). In a similar vein, it was noticed that there were significant differences between groups in terms of the PON activity. As there was a significant difference between the PON activity in Group 2 and the PON activity

Figure 2. Demonstration of the ARE level distribution among groups by Box PLOT graph

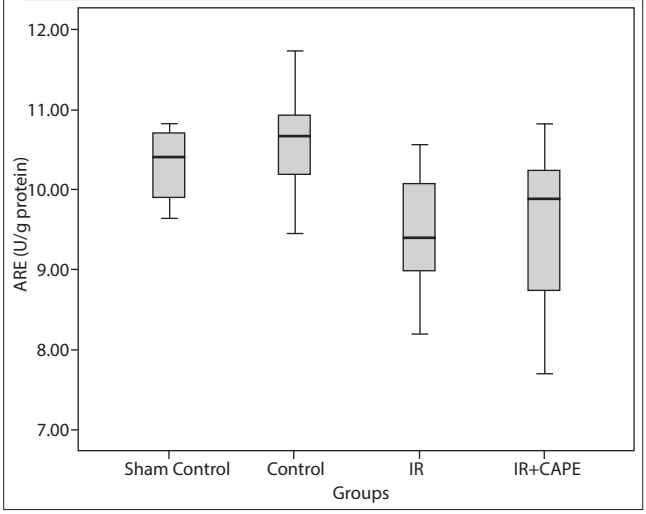


Figure 3. Demonstration of the TOS level distribution among groups by Box PLOT graph

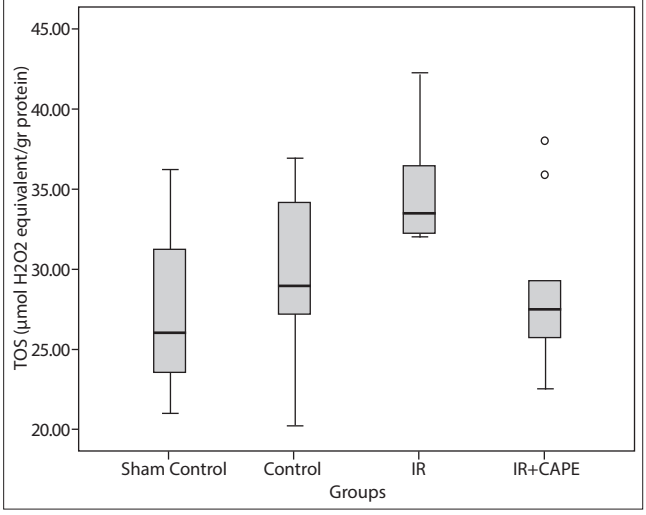


Figure 4. Demonstration of the LOOH level distribution among groups by Box PLOT graph

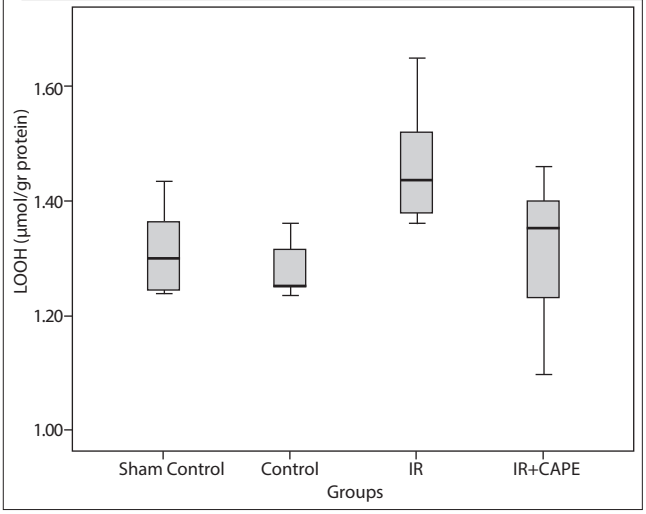
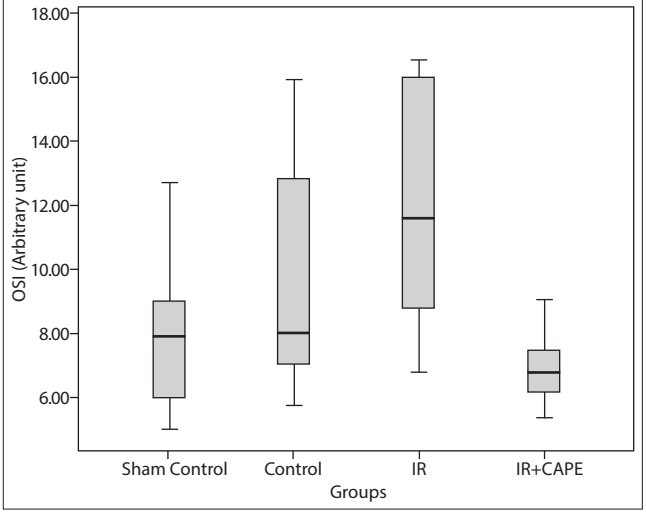


Figure 5. Demonstration of the OSI level distribution among groups by Box PLOT graph

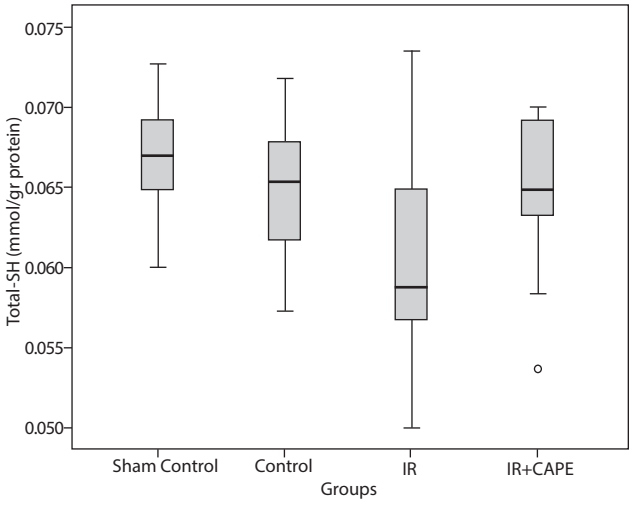


in Group 1, the value was higher in Group 2 ($p<0.05$). This increase in Group 2 was not only to be considered as a significant change in relation to Group 1, but also the others ($p<0.001$). Moreover, the PON activity in Group 3 decreased significantly in comparison with Groups 1, 2, and 4 ($p<0.01$, $p<0.0001$, $p<0.005$, respectively). By the time the total-SH assessment was made, it was observed that there were statistically significant differences between groups ($p<0.05$). The level of total SH in Group 4 was found to be significantly higher than in Group 3. Finally, when groups were compared in terms of the ARE activity and CER level, it was observed that there were statistically significant differences in the ARE activity, whereas there was no such difference in terms of the CER level. The ARE activity in Group 3 was found to be significantly low in comparison with that of Groups 2 and 4 ($p<0.05$). Yet, there were no significant changes detected between Groups 3 and 1 ($p>0.05$).

DISCUSSION

Ionized radiation, radiology in particular, is a risk factor for the personnel working in the radiotherapy and nuclear medicine departments. Such a risk originates from used radioactive materials such as the isotopes of radium, uranium, and thorium (21). Despite its detrimental effects, radiotherapy is one of the most significantly effective treatment modalities. Hence, approximately more than a half of patients with cancer is treated via radiotherapy. During the radiation treatment, an effective dose is to be determined to maximize toxicity upon cancer cells; however, this dose may also show toxic effects on healthy tissues that remain within the application area of radiotherapeutic treatment. Each tissue's sensitivity level is different. Therefore, the extent of damage on a tissue that has been subject to radiation depends on that tissue's sensitivity. These issues reflect the increasing importance of the need for research on the acute effects and af-

Figure 6. Demonstration of the total-SH level distribution among groups by Box PLOT graph



ter-effects of ionized radiation on tissues and cells (6, 12, 22, 23). In accordance, among the scholarly work focusing on relevant radiotherapy studies, the ones researching the oxidative stress occurrence, the consequent free radical generation, and furthermore the interaction of antioxidants with this oxidative mechanism bear vital importance.

It has been reported that accumulated within the cell, ROS damages its components and therefore pave the way for diseases (24, 25). One kind of radiotherapy cell damage is lipid peroxidation, thus the deformation of the epicyte. The failure of its structure and function causes an uncontrolled flow of free radicals coming in and going out of the cell, resulting in damage.

When the cell and the tissue are radiated, the oxidative damage begins within the cell. The degree of this damage changes depending on the balance between the cell's antioxidant defense system and the ROS levels. When this balance is altered in favor of ROS, the degree of damage within the cell increases (26, 27). Categorized by effect types, basic antioxidants within cells are divided into two main groups as enzymatic and non-enzymatic. Enzymatic antioxidants operate by activating enzymes within the cell. These, in short, can be outlined as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and glutathione reductase (GR). Non-enzymatic ones, on the other hand, take effect through inhibiting the ROS production. These can be exemplified as GSH, Vitamins C and E, melatonin, zinc, ginkgo biloba and carotenes (28-31).

In the central nervous system, the amount of endogenic antioxidants is relatively low compared to other tissues, and for this reason, nerve cells are more sensitive to oxidative damages that can potentially increase (32). As in all tissue types, there are ROS cleaner enzymes within the ones in brain. Most important among these are SOD and GSH-Px. SOD converts the superoxide radical ($O_2^{\bullet-}$) that is accumulated within the cell into hydrogen peroxide (H_2O_2). Being different enzymatic antioxidants, GSH-Px and CAT prevent the harm of H_2O_2 by converting it into H_2O and molecular oxygen (O_2).

Scholarly work within the literature reports that SOD may protect the cell from the damage caused by ROS accumulated within (33, 34). In addition, thanks to the available cell culture studies, it is known that SOD shows a protective effect against ROS caused by tumor necrosis factor, interleukin1, and ionized radiation within cells (35). Many studies examined the parameters that display the damage on the brain tissue caused by radiation (11, 36, 37). Among these, the research conducted by Kojima et al. (37) can be pointed out as one of the milestones within this field. In this study, mice of different age (1, 4, and 12 week old, and 1 year old) were subjected to whole-body radiotherapy, and the effect of this application on the lipid peroxidation within the mouse brain tissue was examined. In this approach, 1-week-old mice were considered to be equivalent to newborn humans, 4 week old to adolescents, 12 week old to adults, and 1 year old to the elderly. As a result, when compared to control groups, it was reported that there were no statistically significant changes in SOD, GSH-Px, and CAT activities and MDA levels in the brain tissue of adult (12-weeks-old) mice (37, 38).

Another important study is the one by Collins-Underwood et al. (39), where rats were subjected to cranial radiation therapy, and primary neuronal culture was shown. When cells within the culture reach a sufficient number, the NADPH oxidase activity that converts O_2 to $O_2^{\bullet-}$, an oxidative type, was studied, and as a result, a decrease was observed. When rats were given a NADPH oxidase inhibitor IP before irradiation to verify this finding, it was demonstrated that a ROS increase was substantially prevented within the cell induced with radiation. Moreover, Kojima et al. (37) show that irradiating the mouse brain with a low-dose (50 cGy) gamma ray induces endogenic antioxidant potential. In line with this finding, it is thought that low-dose irradiation can be used in the treatment of neurodegenerative diseases that are induced with ROS accumulated within nerve cells. This issue remains as a new research question.

There are some parameters found in the literature used to assess the oxidative level and lipid peroxidation in the brain tissue. Whereas the MDA level is used to assess lipid peroxidation, SOD, GSH-Px, CAT, and XO activities are acknowledged as valid parameters for the evaluation of oxidative damage. Accordingly, in our study, TAS, TOS, OSI, PON, ARE, CER, and SH parameters in the brain tissue are measured and assessed to detect whether there are any protective effects of CAPE, which has antitumoral and antiinflammatory and antioxidant effects, in averting the impact of locally applied radiotherapy on the oxidant–antioxidant system.

In their study with rabbits, Ilhan et al. (40) researched the protective effect of CAPE and methyl prednisolone on the irradiated spinal cord. The study assessed the spinal cord's post-irradiation MDA level, SOD and CAT activities, and histopathological changes. It is reported that MDA levels are significantly low in the group that was applied CAPE in comparison with the methyl prednisolone group, and compared with the control group, there was no observed tissue damage in the CAPE group. Con-

ducted by Yilmaz et al. (41), in another study, the MDA levels and SOD and CAT activities in the liver of rats with diabetes induced with streptozotocin were researched. As a result, it was reported that MDA levels increased in diabetic rats compared to the control group, and in the group injected with CAPE, it remained on the same level with the control group. Hence there was no significant change observed. Moreover, it is detected that CAPE reduces the SOD and CAT activities in these rats. This is because of CAPE's cleansing of ROS and pressure on the SOD and CAT activities.

To the best of our knowledge, there are no existing studies on TAS, TOS, OSI, PON, ARE, CER, LOOH, and SH parameters in the brain tissue of rats subjected to ionized radiation. In our study where these parameters were assessed in the case of rats subjected to total-head irradiation for the first time, it was observed that there was no significant difference on TAS levels among rat groups. In line with the existing literature, it has been verified that radiotherapy does not affect the anti-oxidative mechanism in the brain tissue. In addition, it has also been verified with a significant increase in the TOS level that radiotherapy generated oxidative damage in rats.

One of the most important outcomes of the ROS damage on tissues is lipid peroxidation. In the recent years, lipid peroxidation has been emphasized as an important topic. LOOHs that emerge at the chain stage of lipid peroxidation are weak outputs, and they form aldehydes, ketones, carboxylic acids, alkanes, alkenes, and various polymerization products as breaks and dissolutions occur on the chain. As a result of this reaction, products such as MDA emerge and determine the degree of peroxidation (42). When assessing our working groups in terms of LOOH levels, a significant difference was detected between Group 4 injected with CAPE and Group 3 subjected to radiotherapy ($p<0.05$). The LOOH level in Group 4 was lower. This finding indicates that CAPE plays a protective role against oxidative damage on rats induced by radiotherapy. The MDA levels increase in Group 3 and a significant decrease in the CAPE+R group supports the claim of CAPE's antioxidant properties.

Being a serum enzyme, PON is related to HDL, and in addition, it was reported that it has an antioxidant function (43). Although PON and ARE activities are considered to be two distinct enzymes within the scholarly literature, advanced molecular studies suggest that the PON enzyme in the human serum shows both the ARE and PON activity (44). These two enzymes are components of the antioxidant enzymatic system that plays a role against oxidant accumulation. Therefore, when oxidative damage increases, to put another way, the balance is altered toward ROS, and it is expected that there will be a decrease in PON and ARE activities. In our study, we aimed to determine whether CAPE had a protective effect by assessing whether there was any significant increase in the IR+CAPE group in relation to IR group. In this sense, examining Figure 4, it can be observed that the PON activity in Group 3 decreased significantly in comparison with its control group (Group 1). Although a dramatic increase was observed in the IR+CAPE group contrary to expectations, the difference between the IR+CAPE and IR groups is statistically significant. Moreover, when groups are evaluated in terms of the ARE activity, whereas the ARE activity in the IR group decreased significantly in relation to control groups, the ARE activity in the IR+CAPE group is found to be significantly high in comparison with the IR group. The ARE finding indicates that CAPE is protective against ROS induced by radiotherapy.

Finally, this study assessed CER and total SH levels for examining whether CAPE is protective against ROS accumulation induced by total-head irradiation. Being an important antioxidant, CER resembles SOD in terms of its mechanism of action. In sum, its most important physiological task within the organism is to transform the ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}). In doing so, it prevents the hydroxyl radical ($OH^{\cdot}$) generation through halting the Fenton reaction within the cell (45, 46). Hence, the decrease in the CER level increases the free $OH^{\cdot}$ oscillation. In this study, whereas there is no significant difference between groups in terms of CER, the total SH level in the IR+CAPE group was found to be significantly higher than in the IR group ($p<0.05$).

Many agents that decrease the cellular toxicity of ionized radiation within the normal tissue have been used before the application in organs such as the brain, heart, bladder, kidney, etc. to prevent early and late complications caused by ionized radiation (47). In these studies, it was reported that enzymatic and non-enzymatic antioxidants decreased as a result of overproduction of free radicals within the brain tissue, linked to ionized radiation. For this reason, studies suggest either a treatment aiming to raise the antioxidant enzyme activity of the tissues subjected to ionized radiation or using agents that increase the antioxidant enzyme activity to avert the rise of oxidative stress seen in patients receiving radiotherapy (48).

There are many studies conducted with a variety of antioxidant materials believed to have preventive or reducing effects regarding the tissue or organ damage in radiotherapy. The preventive role of CAPE on the development of tissue and organ damage might be inferred because of the antioxidant effect.

One of the major limitations of this study is the lack of histological evaluation. Although biochemical analyses suggest that CAPE exhibits radioprotective effects against oxidative damage in the brain tissue of irradiated rats, it may be reasonable to support these data with histological evaluations. Moreover, radioprotectors are ideally expected to have selectivity for normal tissues, but not for tumor tissues from the effect of radiotherapy. However, the study does not provide any data for such comparison with CAPE. This is another limitation of our study.

CONCLUSION

By reducing the formation of TOS, LOOH, and OSI, oxidant stress parameters, and increasing the ARE and PON activity, total-SH levels, and antioxidant parameters, CAPE reduces irradiation-induced oxidative stress in the rat brain tissue. Since free radicals are the major mediators for radiation-induced damage, a treatment combining radiation with an antioxidant might provide a strategy for preventing radiation injury to normal tissues.

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

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

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Original Research

Shear Bond Strength of Aged Composite Restorations Repaired with a Universal Injectable Composite

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ABSTRACT

Objective: The purpose of this study was to analyze the shear bond strength of a universal injectable composite used in the repair of aged composites.

Methods: A total of 100 disk-shaped specimens (8 mm×2 mm) were produced using five different composites (n=20) (Gradia Direct Posterior, Tetric N Ceram BulkFill, Filtek Z250, SonicFill and Filtek BulkFill Posterior). Specimens were polymerized using an LED light curing unit for 20 s and stored at 37ºC in distilled water for 3 weeks. Specimens were subdivided into two groups per composite for repair using either the same composite used for the specimen or G-aenial Universal Flo. Following acid-etching and silane application, a universal adhesive (G-Premio BOND) was applied and light-cured. The repair materials were placed on the bonded surfaces of the specimens and polymerized in silicone molds (2 mm×2 mm). After thermocycling to simulate aging, shear bond strength (SBS) was tested using a universal testing machine at a crosshead speed of 1 mm/min. Failure modes were examined using a stereomicroscope at ×40 magnification.

Results: No statistically significant differences were found among the tested composites repaired with their own substrates. However, the SBS SonicFill and Filtek Bulk Fill Posterior groups had significantly lower bond strengths when repaired with G-aenial Universal Flo in comparison to repairs made with their own substrates ($p<0.05$).

Conclusion: When repaired with their own substrates, reliable bond strengths were obtained for all the composites tested.

Keywords: Bulk fill composites, dental materials, shear bond strength, repair

INTRODUCTION

Over the last decade, dental resin based composites have risen in popularity in response to growing needs of patients (1, 2). Dynamic changes in pH and temperature in the oral cavity caused by saliva, diet, and aging result in degradation of resin composites (3). Despite recent improvements in material performance, clinical problems such as fractures, micro leakage, chipping, discoloration, wear, and other restoration defects may occur (4). When esthetics is compromised, the clinician must replace or repair the restoration using one of the various alternatives available. In the past, replacement was the only option available; however, it resulted in an undesirable loss of dental structure and extension of the cavity (5). In line with the concept of minimally invasive dentistry, several clinical studies have reported that the more conservative option restoration repair is able to increase the restoration longevity, while preserving dental structures and reducing operative trauma (4, 6).

An important factor influencing the repair success is the interfacial bond between the old and new composite resins (7). In clinical practice, the presence of an oxygen inhibition layer maintains the bond between the two layers of composite (4, 6). Various chemical and micromechanical methods such as mechanical roughening, etching with hydrofluoric or phosphoric acid, air abrasion, resin coating, and silanization may be used, either alone or in combination, to improve bonding between old and new resin composites (4). Studies have reported repair strengths ranging from 25% to 82% of the composite substrate shear bond strength (SBS) values (7).

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While there are many data available on the SBS of conventional composites, few studies have examined the SBS of bulk-fill composites, which were more recently introduced into clinical use to facilitate the application process. In comparison to conventional composites, bulk-fill composites can be applied in deeper layers, and studies have demonstrated an adequate polymerization of layers up to 4 mm in thickness (8). Various strategies have been applied to increase the depth of polymerization of bulk-fill composites, including adding a non-camphorquinone initiator and increasing translucency by changing the filler size, concentration and refractive index (9).

G-aenial Universal Flo (GC Corporation, Tokyo, JAPAN) is a high-fill injectable composite that has recently come into clinical use. Due to its high viscosity and improved mechanical properties, it is similar to conventional composites (10). According to the manufacturer, G-aenial Universal Flo consists of a revised formulation of strontium glass which filler particles have been reduced in size to 200 nanometers. The application of silane to the nano-sized glass surface enhances the adhesion between the glass particles and the resin matrix to provide greater durability and hydrolytic stability (11).

The aim of this in vitro study was to analyze the shear bond strengths of composites used in the posterior region when repaired using their own substrates or with G-aenial Universal Flo. The null hypothesis tested was that there would be no difference in the shear bond strength of composites to their own substrates and to an injectable universal composite.

METHODS

The compositions of the materials and manufacturer details are listed in Table 1.

Sample Preparation

Using a Teflon mold (8 mm×2 mm), 20 disk-shaped specimens were created from five different resin composites (Filtek Bulk Fill Posterior, Filtek Z250, Gradia Direct Posterior, SonicFill, and Tetric N-Ceram Bulk Fill), for a total of 100 specimens. The resin composite was condensed with a filling instrument and covered with a Mylar strip and pressed with glass coverslips to create a smooth surface. Polymerization was performed with a third-generation light curing unit (VALO; Ultradent, Utah, USA) for 20 seconds in standard mode. The light intensity was periodically checked by a radiometer (LED Radiometer, SDI, Australia) after the processing of every five specimens, and it was verified to be higher than 1000 mW/cm^2.

Specimens were removed from the molds, roughened with 600 and 1,200 grit silicon carbide paper, and then cleaned with an ultrasonic device for 10 minutes. Similar to the previous studies, aging was simulated by storing all samples in distilled water at 37°C for 3 weeks (12-14). Prior to the repair procedure, as stated in some studies, the samples were etched with 37% phosphoric

Table 1. Chemical compositions and manufacturers of the tested composites

Material	Resin matrix	Fillers	wt.% /vol. %	Manufacturer
Filtek Z-250	Bis-GMA, Bis-EMA, UDMA, TEGDMA	Zirconia/silica	82/60	3M ESPE (St Paul, MN, USA)
G-aenial universal flo	UDMA, Bis-MEPP, TEGDMA	Silicon dioxide, strontium glass	69/50	GC Corp. (Tokyo, Japan)
Gradia direct posterior	UDMA co-monomer matrix	Silica, prepolymerized fillers, fluoroalumino-silicate glass	80/-	GC Corp. (Tokyo, Japan)
Tetric N-ceram bulk fill	Modified , Bis-GMA, UDMA, Bis-EMA	Barium, ytterbium, spherical mixed oxide, prepolymer fillers	79-81/60-61	Ivoclar Vivadent AG, Schaan, Lichtenstein
Filtek TM bulk fill posterior restorative	AUDMA, UDMA, DDDMA	Silica, zircon, YbF_3	76.5/58.4	3M ESPE (St Paul, MN, USA)
SonicFill TM	Bis-GMA, TEGDMA, Modified Bis-EMA	SiO_2, glass, oxide	83.5/66	Kerr (Orange, CA, USA)
G-premio bond	Main Components: MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiators, silica			GC Corp. (Tokyo, Japan)
GC ceramic primer-II	Main Components: Silane, phosphate monomer, methacrylate, ethanol			GC Corp. (Tokyo, Japan)
Bisco select HV etch	35% phosphoric acid			BISCO Inc., Schaumburg, USA

All data were supplied by manufacturers

BIS-GMA: bisphenol A dimethacrylate; BIS-EMA: bisphenol A polyethylene glycol diether dimethacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethyleneglycol dimethacrylate; BIS-MEPP: 2,2-bis(4-methacryloxyethoxyphenyl) propane; DDDMA: 1,12-dodecane dimethacrylate; YbF_3: ytterbium trifluoride; SiO_2: silicon dioxide; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; 4-MET: 4-methacryloxyethyl trimellitic acid; MEPS: methacryloyloxalkyl thiophosphate methylmethacrylate

acid (Bisco Select HV etch, Schaumburg, USA) for 15 s, rinsed with water, and air-dried (15, 16). A silane coupling agent (GC Ceramic Primer II; GC Corporation, Tokyo, JAPAN) was then applied and air-dried for 10 s, and a universal adhesive (G-Premio Bond; GC Corporation, Tokyo, Japan) was applied in accordance with the manufacturers' recommendations and polymerized with the same LED unit (VALO) for 10 s in the standard mode.

Each group of composite specimens was then divided into two subgroups according to repair material (either their own substrate or G-aenial Universal Flo). A silicone mold (2 mm×2 mm) was placed over the composite sample, which was filled with the repair material and vertically photopolymerized for 10 s. Samples were then stored in distilled water at 37°C for 48 hours, and they were subjected to 500 thermocycles in water between 5°C and 55°C at a dwell time of 30 s, as stated in previous studies (17, 18).

Figure 1. The universal testing machine

Ethics committee approval was not taken due to in vitro design of the study. This study does not include human participants. Thus, no consent form was required.

Bond Strength Testing

Bond strengths of samples were tested using a universal testing machine (LRX Plus 6; Llyod Instruments, Leicester, UK) (Figure 1). Specimens were screwed to the lower compartment of the testing machine and subjected to a load of 5 kN at a 90° angle and a cross-head speed of 1 mm/min until fracture. The load required to dislodge each specimen was recorded in Newtons and then converted into megapascals (MPa) by dividing the fracture load (Newton) by the repair surface area. The failure type was identified using a stereomicroscope (Nikon SMZ 1500; Nikon Instruments Inc., Tokyo, Japan) at a magnification of ×40 and classified as adhesive failure (fracture between the composite and adhesive); cohesive failure (fracture within the composite); or mixed adhesive and cohesive failure (both composite and adhesive residue detected on the surface).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software, version 17.0 (SPSS Inc.; Chicago, IL, USA). The SBS means and standard deviations were calculated for all groups. Differences in the mean SBS between groups were compared by two-way analysis of variance and a post-hoc Tukey test, and differences in the failure mode distribution were identified by the Chi-square test, with the level of significance set at $p<0.05$.

RESULTS

The SBS values of tested materials are presented in Table 2. No statistically significant differences were found among the tested composite groups when repaired with their own substrates. However, when repaired with G-aenial Universal Flo, the Filtek Z250 group exhibited the highest SBS values (34.14±14.89), and the Sonic Fill group had the lowest SBS values (21.12±7.95). The difference between the two groups was statistically significant ($p<0.05$).

Table 2. Mean±standard deviation of microshear bond strength (MPa) of the study groups

	Composites' own substrates	G-aenial universal flo
n=10	Mean±standard deviation	Mean±standard deviation
Gradia direct posterior	$29.25^{a,A}$±6.91	$33.16^{a,AB}$±10.99
Tetric N-ceram bulk fill	$33.00^{a,A}$±13.81	$31.95^{a,AB}$±4.87
Filtek Z-250	$33.43^{a,A}$±9.62	$34.14^{a,A}$±14.89
SonicFill™	$31.52^{a,A}$±7.72	$21.12^{b,B}$±7.95
Filtek™ bulk fill posterior restorative	$41.01^{a,A}$±11.59	$29.73^{b,AB}$±6.58

*Different superscript lowercase letters in rows and uppercase letters in columns indicate statistically significant differences

Table 3. Distribution of failure modes for all experimental groups

	Failure mode					
	Composites' own substrate			G–aenial universal flo		
	Adhesive	Cohesive	Mixed	Adhesive	Cohesive	Mixed
Gradia direct posterior	5	4	1	3	5	2
Tetric N–ceram bulk fill	3	3	4	2	5	3
Filtek Z–250	3	5	2	5	5	0
SonicFill	9	1	0	10	0	0
Filtek bulk fill posterior restorative	5	2	3	2	5	3

Intragroup comparisons showed the mean SBS of Sonic Fill and Filtek Bulk Fill Posterior to decrease significantly when repaired with G-aenial Universal Flo as compared to their own substrates ($p<0.05$), whereas the bond repair material did not significantly affect the mean SBS values of Gradia Direct Posterior, Tetric N-Ceram Bulk Fill, or Filtek Z250.

The distribution of failure modes for all groups is shown in Table 3. Adhesive failures were more frequent in both the Sonic Fill group repaired with its own substrate and the Sonic Fill group repaired with G-aenial Universal Flo ($p<0.05$).

DISCUSSION

This study assessed bond strengths of new composites used in the posterior region when repaired with their own substrates and an injectable universal composite. Both Sonic Fill and Filtek Bulk Fill had mean bond strengths that were significantly lower when repaired with G-aenial Universal Flo as compared to their own substrates; thus, the null hypothesis that there would be no differences in the shear bond strength of the tested composites when repaired with their own substrates or with an injectable universal composite was partially rejected.

The repair of composite restorations is considered a conservative option that offers the advantages of increased durability and longevity, preservation of dental structure, faster treatment, and less strain on the patient during treatment (2). However, in addition to these advantages, repair entails the risk of weakening the restoration. Different studies have reported interfacial bond strengths ranging between 25% and 80% of the cohesive strength of the substrate materials (4, 19). Factors such as chemical differences between different resins used in repair process, surface treatment, and the length of time between initial restoration and repair have a significant effect on bond strength of repaired restorations (1, 4). Under clinical conditions, the type of composite used for the initial restoration is generally unknown to the operator performing the repair. For this reason, this study examined repairs made with the same material as the original composite substrate, as well as repairs made with a high-fill flowable composite (G-aenial Universal Flo).

Although the shear bond strength testing is frequently criticized for its nonhomogeneous stress distribution at the interface (20), it is still the most widely used method for evaluating the bonding effectiveness of restoration repairs (7, 20) because it is easy to prepare samples and implement the test protocol (5, 21) and because it imitates oral clinical conditions better than other methods (22, 23).

Flowable composites are widely used in clinical practice today. High-fill flowable composites are particularly recommended for posterior restorations. Kitasako et al. (10) reported the clinical performance of the high-fill flowable composite G-aenial Universal Flo used in the posterior region to be comparable to that of a conventional composite after 36 months.

Bulk-fill materials have grown in popularity due to their ease of application (24). The particular advantages offered by bulk-fill composites when used in the posterior, stress-bearing region make their mechanical properties especially important. However, there is little information available in the literature about their clinical performance and repair (25, 26).

Previous studies have shown that the original filling material has a greater effect on the bond strength than the repair material (27). As the composite ages, the number of free radicals within the resin structure that provide adhesion between the different composite layers decreases (28). Successive changes in temperature that occur in the oral environment also weaken the bond between the resin matrix and filler (5). Due to the differences in thermal expansion coefficients, the composite resin matrix and inorganic fillers are affected at different rates, resulting in weaker interfacial bond strength (2). Because the length of time and environmental conditions of clinical service also affect the outcome of composite repair, in vitro studies need to take these conditions into consideration (5).

The preferred methods for simulating aging and interfacial bond stressess are storage in water and thermal cycling (2, 29), which tests thermal stress caused by contact with liquid and temperature changes between 5°C- and 55°C. In this study, samples were stored at 37°C in distilled water for 3 weeks (4, 30) and thermocycled for 500 cycles to simulate thermal strain caused by the exposure to liquids and temperature changes.

Papacchini et al. (1) stated that higher composite-to-composite bond strength is obtained with a flowable resin, and thus a flow-

able resin is recommended for use as an intermediate agent in composite repair. For this reason, G-aenial Universal Flo, a highly filled flowable composite, was tested as a repair material in this study.

Repair of a composite resin restoration generally requires partial removal of both the restoration and adjacent enamel and dentin (7). In clinical practice, acid etching is performed to remove the smear layer and expose the filler and underlying surface, increasing the surface area so that the stress is distributed across the interface of the two bonded substrates (6). For this reason, phosphoric acid etching was performed to roughen the specimen surfaces.

In the present study, a silane solution was applied to specimens after surface treatments. Various studies have reported that treatment with silane improves surface wettability and promotes chemical bonding between the resin matrix and fillers (7, 20). The silane molecule contains both silanol, which bonds to the silica particles of the composite, and an organofunctional group, which bonds to the methacrylate of the bonding agent (6, 23).

Brosh et al. (31) have mentioned three important mechanisms of the repair process to achieve an ideal bonding between the old and new composite: (1) micromechanical bonding of the treated surface, (2) chemical bonding of the organic matrix, and (3) chemical bonding of the exposed filler particles. In the present study, given that the surface treatment of the specimens was standardized, differences in micromechanical bonding cannot explain the differences in the bond strength of tested composites; rather, the differences can be explained by differences in chemical bonding between the organic matrix and/or filler particles.

According to Baur and Ilie (2), the weak bond demonstrated by adhesive failure may be related to technical faults such as voids and porosities, the chemical structure of the adhesive system, and low wettability of the composite. These authors also state that the low repair bond strength of high-filled composites is due to their low wettability. The present study's finding that Sonic Fill, which contains the highest amount of inorganic fillers of all the composites tested, had a lower mean bond strength when repaired with G-aenial Universal Flo in line with this assertion. The lower repair bond strength values of both Sonic Fill and Filtek Bulk Fill when repaired with G-aenial Universal Flo may also be related to differences in the monomer composition of the composite and the repair material. Filtek Bulk Fill contains high-molecular-weight resin monomers such as AUDMA and additional fragmentation monomer, which could be responsible for the low- bond- strength values obtained when the Filtek Bulk Fill specimens were repaired with G-aenial Universal Flo. Conversely, the similarities in the bond strength of the Gradia Direct Posterior, Tetric N- Ceram Bulk Fill, and Filtek Z250 specimens repaired with their own substrates and those repaired with G-aenial Universal Flo may be due to the similarity in the monomer structures of the composite substrates and repair material. Despite the fact that previous studies have reported that superior interfacial coupling in bonding does not require similarities in chemical composition between the substrate and repair material (1), the fact that all the composites in the study had higher bond strengths when repaired with the same substrate as compared to repair with another material suggests that similarity in the composition of the resin matrix and inorganic fillers in the original and repair materials is important for achieving a strong bond. Future in vitro studies are needed to better understand how different monomers affect the repair bond strength of composites.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the substrate has a greater effect than the repair material on the bond strength between an aged composite restoration and its repair. While the use of homologous repair materials generally offers more reliable results in terms of bond strength, the fact that clinicians are seldom aware of the substrate material makes it difficult to predict the success of the repair process. However, based on the data obtained from our study, the use of a universal injectable composite is not recommended for the repair of Filtek Bulk Fill and Sonic Fill restorations.

Ethics Committee Approval: Ethics committee approval was not taken due to in vitro design of the study.

Informed Consent: This study does not include human participants. Thus, no consent form was required.

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Original Research

The Effects of Some Phytotherapeutic Plants on *Escherichia coli* spp. that are Exposed to Different Doses of Gamma Radiation

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ABSTRACT

Objective: The aim of the present study was to determine the antioxidant effects of phytotherapeutic plants on bacterial groups to which gamma radiation at different doses was applied. By this way, the effects of antioxidant ethanolic extracts of plants and the effect of gamma radiation on *Escherichia coli* spp. *(E. coli)* were investigated.

Methods: In the present study, *E. coli* spp. in the areas of agar and agar+plant extracts (83 µl) was irradiated by increasing gamma radiation (100, 200, 500, 1000, 3000, and 6000 cGy). In our study, six plants (carob (*Ceratonia siliqua* L.), basil (*Ocimum basilicum* L.), ginger (*Zingiber officinale*), rosemary (*Rosmarinus officinalis* L.), yarrow (*Achillea millefolium* L.), and cumin (*Cuminum cyminum* L.)) were found to be consumed by people, and their phytotherapeutic effects were investigated.

Results: In the present study, a decrease in the number of colonies of *E. coli* spp. was demonstrated due to the increasing gamma radiation dose. It has been assumed that the ethyl alcohol components of phytotherapeutic plants cannot be assessed but are distributed and clustered on the Petri dish and plant extracts may show antioxidant effects.

Conclusion: It is understood that some phytotherapeutic plants used in gamma radiation applications may show a protective effect.

Keywords: Antioxidant, *Escherichia coli* spp., gamma radiation, phytotherapeutic plants, plant extract

INTRODUCTION

Radiation is defined as the spreading of energy from a source and is divided into ionizing and non-ionizing radiation. Effective protection measures against the effects of ionizing radiation are very important (1). Gamma radiation is defined as a strong carcinogen due to the potential for oxidative damage. It causes DNA damage that is contained in various bond breaks (2). Microbiological (e.g., colony count and growth rate), biochemical (ATPase activity), and biophysics (H^+ fluxes along the cytoplasmic membrane of bacteria) methods are used to evaluate the effect of radiation on bacteria (3). The intensity of the biological damage that the radiation makes at the cellular level depends on the intensity of ionization of the radiation species. Thus, more intense ionizing radiation can cause more damage (4).

Bacteria are produced in liquid and solid mediums. Bacteria, such as *Escherichia coli*, which show a logarithmic proliferation, cloud the fluid medium in 2-3 h. The growth of bacterial is determined by various methods. These; spectrophotometric turbidity, the volume in centrifuge and total nitrogen determination. (5). Over the past 20 years, it has become increasingly common to work with *E. coli* and organisms that are evolutionarily close to it. Owing to its small size, the absence of pathogens for any living organism and the ease of production in laboratory conditions make *E. coli* the most widely studied organism, except for human beings (6).

Medical plants have been used in developing countries for centuries for the treatment of diseases. Phenolic compounds and flavonoids, which contribute to human health, are used in the treatment and prevention of various diseases (7). It is seen that these plants are used in different regions, such as ancient Egypt, China, India, and Mesopotamia, and in most countries, in kitchens, cosmetics, after medical application, and in traditional medicine applications. Many biological activities of rosemary have been described, including antioxidant, antibacterial, antifungal, and anti-cancer (8). Ginger has been widely used for many years in beverages and foods (9). Cumin is one of the oldest medical

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food plants grown in Asia, Africa, and Europe and is also cultivated in our country (10). Gamma radiation is used during a safe food processing method, and basil has been reported to increase nutritional value after irradiation (11). Owing to the antioxidant properties of carob, it exhibits many protective properties against oxidative stress (12). Both animal and preclinical studies have highlighted that yarrow has anti-inflammatory, anti-ulcer, hepatoprotective, anxiolytic, and perhaps antipathogenic activities and is widely used worldwide for wounds, digestive problems, respiratory infections, and skin disorders (13).

METHODS

Preparation of Bacteria Used in the Study, Phytotherapeutic Plants, and Irradiation Center

In our study, *E. coli* spp. strains obtained from the Microbiology Laboratory of Dicle University School of Medicine were used. Both liquid and solid mediums were studied. Preparation of the extracts of the phytotherapeutic plants used in our study was performed in the Chemistry Department Laboratory of Dicle University Science Faculty. The gamma irradiation used in the study was held at the Department of Medical Oncology at the Dicle University Oncology Hospital. Ethics committee approval was not taken due to in vitro design of the study. This study does not include human participants. Thus, no consent form was required.

Liquid Mediums (Mueller Hinton Broth: Merck, cat no. 1.05437, KGaA, Darmstadt, Germany)

These materials (beef infusion solids: 4 g/l, starch: 1.5 g/l, casein hydrolysate: 17.5 g/l, distilled water: 1000 mL, pH: 7.1±2) were left in a glass flask at the indicated ratios, then thoroughly mixed, and allowed to boil for 1 h in a water bath. The suitability of the mixture for pH was checked. Cylindrical glass tubes prepared for the study were sterilized at 170°C in a Pasteur oven (Heraeus; Thermo Scientific, Waltham, MA, USA). Then, a 5 cc mixture was transferred to each glass tube through a glass pipette, sterilized in an autoclave (Model HA-300MII, Hirayama, Tokyo Japan), and stored in a refrigerator.

Agar Mediums (Blood Agar Base: Merck, cat no. 1.10886.0500, KGaA, Darmstadt, Germany)

These materials (peptone: 10.0 g, beef extract: 9.9 g, sodium chlorite: 5.0 g, agar: 12.0 g, distilled water: 1000 ml, pH: 7.1±2) were mixed in a glass balloon at the specified ratios and allowed to boil for 1 h in a water bath. The suitability of the mixture for pH was checked. Cylindrical glass tubes were then sterilized in an autoclave at 121°C for 15 min. While hot, the medium is expected to solidify by pouring 15-20 mL into sterile petri dishes before the solidification. Before work, the proliferation was examined for growth in Petri dishes at 37°C, and non-reproductive was used.

Collecting, Drying, and Extracting Plants

Six plants assumed to have antioxidant effects were used in the study. The flowers, leaves, and other parts of the six plants (carob, basil, ginger, rosemary, yarrow, and cumin) were collected, dried, and extracted. Plant specimens were pulverized using a mechanical shredder (hand mill and mixer) after weighing 5 g by precise weighing (Vibra Shinko Denshi, Japan) from each plant sample. The pulverized plants were then dissolved in ethanol and incubated. Filtration was applied to eliminate plant residues. After filtration, the ethanol was removed by an evaporator (Heidolph Laborota 4000; Germany) at 50°C at 200 rpm as seen in Figure 1. The samples were transferred to sterile vial tubes (50 mL) with plastic cap and kept in a refrigerator at +4°C until the examination stage. All these operations were performed indoors and under normal conditions.

Preparation of *E. coli* Cultures and the Addition of Extracts to the Agar Medium

In our study, five cc of *E. coli* spp. bacteria was transferred to glass tubes and left in an incubator for 18 h at 37°C. Then, 0.5 cc was extracted from the bacterial culture via a micropipette, and 5 cc of liquid nutrient was transferred to the tubes under sterile conditions, and a dilution of 10^6 was obtained. It was standardized according to 0.5 McFarland (14). In the present study, a standard curve was drawn, absorbance and transmittance values were determined, and bacterial counts were found in the liquid medium. After incubation, the plant extracts were sterilized and added to 20 mL of uncooled agar medium, separately to give 83 µl.

Gamma Irradiation

In our study, gamma radiation was applied at doses of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy. For the groups outside the control group, gamma radiation was applied using the Co 60 teletherapy device (General Electric Alcyon II, Buc,. France) in the Oncology Hospital of the School of Medicine of Dicle University. The activity of the device was 195 cGy/min, and the surface area of the samples was 80 cm on a 32×32 cm^2 area. *E. coli* spp. was irradiated in logarithmic phase (1-8 min). After incubation, incubation was allowed for 18 h at 37°C. Colony counts were made at the end of the incubation period. Figure 2 shows the irradiation process.

Streaming

In the study, seven groups including one control and six experimental groups were formed. Then, the groups were created to in-

Figure 1. Removal of ethanol by an evaporator after filtration (Evaporator, Heidolph Laborota 4000, Germany)

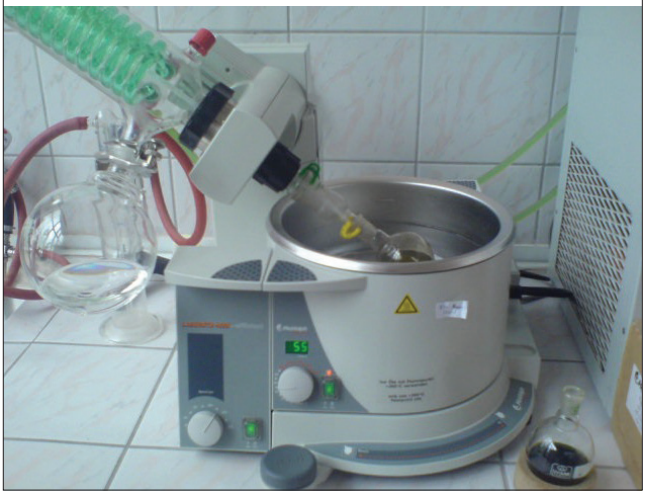


Figure 2. The device that the irradiation is applied to (General Electric Alcyon II, Buc,. France)

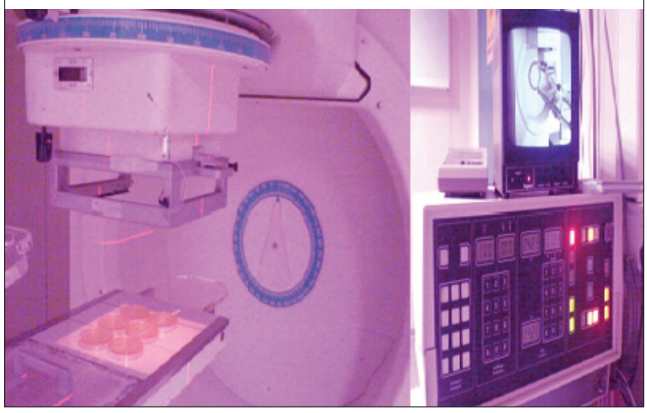
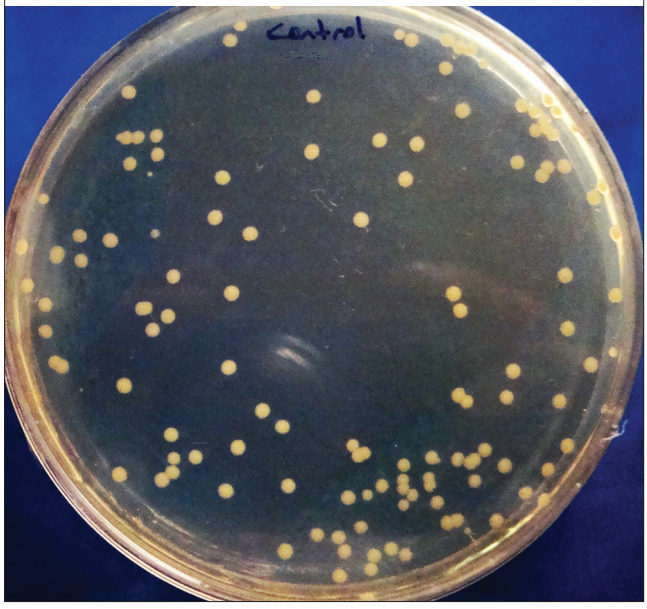


Figure 3. Colony appearance of the control group



vestigate the effect of radiation without adding any plant extracts. The control group was not irradiated, whereas the other groups were irradiated with gamma radiation of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy in the form of agar+plant extracts (carob: *Ceratonia siliqua* L., basil: *Ocimum basilicum* L., ginger: *Zingiber officinale*, rosemary: *Rosmarinus officinalis* L., yarrow: *Achillea millefolium* L., and cumin: *Cuminum cyminum* L.).

Statistical Analysis

Data were assessed using the Statistical Package for Social Sciences software, version 11.5 (SPSS Inc.; Chicago, IL, USA) for statistical analysis. The average and standard deviations of the colony counts produced by the bacteria in the Petri dish were calculated. Kruskal-Wallis ANOVA test, a non-parametric test, was used for statistical evaluation between the control group and the experimental groups. Mann-Whitney U test, a non-parametric test, was used to compare each of the experimental groups with the control group.

Figure 4. Colony appearance of *E. coli* spp. after gamma irradiation (group 1, 100 cGy)

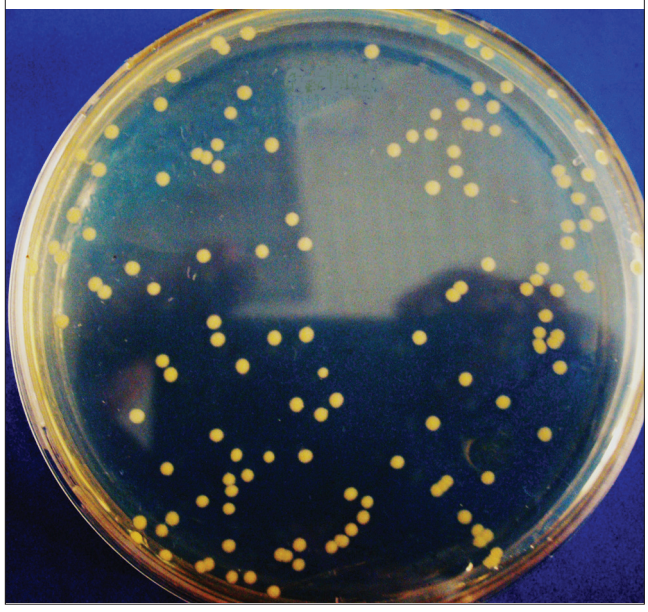
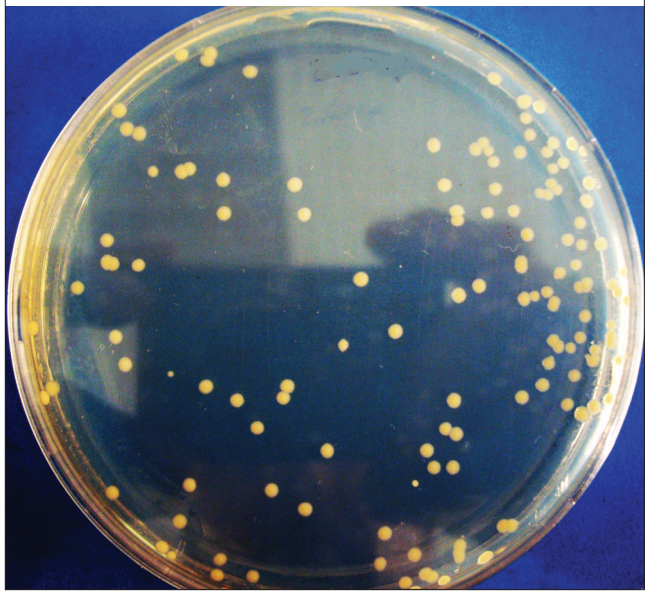


Figure 5. Colony appearance of E. coli spp. after gamma irradiation (group 2, 200 cGy)



RESULTS

In the study, gamma radiation was applied at doses of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy, and the number of colonies and images formed after incubation were obtained.

Gamma radiation significantly reduces the number of bacterial colonies at increasing doses, and radiation at high doses appears to negatively affect bacterial proliferation. It is assumed that the phytotherapeutic plant-containing extracts we use are not fully

Figure 6. Colony appearance of *E. coli* spp. after gamma irradiation (group 3, 500 cGy)

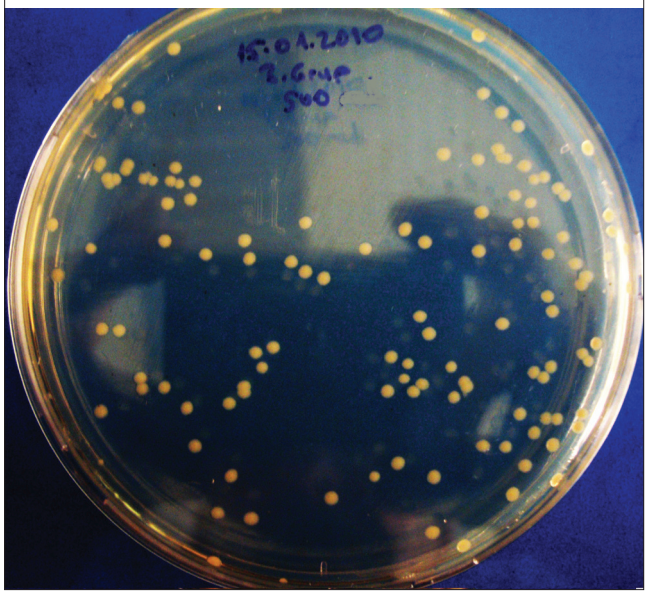


Figure 7. Colony appearance of *E. coli* spp. after gamma irradiation (group 4, 1000 cGy)

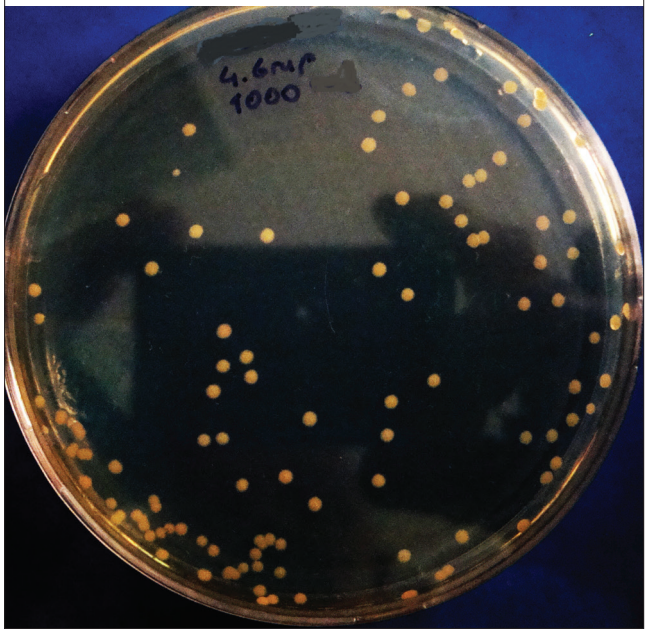


Figure 8. Colony appearance of *E. coli* spp. after gamma irradiation (group 5, 3000 cGy)

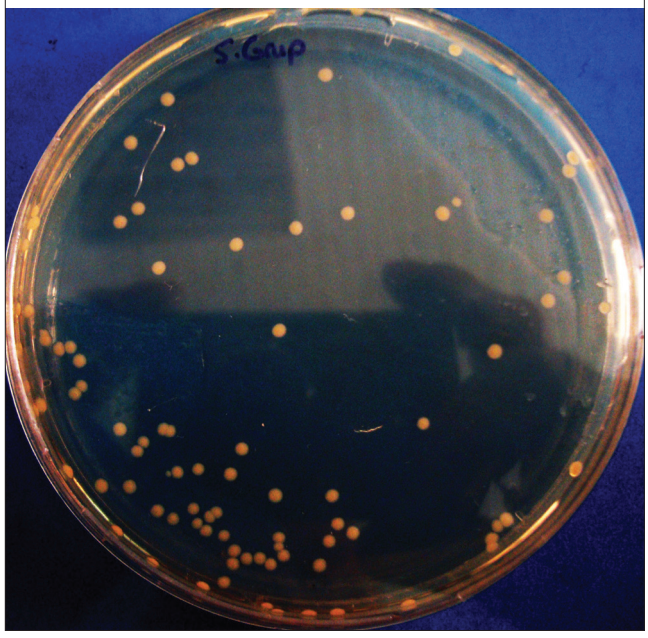
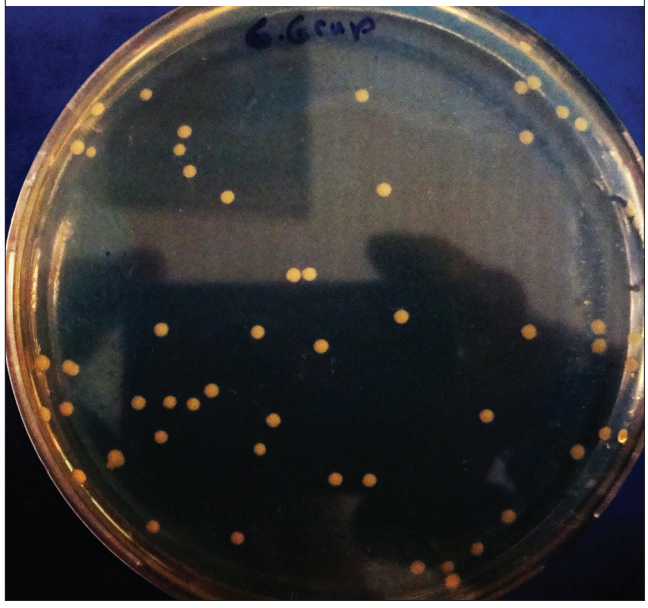


Figure 9. Colony appearance of *E. coli* spp. after gamma irradiation (group 6, 6000 cGy)



counted in the colony counts but may be antioxidant by looking at colony appearance and colony spreads. The number of colonies after irradiation is seen in Table 1. The image of the control group is shown in Figure 3. Figures 4-9 show the number of colonies after 100-6000 cGy radiation.

DISCUSSION

In recent years, serious work has been done on cancer that threatens human life. It has been known for a long time that antioxidant substances are used in cancer treatment. In our study, the effects of changes of gamma radiation we applied on *E. coli* spp. and the possible protective effects of phytotherapeutic plants (basil, carob, rosemary, ginger, cumin, and yarrow) were investigated.

For many years, studies have concluded that ionizing radiation forms reactive oxygen species in cells, which in turn damages the biological system (15). Free radicals are composed of different physiological and pathological conditions and are formed by normal metabolism. When free radicals are formed, the condition between the antioxidant and the oxidant balance deteriorates in

Table 1. Colony counts after incubation of *E. coli* spp. after irradiation

Groups	No. of colonies Mean±SD	Radiation dose (cGy)	p	p
Control	130.3±5.13	0	–	–
Group 1	129±2.00	100	1.00^{ns}	0.008**
Group 2	118±20.22	200	0.376^{ns}	
Group 3	117±2.00	500	0.05*	
Group 4	93.3±9.45	1000	0.05*	
Group 5	75.6±3.78	3000	0.05*	
Group 6	64±6.08	6000	0.05*	

ns: p>0.05, insignificant; *=p<0.05, significant; **=p<0.01, very important

the organism. Thus, lipids, macromolecules, proteins, and nucleic acids will lead to strong damage. It is stated that it may cause different diseases, such as cancer and tissue damage (16). In recent years, extracts and essential oils of many plant species have been used in the treatment of different diseases. Owing to the bioactive nature of these plants, many nutrient and drug applications are increasingly being used. They contain a wide variety of free radical scavenging molecules, such as plants (fruits, vegetables, and medicinal herbs), terpenes, vitamins, nitrogen compounds, phenolic compounds, and other endogenous metabolites. This is due to their rich antioxidant properties (17).

The study of agar+phytotherapeutic plant groups was prepared on the same conditions (e.g., quantity and pH). We observed that irradiation of 6000 cGy on agar+basil group bacteria changed the radiation effect of basil in the medium after comparing with the control group. Carob, rosemary, ginger, cumin, and yarrow also appear to reduce the effect of radiation. Radiation exposure was applied to the bacteria that we received at the same dilution rate. In the case of agar+phytotherapeutic plant, it was observed that the colons dispersed and aggregated on the Petri surface, whereas the columns that formed in the agar environment were countable and fell into a single Petri dish.

Fathiazad et al. (18) stated that ethanolic extracts made from basil leaf extracts may have a cardioprotective effect due to their rich antioxidant. Mahtout et al. (12) reported that the carob is a natural antioxidant because of its potential health benefits. Scientific studies on rosemary and its compounds have been increasing in recent years. It was emphasized that rosemary extracts were effective in modulating irregular signaling pathways in blood cancers (19). Ginger extract (GE) has been reported to have antioxidant properties. GE was evaluated for its activity at different temperatures. It has been found that it has a strong antioxidant effect at high temperatures (20). Owing to the antioxidant effects of essential oils obtained from cumin, it is accepted that it is a useful antioxidant compound in the food industry (21). Navaie et al. (22) used the extracts of the *A. millefolium* L. (yarrow) on breast cancer cell lines. Yarrow extracts can have a potential chemotherapeutic activity for breast cancer treatment. Scientists have shown that they prevented the proliferation of ovarian cancer cell lines by affecting the cell cycle at multiple phases (23). Traditionally, the use of ginger has attracted the attention of scientists, and it has been emphasized that ginger has chemopreventive and anticancer effects due to studies performed in the laboratory environment (9).

In our study, we found that gamma radiation significantly reduces the number of colonies at increased doses, and that radiation is harmful to high doses in bacterial growth. At the same time, plant extracts were added to the agar medium and exposed to gamma radiation at increasing doses. In the agar+plant extract applications, rosemary and yarrow can show antioxidant effects, and carob relatively, basil, ginger, and cumin can have significant phytotherapeutic effects at a certain level. As a result, the size, spread, and distribution of the colonies suggest that these plants have protective effects and are consistent with the literature.

CONCLUSION

The effects of radiation and plants are shown on the bacterium (*E. coli*). It did not fully reflect the effects on human beings. More studies are necessary before clinical use of these plants. The uncontrolled use of herbal products may have unintended consequences. After being proven to be harmless and usable in the clinic, the benefits to human health come before economic benefits.

Ethics Committee Approval: Ethics committee approval was not taken due to in vitro design of the study.

Informed Consent: This study does not include human participants. Thus, no consent form was required.

Peer-review: Externally peer-reviewed.

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

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

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Original Research

Effect of Agitation of Ethylenediaminetetraacetic Acid with Sonic and Photon–Initiated Photoacoustic Streaming Tecniques on Dentin Microhardness

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ABSTRACT

Objective: The aim of this study was to evaluate and compare the effect of the ethylenediaminetetraacetic acid (EDTA) agitation with a sonic system and the photon-initiated photoacoustic streaming (PIPS) technique on the microhardness of the root canal dentine.

Methods: A total of 24 extracted single-rooted human mandibular incisor teeth were collected. The root canals were instrumented according to the manufacturer's instructions using the reciprocating single-file system Reciproc. The teeth were decoronated, and the roots were longitudinally split into two halves. Following initial microhardness measurements of root canal dentine, the halves were connected, and the samples were divided into four groups, according the final irrigation protocol (Group 1, only distilled water; Group 2, EDTA; Group 3, EDTA+sonic agitation, Group 4: EDTA+PIPS agitation). Final microhardness values were calculated after the irrigation procedures. The Shapiro–Wilk test was used to evaluate the normal, or abnormal distribution of the values. For multi-comparison of the groups, one-way analysis of variance and post-hoc Tukey tests were used.

Results: The EDTA significantly reduced microhardness compared to the distilled water group ($p<0.001$), while the results of the sonic and PIPS activation groups were statistically similar to the EDTA alone group ($p=0.053$ and 0.266, respectively). No significant difference was found between the agitation groups ($p=0.853$).

Conclusion: The results of the present study revealed that neither sonic nor PIPS agitation resulted in further microhardness reduction.

Keywords: Dentin, ethylenediaminetetraacetic acid, laser, microhardness

INTRODUCTION

The success of a root canal treatment depends on the removal of microorganisms and organic and inorganic tissue remnants from the root canal system. Particularly, the removal of a smear layer formed during canal preparation is important because of its microbial and necrotic tissue content (1). Different irrigation solutions have been employed for this purpose.

Ethylenediaminetetraacetic acid (EDTA) is a widely used chelating solution to remove the inorganic content of the smear layer, and it acts by decalcifying calcium ions from the root dentin and smear layer (2). However, this mechanism of action may cause alterations in the physical properties of dental hard tissues (3). Previous studies revealed that EDTA resulted in a significant reduction of root dentin micro hardness (3-5). Furthermore, the effectiveness of EDTA in the apical region during irrigation with conventional syringes is limited due to a decreased flow of the solution to this zone (1). To overcome this problem, sonic and ultrasonic devices and lasers have been used to increase the efficiency of intra-canal solutions by agitation of the solutions. In a previous study, it was demonstrated that the EDTA agitation with a diode laser may result in a further decrease of root dentin micro hardness (6). While sonic activation agitates the intra-canal fluids by using vibrating devices with a frequency of 2-3 khz (7), photon-initiated photoacoustic streaming (PIPS) is a novel technique that constitutes of an Er:YAG laser equipped with a specially designed tip. It agitates the intra-canal solutions by producing photoacoustic shock waves (8). Although it was stated that PIPS enhances the smear layer by removing the efficiency of intra-canal EDTA, to the best of our knowledge, no data are available regarding whether this technique may lead to further decrease in the micro hardness of root dentin.

Thus, the present study aimed to evaluate micro hardness changes in the root canal dentin after the agitation of EDTA with PIPS and sonic activation and to compare the results when EDTA is used alone without agitation.

METHODS

The present study was approved by the Ethical Committee of İzmir Katip Çelebi University School of Medicine numbered/

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dated 157/08.22.2017. This study is an *ex vivo* study and does not include human participants. Thus, no consent form was required. The teeth were randomly collected following the tooth extraction procedure of a patient who attended the clinic due to periodontal, orthodontic, or prosthetic problem at the İzmir Katip Çelebi University School of Dentistry, Department of Oral and Maxillofacial Surgery. Twenty-four freshly extracted, human mandibular incisor teeth from patients of both sexes aged 40-50 years with single canals free of any cracks, fractures, hypoplastic defects, and resorption were included. After the removal of soft tissue remnants with a scaler, the teeth were kept in +4°C distilled water until they were used. Coronal access cavities were achieved conventionally with a high-speed round carbide bur (Diatech; Coltene Whaledent, Altstatten, Switzerland) under water cooling. The canal patency was established with a #15 K-file (Dentsply Maillefer, Ballaigues, France), and working length was determined by subtracting 1 mm from the point it was viewed at the apex. The root canal preparation was performed with Reciproc R25 (25.08) and R40 (40.06) (Reciproc; VDW, Munich, Germany) instruments according to the manufacturer's recommendation using VDW Silver Reciproc endodontic motor (VDW, Munich, Germany) with settings "Reciproc ALL" program, special to Reciproc files.

The teeth were decoronated from the cemento-enamel junction, and split longitudinally into two halves. In this manner, 48 samples were obtained. Each sample was embedded into acrylic block as the root canal surface is visible at the top. Initial microhardness measurements were performed throughout the mid-root region at a constant mark by using a Vickers indenter (HMV-G20 Series; Shimadzu Corp., Shiga, Japan) using a 50 gr load for 15 seconds following surface polishing with 500, 800, 1000, and 1200 grit abrasive papers with a grinding machine (Microtest grinder-polisher; Microtest Ltd., İstanbul, Turkey). The average of three measurements was accepted as the microhardness value and recorded.

After initial microhardness measurements, the two halves of each tooth were reconnected with boxing wax to perform the intra-canal procedures. All teeth were then divided into four groups according to the irrigation protocol:

Group 1 (negative control; n=3 teeth; 6 halves): Each canal was rinsed with 5 ml distilled water.

Group 2 (n=7 teeth; 14 halves): Each canal was rinsed with 5 mL 17% EDTA for 60 seconds, 5 mL 5% NaOCl for 60 seconds, and 5 ml distilled water, respectively.

Group 3 (n=7 teeth; 14 halves): Each canal was rinsed in 2.5 mL 17% EDTA for 15 seconds followed by 15 seconds of sonic activation with activator (EndoActivator; Dentsply Tulsa, OK, USA) equipped with the blue tip (30/0.6) at a distance 2 mm short of the working length. Its frequency was 10,000 cycles (in-and-out motion) per minute. This was repeated 2 times, and by this way, an irrigation with 5 mL 17% EDTA for 60 seconds (30 seconds irrigation + 30 seconds sonic activation) was achieved. Following 17% EDTA, 5 mL of 5% NaOCl irrigation for 60 seconds and irrigation with 5 mL distilled water was also performed like in other groups.

Group 4 (n=7 teeth; 14 halves): Each canal was rinsed with 1 mL 17% EDTA for 6 seconds, and intra-canal EDTA was agitated with a 12-mm-long, 300 nm quartz-stripped tip attached to the Er:YAG laser (Fidelis AT; Fotona, Ljubljana, Slovenia) (0.3 W, 15 Hz, and 20 mj) for a further 6 seconds. Following a 10-second break, the same procedure was repeated 5 times. In this way, the irrigation with 5 mL 17% EDTA for 60 seconds (30 seconds irrigation+30 seconds PIPS activation) was achieved. Again, 5 mL 5% NaOCl irrigation for 60 seconds, and irrigation with 5 mL of distilled water was performed as the final irrigation.

Irrigations was performed by using a 30-gage double-side vented, close-ended irrigation needle (C-K Endo Needles; C-K Dental, Korea) for all groups. Following irrigation, the teeth were re-split, and the last indentations were performed at the same previous marks. The differences between the initial and the last indentations were recorded as the change in the microhardness value. The mean changes in microhardness as percentage were calculated and recorded for each specimen.

Statistical Analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 20.0 (SPSS IBM Corp.; Armonk, NY, USA). The standard descriptive methods such as the mean, standard deviation, median, frequency, and minimum and maximum were applied to determine the characteristics of the sample. The Shapiro-Wilk test was used to evaluate the normal or abnormal distribution of the values. For multi-comparison of the groups, one-way analysis of variance and post-hoc Tukey tests were used. The significance was set to <0.05.

RESULTS

The mean difference in microhardness and standard deviations are presented in Table 1. The negative control group (distilled water) revealed a significantly smaller microhardness change compared to the other groups ($p<0.001$). Group 2 (EDTA), Group 3 (EDTA with sonic activation), and Group 4 (EDTA with PIPS activation) had statistically similar results ($p>0.05$).

Table 1. Mean values of microhardness change and their standard deviations (SD) shown as percentage

Groups	N	Change in Microhardness (%)±SD
Group 1	6	4.04±3.31[a]
Group 2	14	16.70±4.60[b]
Group 3	14	22.88±7.90[b]
Group 4	14	21.01±6.41[b]

Different lowercase letters represent the statistically different groups

DISCUSSION

According to the results of the present study, EDTA resulted in a significant decrease in the microhardness of root dentin in accordance with the results of previous studies (4, 5). However, neither sonic nor PIPS agitation resulted in a further decrease in microhardness compared to the not agitated EDTA irrigation. The rationale for the use of these irrigation agitation protocols is to increase their effectiveness particularly in the apical region. The EndoActivator is a sonic activation device. It acts by agitating intra-canal fluids with an in-and-out motion, and in this way, it generates a hydrodynamic activation (7, 9). This synergistic effect provides more penetration of irrigation solution in the root canal system. PIPS is another irrigating agitation technique that uses laser energy to generate photoacoustic shock waves, and in this way, it increases the efficiency of intra-canal irrigants. Its most prominent advantage compared to other agitation techniques is its ability to act by placing the tip only in the coronal part of root canals (10), while the sonic activation requires the advancement of the tip closer to the apex. However, according to our results, the effect of both agitation techniques in terms of a further decrease in microhardness compared to EDTA alone is slight and insignificant. We assume that the agitation of EDTA only enhances the penetration capacity and flow features of solution, and it cannot alter the demineralization mechanism or capacity of the solution. However, slightly more decreased microhardness values of dentin in the agitation groups than EDTA alone may have been due to an increased surface contact of the EDTA solution with dentin in agitation applications. This is in accordance with the study by Arslan et al. (6) who found that the ultrasonic activation of EDTA did not cause an additional decrease in microhardness. We assume that similarity among the groups (except the control group) of the present study is related to the same application interval of EDTA (60 seconds for each group), regardless of the activation protocol. In a previous study, it was stated that the EDTA exposure time had a significant effect on dentin microhardness and in turn, fracture resistance (11). This is related with the organic-inorganic composition of dentin. Particularly, the calcium-to-phosphorus ratio is a critical determinant for the psychical properties of dentin, which is affected from the contact time of EDTA with dentin (12). In the study by Gurbuz et al. (13), it was demonstrated that laser irridation reduces this ratio in dentine. However, in the present study, similar durations of EDTA application may have resulted in similar calcium absorption and thus similar microhardness changes. In other words, it can be stated that the effect of the sonic activation and PIPS is to increase the penetration of EDTA in the root canal system, not to aggravate its mechanism of action. On the other hand, the study by Arslan et al. (6) revealed that the agitation of EDTA with a 2 watt diode laser resulted in a significant decrease when the duration of laser application reached 40 seconds, while shorter durations (10, 20, and 30 seconds) did not resulted in a significant decrease compared to EDTA alone. They correlated their results to the morphological and chemical changes of dentin, as stated by Al-Omari and Palamara (14), who reported that the laser irridation vaporized the organic matrix of dentin, and such a disintegration causes more reduction in microhardness because collagen constitutes nearly one-fifth of dentin and provides resistance to dentine by reducing stress (15). This may explain why PIPS technique did not resulted in further decrease in the present study because the effect of laser in the PIPS technique is obtained by its effect on the intra-canal irrigation solution rather than directly on dentin. Thus, its effect on dentin may have remained limited. To the best of our knowledge, no previous studies evaluated the effects of the PIPS technique on the microhardness reduction. Our results showed that the PIPS technique did not result in a further microhardness decrease. Furthermore, those previous studies showed that microhardness was significantly reduced following laser irridation with an output power ranging between 2 W and 4.5 W during a time interval ranging from 40 seconds to 2 minutes, which may indicate that both the output power and duration may affect microhardness (6, 14). Al-Omari and Palamara (14) stated that the reduction in microhardness is directly proportional to the output power. This may be another explanation why our PIPS groups did not significantly reduce microhardness because it was used with a lesser output power (0.3 W) for lesser duration (30 seconds).

As in the PIPS group, the sonic activation of EDTA did not result in a further microhardness decrease. This is presumably related to its mechanism of action, which is only mechanical. It agitates intra-canal fluids and thus provides more penetration of irrigation solutions. Although it enhances the efficiency of EDTA (16), it does not cause any alteration in the chemical composition of root dentine due to its limited mechanical effect. That is why the sonic activation group did not show a significant microhardness reduction compared to the EDTA alone group.

CONCLUSION

In the present study, EDTA decreased the microhardness of root dentine in accordance with the previous studies. Sonic and PIPS agitation techniques did not result in further reduction in microhardness. Further studies may be beneficial to better clarify this issue.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of İzmir Katip Çelebi University School of Medicine (numbered/dated 157/08.22.2017).

Informed Consent: This study is an ex vivo study and does not include human participants. Thus, no consent form was required.

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

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Original Research

One-Year Outcomes of Femoropopliteal Chronic Total Occlusions Treated With Percutaneous Provisional Approach: A Single Center Experience (Percutaneous Treatment of Femoropopliteal CTO)

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ABSTRACT
Objective: The aim of the present study was to evaluate the results of percutaneous treated femoropopliteal chronic total occlusions (CTOs).
Methods: A total of 204 limbs (167 patients) that were treated with drug-coated balloon angioplasty and/or self-expandable stent implantation between January 2015 and December 2017 were assessed. Immediate and follow-up features were expressed with frequency tables, and Kaplan–Meier analysis was calculated for primary, primary-assisted, and secondary patency rates.
Results: In 202 (99%) cases, optimal targeted success was achieved. Death, arterial rupture requiring surgery, and myocardial infarction were not observed in the hospital period. In only 6 (3%) cases, an entry site hematoma developed but recovered without any intervention. Restenosis in 22 patients (5 in the first 6 months and 17 in the second 6 months) and reocclusion in 8 patients (2 in the first 6 months and 6 in the second 6 months) were observed in a 1-year follow-up. Primary, primary-assisted, and secondary patency rates were found to be 85.1%, 96%, and 98%, respectively, at the end of the first year.
Conclusion: Percutaneous revascularization of femoropopliteal CTOs appears to be safe and effective.
Keywords: Chronic total occlusion, drug coated balloon, peripheral artery disease

INTRODUCTION

Peripheral arterial disease (PAD), which is quite common worldwide, constitutes most of the circulatory problems in addition to coronary and cerebral arteries. Patients with PAD are presented with a wide range of clinical manifestation, such as claudication, rest pain, ischemic ulcer, and tissue loss. Owing to these clinical problems, a decrease in the quality of life, depression, and loss of functionality can be observed. To treat this disease, management of risk factors, exercise programs, antithrombotic therapy, and surgical or percutaneous intervention are applied in case of inevitable situations, such as critical limb ischemia (1-3). In developing world, percutaneous interventions with low complication rates and short hospitalization time are becoming increasingly more attractive than surgical treatment options for PAD. In cases of high risk of surgery or when femoropopliteal lesions are ≤25 cm or when there is no appropriate vein graft, percutaneous interventions are recommended as the first choice for all of Trans-Atlantic Inter-Society Consensus Document II (TASC II) lesions (4). Moreover, due to the developments in techniques and materials which are being used, long segment and calcified lesions can be revascularized percutaneously with high success rates. Some recently published series show that long superficial femoral artery (SFA) lesions have been treated percutaneously with high success and low periprocedural complication rates (5-7).

The aim of the present study was to determine the success of percutaneous femoropopliteal chronic total occlusion (CTO) treatment and to present the 1-year follow-up results in our center.

METHODS

Patient Selection

A total of 204 limbs (167 patients; 142 patients with claudication Rutherford category 2-3, 17 patients with Rutherford cate-

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gory 4, and 8 patients with Rutherford category 5-6) with femoropopliteal CTO which were treated percutaneously between January 2015 and December 2017 were prospectively included in the study. Patients who had previously undergone surgery and/or percutaneous intervention in the iliac and more distal arterial segments were excluded from the study. In addition, patients with glomerular filtration rate <90 mL/min (calculated by Cockcroft & Gault formula) and women with suspected pregnancy were also excluded. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all of the patients. Ethics committee approval was received for this study from the ethics committee University of Health Sciences Elazığ Training and Research Hospital, on 12.11.2014 and number 8419.

Procedure

Six hundred mg clopidogrel and 100 mg acetylsalicylic acid (ASA) were administered orally to all patients prior to the procedure. In the first month, dual antiplatelet (ASA+clopidogrel) and, after that, single antiplatelet (ASA) were given to all patients. Moreover, for patients who needed oral anticoagulant (OAC) therapy, in the first month, dual therapy (OAC+ASA) and, after that, only OAC were administered. Percutaneous interventions were performed by an interventional cardiologist under local anesthesia. To reach the responsible lesion, a crossover long arterial 6-French (F) sheath was used from the contralateral extremity common femoral artery to the related limb artery (45 cm) (Destination, Terumo, Japan). To pass the lesions, antegrade intimal wiring, subintimal tracking and reentry (STAR), or subintimal arterial flossing with antegrade and retrograde intervention (SAFARI) techniques were applied. In some cases, to do distal access, distal SFA, popliteal artery, and below the knee (BTK) arteries were punctured with a 21-gauge needle and were placed in a 4F radial sheath (7 cm) (Radifocus Introducer II transradial kit; Terumo, Japan). In general, the majority of cases, 0.035" hydrophilic wire (Radifocus; Terumo, Japan) and 4F 100 cm support catheter (Tempo Acqua Berenstain II; Cordis, USA) were used to pass the lesions. Astato 30 g (Asahi Intecc., Japan) extrastiff wire was used in cases where the hydrophilic wire could not pass the true lumen. No reentry device was used, and all of the lesions were passed through the wire, support catheter, and balloon. After the wire passage was achieved, all lesions were dilated for 5 min with a paclitaxel-coated balloon (Inpact Pacific; Medtronic, USA), which is appropriate to the distal and proximal reference vessel diameter measured by quantitative angiography. In case of flow limiting dissection, a bare self-expandable metal stent (Supera Stent; Abbott Vasc., USA) that has the same diameter with the vessel was implanted at dissected segments (provisional stenting). In the event that residual stenosis remained >30% on the treated lesion, post-dilation was performed with the same balloon catheter.

Technical success for all cases was defined as residual stenosis <50% after the procedure. Primary patency rate was defined as the percentage of patients without any restenosis or occlusion in the arterial segment undergoing intervention during the follow-up period. Primary-assisted patency rate was defined as the percentage of patients without restenosis or occlusion and patients who achieved patency via additional endovascular interventions in the arterial segments suffering restenosis. Secondary patency rate was defined as the percentage of patients without restenosis or occlusion and patients who achieved patency utilizing additional endovascular interventions in the occluded arterial segments. Restenosis was defined as >50% luminal diameter loss which is seen on angiography or duplex scanning (8).

Follow-Up

Before discharge, on months 1, 6, 9, and 12, all patients were called for control and evaluated clinically and ultrasonographically.

Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) program (SPSS Inc.; Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation, and categorical variables were expressed as number and percentage (%). The demographic and comorbidity data were calculated for each patient, and patency data were calculated for each limb. Primary, primary-assisted, and secondary patency rates were calculated by Kaplan-Meier analysis using Log-rank test. A p value <0.05 was considered statistically significant.

RESULTS

Patients and Lesions Characteristics

A total of 204 femoropopliteal occlusions (167 patients) underwent percutaneous intervention. The mean age of the patients was 62.9±9.4 years, and most of the patients were male (82%). Of the 167 patients, 67 (40.1%) had diabetes mellitus, and 116 (69.4%) had hypertension. All demographic characteristics and baseline clinical and angiographic features are given in Table 1. The mean lesion length was 124±51.4 (19-279) mm, and all lesions were totally occluded before the procedure. Of the 202 lesions, 187 (92.1%) were in only SFA, 14 (7.3%) were in only popliteal artery, and 1 (0.5%) was in both arteries. According to the TASC II classification lesions, 7 (3.4%) of them were classified in class A, 112 (55%) in class B, 64 (31.4%) in class C, and 21 (10.3%) in class D. Moreover, there were 10 (4.9%) extremities with distal run-off one-vessel occlusion, 7 (3.4%) extremities with two-vessel occlusion, and 3 (1.5%) extremities with three-vessel occlusion.

Immediate Results after the Procedure

The lesions were passed via intimal wiring in 68 (33.7%) cases, STAR technique in 124 (61.4%) cases, and SAFARI technique in 10 (5%) cases. While in 28 (13.7%) cases, bail-out stenting was performed due to flow limiting dissection or recoil of the lesion following balloon angioplasty, in 176 (86.3%) cases, optimal results were achieved only for balloon dilatation (Figure 1). Post-dilation was performed in 6 (2.9%) cases. In 2 (1%) cases, distal BTK embolization was observed, but both did not lead to clinical signs. Optimal targeted interventional success was achieved in 202 (99%) cases (1 case had no distal flow and 1 case had >50% residual stenosis). Death, major arterial rupture requiring surgery, and myocardial infarction were not observed in any cases. In only 6

Table 1. Demographic features and baseline characteristics

No. of patients (extremity)	167 (204)
Age (mean±SD)	62.9±9.4
Male (%)	137 (82)
Diabetes (%)	67 (40.1)
Hypertension (%)	116 (69.4)
CAD (%)	95 (56.9)
Hyperlipidemia (%)	88 (52.7)
Carotid artery disease (%)	5 (3)
Statin use (%)	31 (18.5)
CVA (%)	5 (3)
MI (%)	9 (5.4)
CHF (%)	4 (2.4)
COPD (%)	24 (14.4)
Smoking (%)	129 (77.2)
Rutherford claudication class (%)	
2	11 (6.6)
3	131 (78.4)
4	17 (10.2)
5	5 (3)
6	3 (1.8)
Bilateral disease (%)	131 (78.4)
Severe calcification (%)	12 (5.9)
Target BTK disease (%)	39 (19.1)
Run-off vessel occlusion (%)	
1 vessel	10 (4.9)
2 vessel	7 (3.4)
3 vessel	3 (1.5)
Lesion length (mm)	124.5±51.4
Artery	
SFA (%)	188 (92.1)
Popliteal (%)	15 (7.3)
SFA+popliteal (%)	1 (0.5)
TASC II classification	
A (%)	7 (3.4)
B (%)	112 (55)
C (%)	64 (31.4)
D (%)	21 (10.3)

SD: standard deviation; CAD: coronary artery disease; CVA: cerebrovascular accident; MI: myocardial infarction; CHF: congestive heart failure; COPD: chronic obstructive pulmonary disease; BTK: below the knee; SFA: superficial femoral artery; TASC: trans-atlantic inter-society consensus

(3%) cases, entry site hematoma developed but spontaneously recovered without any intervention. In 2 (1%) cases, contrast nephropathy was observed without requiring dialysis. Basal renal functions were obtained with only fluid replacement. Life-restraining claudication and rest pain disappeared or receded to a mild level in all patients who underwent revascularization before discharge. One patient who had no reflow after balloon angioplasty had knee amputation on day 14, and one patient who had distal run-off three-vessel occlusion had knee amputation on day 23. The list of details of the interventions and follow-up are given in Table 2.

Table 2. Immediate results after the procedure and 1-year follow-up parameters

Time of procedure (min, IQR)	36 (31–42)
Type of recanalization	
Antegrade intimal	68 (33.7)
Antegrade STAR	124 (61.4)
SAFARI	10 (5)
Bail-out stenting (%)	28 (13.7)
Post-dilation (%)	6 (2.9)
Fluoroscopy time (min, IQR)	28 (23–34)
Opaque amount (mL, IQR)	179 (159–204)
Balloon diameter (mm, IQR)	6 (5–6)
Flow limited dissection (%)	22 (10.8)
Residual stenosis >50% (%)	2 (1)
Distal embolization (%)	2 (1)
Intervention success (%)	202 (99)
Target limb amputation at follow-up (%)	2 (1)
Primary patency, 12 months (%)	172 (85.1)
Primary-assisted patency, 12 months (%)	194 (96)
Secondary patency, 12 months (%)	198 (98)
CV death, 12 months (%)	1 (0.5)
All cause death, 12 months (%)	2 (1)
Major adverse event, 12 months (%)	11 (5.4)
Thrombosis, 12 months (%)	1 (0.5)
Contrast-induced nephropathy	2 (1)
Hematoma	6 (3)

IQR: interquartile range; CV: cardiovascular

Follow-Up Results

All patients were called for control on months 1, 6, 9, and 12, except for one patient who died because of anterior myocardial infarction on month 5 and one patient who died because of pancreatic cancer on month 9. Clinical and ultrasonographic examinations were performed for all patients. The restenotic and reoccluded lesions were revascularized, and those cases' follow-up program was continued until on month 12.

Acute thrombosis developed in one patient on month 2, and that problem was solved with manual thrombectomy through 6F multipurpose catheter (Boston Scientific, USA) in 7F long

Figure 1. a-f. Example from the study, 34th case. (a, b) Basal angiograms; (c, d) angioplasty with DCB; (e, f) final angiograms

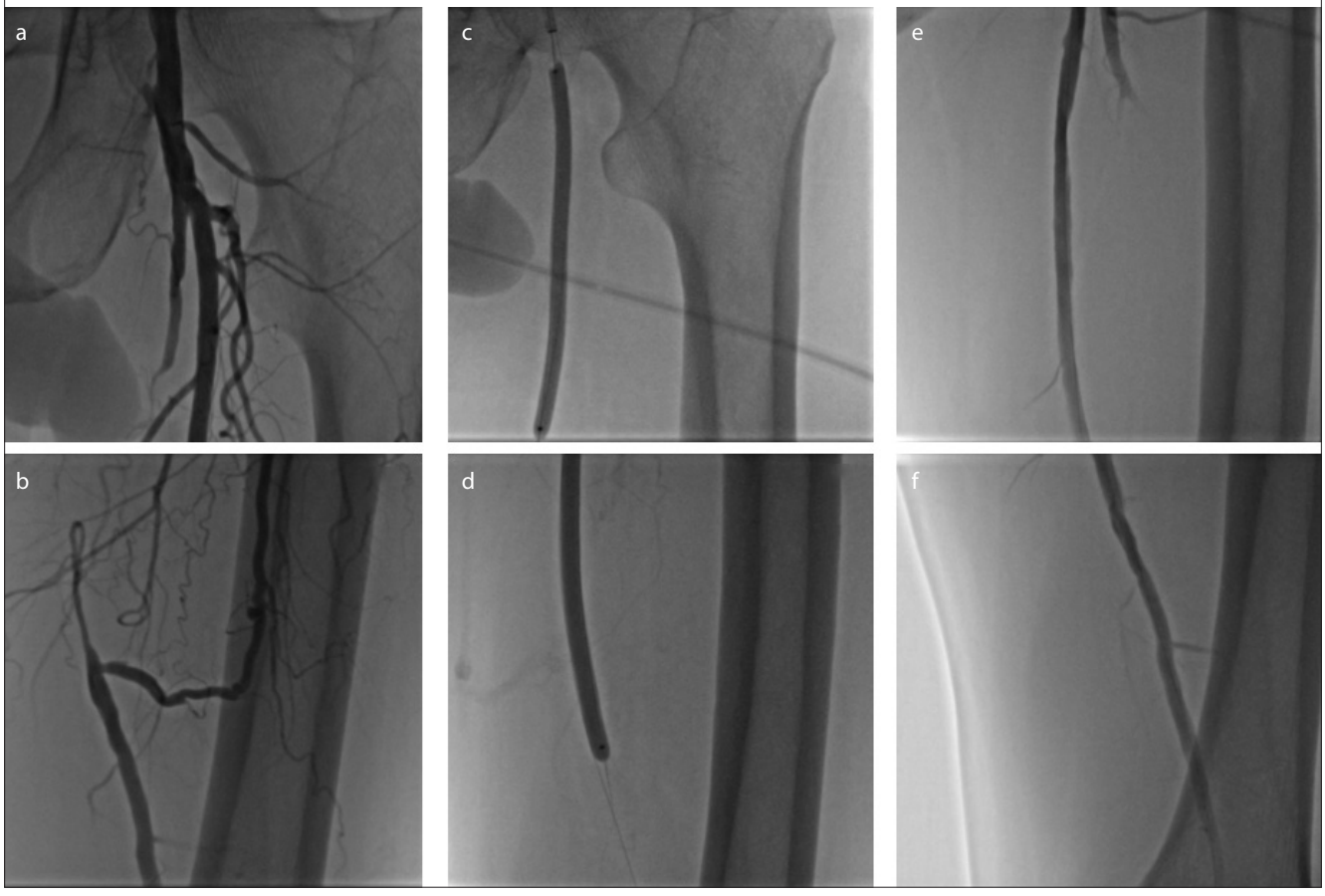
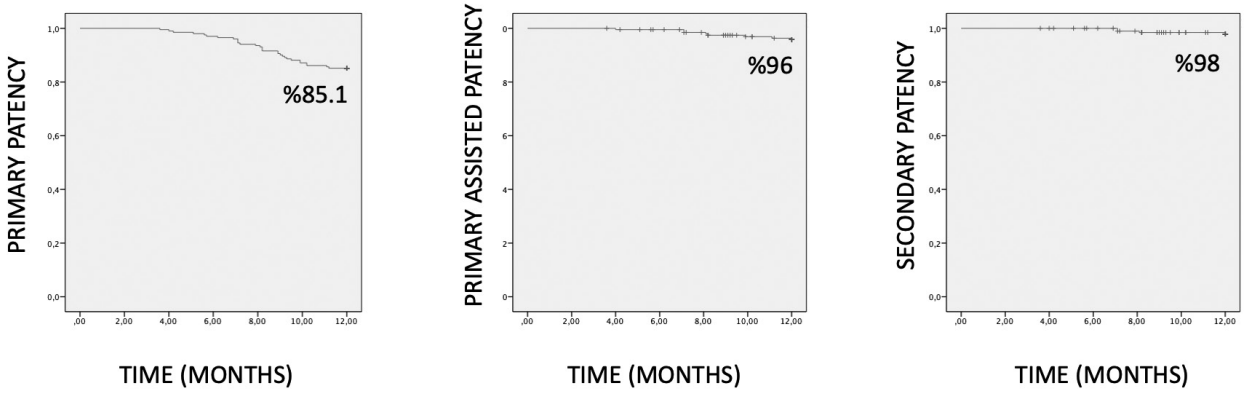


Figure 2. For the first year after intervention, primary, primary-assisted, and secondary patency rates



sheath (Destination, Terumo, Japan) and self-expandable stent implantation (Supera; Abbott, USA).

In a 1-year follow-up, 22 restenoses (5 in the first 6 months after the procedure and 17 in the second 6 months after the procedure) and 8 reocclusions were observed. On month 12, primary patency rate was 85.1%, primary-assisted patency rate was 96%, and secondary patency rate was 98% (Figure 2).

In addition, three patients underwent percutaneous angioplasty and stent implantation more than once during follow-up for optimal revascularization of the target extremity.

DISCUSSION

In this prospective observational study, 98% of the cases were successfully revascularized, and nearly all patients were followed up for 1 year. Since the primary aim of our study was to determine the patency and durability of the successful revascularization in the femoropopliteal CTO, the primary, primary-assisted, and secondary patency rates were calculated by using only the data of successfully revascularized patients although all patients were followed up for 12 months.

In recent years, the treatment approach in peripheral CTOs has changed due to advances in endovascular techniques, materials, and experiences (9-11). The endovascular approach has begun to surpass surgery worldwide, and the results are satisfactory, especially in femoropopliteal lesions (12-15). Xu et al. (16) explained primary patency rate as 84.1% for 12 months in femoropopliteal lesions in which drug-coated balloon (DCB) angioplasty was performed in their study. Moreover, Schienert et al. found that primary patency rate is 91.1% for 12 months in long lesions (>15 cm) treated with DCB and provisional (bail-out) stenting similar to our study (17). The primary patency rate of our study was 85.1% for 12 months, which is relatively lower than that study. However, the fact that all lesions in our study were CTO may be the main reason of this difference. In addition, primary-assisted patency and secondary patency rates in our study were as satisfactory at 96% and 98%, respectively. We think that patients were followed up closely and repeat revascularized if necessary is the main reason for these results. Therefore, in our point of view, percutaneous revascularization will be more useful and effective in daily practice without exposing to the morbidity and long hospitalization period of surgery in femoropopliteal CTOs.

Another issue which we want to focus is that provisional or primary stenting in femoropopliteal CTOs is still controversial. While some authors claim that routine stenting is not useful, many studies advice primary stenting, especially in long lesions. Surowiec et al. (18) showed that both approaches are not different with respect to patency and extremity revascularization. However, a previously published meta-analysis recommends primary stenting, especially in long lesions (19). Nevertheless, it is clear that there is a need for large-scale randomized studies to make a clear judgment on this issue.

Another remarkable issue which came up with the meta-analysis published in 2018 by Katsanos et al. (20), the risk of death following the application of paclitaxel-coated balloons and stents in the femoropopliteal artery has increased. According to this research, in which 28 randomized clinical trials were assessed, there is an increased risk of death with performed DCBs and stents. However, Schneider et al. (21) showed that there is no correlation between any level of paclitaxel exposure and mortality in their published meta-analysis.

When it comes to the restrictions of this real-life recording study, first, since the follow-up period was shorter than the international recording studies, it was not possible to have an idea about the durability of the femoropopliteal CTO interventions after 12 months. Second, because there were no patients with primary stenting as a control, a comparison could not be made between the two approaches. Third, this study has a limited number of diseased limbs because of being a low volume single center.

CONCLUSION

Percutaneous revascularization of femoropopliteal CTOs appears to be safe and effective. Provisional stenting is of acceptable results in these areas. Although restenosis is a common clinical entity, these problems can be easily solved thanks to close follow-up and re-intervention possibilities.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee University of Health Sciences Elazığ Training and Research Hospital (date: 12.11.2014; number: 8419).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - O.T.; Design - O.T.; Supervision - O.T.; Data Collection and/or Processing - O.T., K.K.; Analysis and/or Interpretation - O.T., K.K.; Literature Search - O.T.; Writing Manuscript - O.T.; Critical Review - O.T., K.K.

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

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Original Research

An Anatomic Study of the Supratrochlear Foramen of the Humerus and Review of the Literature

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ABSTRACT

Objective: The coronoid fossa and the olecranon fossa located on the distal end of the humerus are separated by a thin bone septum. This septum may be translucent or opaque. In some cases, this septum may become perforated, and it is called supratrochlear foramen. The aim of the present study was to describe the morphology of the supratrochlear foramen of the humerus.

Methods: This study was conducted on 108 dry humeri (right (R): 56, left (L): 52) belonging to adults whose age, gender, and racial properties are unknown. They were examined to determine the presence of the supratrochlear foramen. The shapes of the supratrochlear foramen were determined, and their diameters were measured.

Results: The supratrochlear foramen was observed in 11 cases on the right side and 11 cases on the left side. On the right side, 5 foramens were detected to be round-shaped, 3 oval-shaped, and 3 kidney-shaped, whereas on the left side, 6 foramens were detected to be oval-shaped and 5 round-shaped. Of the 86 dry humeri with no supratrochlear foramen, 57 (R: 30, L: 27) had a translucent septum, and 29 (R: 15, L: 14) had an opaque septum.

Conclusion: It is apparent that the supratrochlear foramen has been evaluated on bones generally in the literature, and there are differences in incidence rates. Owing to the clinical significance of this formation, it is thought that studying on a wider population of living individuals using radiologic imaging methods will contribute to the literature. In addition, although there are different terms used to express this formation in the literature, it is thought that adopting the name, which is commonly used as supratrochlear foramen, is most appropriate.

Keywords: Humerus, intercondylar foramen, septal aperture, supratrochlear aperture, supratrochlear foramen, terminology

INTRODUCTION

The coronoid fossa and the olecranon fossa located on the distal end of the humerus are separated by a thin bone septum (lamina) (1, 2). This septum is lined by the synovial membrane. The septum may be translucent or opaque. This septum may become perforated in some cases (1, 3). The perforated septum has many alternate names, such as supratrochlear foramen, septal aperture, supratrochlear aperture, intercondylar foramen, epitrochlear foramen, or olecranon foramen. Although supratrochlear foramen is the most commonly used term in the literature, there is no definite name that is accepted for this condition. De Wilde et al. (4) stated that this anatomic variation may be able to overextend the elbow joint. Erdogmus et al. (5) reported that the supratrochlear foramen has been neglected in the orthopedics and standard anatomy books.

The aim of the present study was to describe the morphology and morphometry of the supratrochlear foramen of the humerus and to compare with the literature in detail.

METHODS

This study was conducted in the laboratory of the department of anatomy, Gaziantep University School of Medicine. This study was performed in a manner to confirm with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. A total of 108 dry humeri (right (R): 56 and left (L): 52) belonging to adults whose age, gender, and racial properties are unknown. They were examined to determine the presence of the supratrochlear foramen. The shapes of the supratrochlear foramen were determined, and their diameters were measured. The distance between the medial edge of the supratrochlear foramen and the outer border of the medial epicondyle, as well as the distance between the lateral edge of the supratrochlear foramen and the outer border of the lateral epicondyle, was measured by a digital vernier caliper (Mitutoyo Digital Caliper, Kawasaki, Japan). The septum was classified as translucent or opaque in the humerus where the supratrochlear foramen was absent.

RESULTS

In the present study, 22 (20.37%) supratrochlear foramens were identified in 108 dry humeri (R: 56 and L: 52). The supratrochlear foramen was observed in 11 (19.64%) cases on the right side and 11 (21.15%) cases on the left side. On the right side, 5 (8.93%) fo-

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Figure 1. a-c. Different shapes of the supratrochlear foramen on dry bone and illustration (a: round-shaped, b: oval-shaped, c: kidney-shaped)

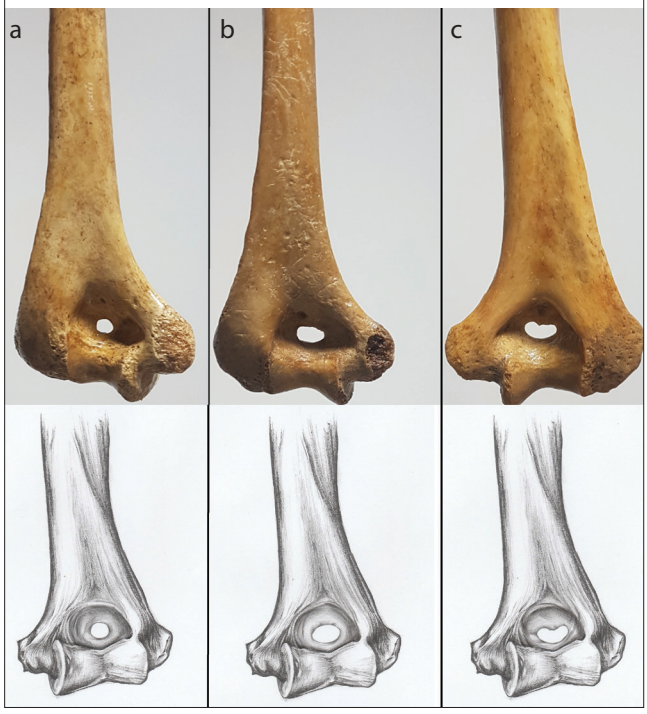


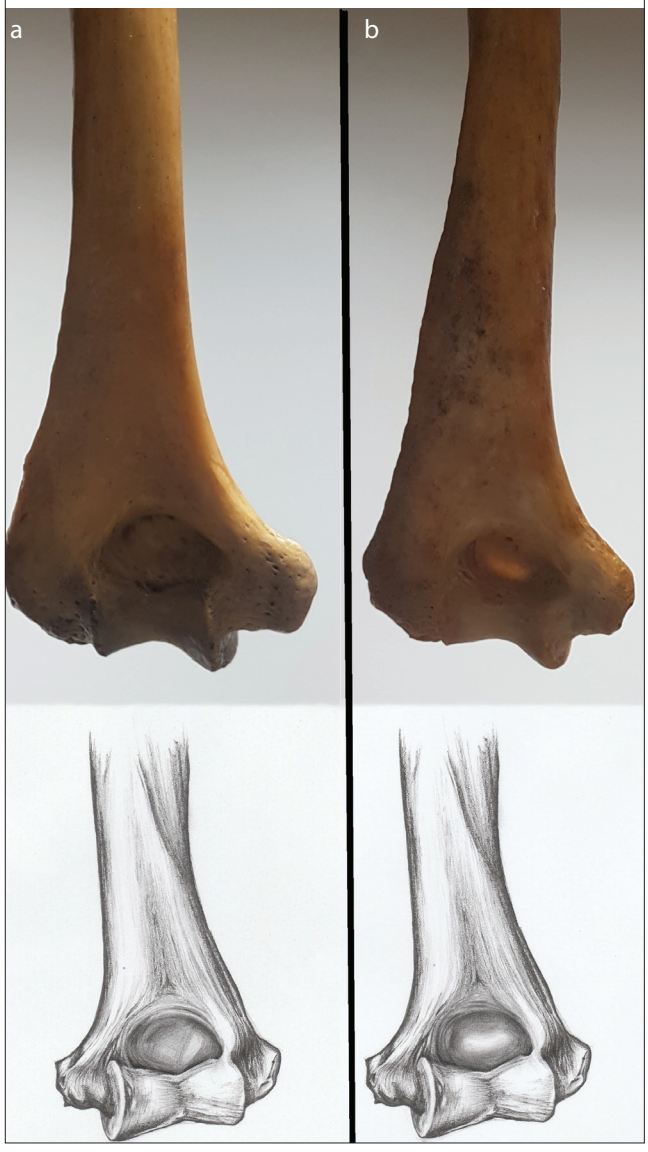
Table 1. Evaluation of the diameters of the supratrochlear foramen

Side	n (%)	Transverse diameter (mm)	Vertical diameter (mm)
Right	11 (19.64)	6.55±2.84	4.81±1.38
Left	11 (21.15)	5.64±1.96	4.82±1.33
Total	22 (20.37)	6.09±2.43	4.86±1.32

ramens were detected to be round-, 3 (5.36%) oval-, and 3 (5.36%) kidney-shaped, whereas on the left side, 6 (11.54%) foramens were detected to be oval- and 5 (9.62%) round-shaped (Figure 1). The diameters of the supratrochlear foramen are shown in Table 1. The distances between the medial edge of the supratrochlear foramen and the outer border of the medial epicondyle were 25.00±3.07 mm on the right side and 24.73±3.04 mm on the left side, whereas the distances between the lateral edge of the supratrochlear foramen and the outer border of the lateral epicondyle were 26.19±2.64 mm on the right side and 26.91±1.97 mm on the left side. No statistically significant difference was detected by the side (p=0.122 and p=0.80, respectively).

The supratrochlear foramen was absent in the 86 dry humeri. The septum of these humeri was classified as translucent or opaque (Figure 2). Of the 86 dry humeri, 57 (66.28%, R: 30 and L: 27) had a translucent septum, and 29 (33.72%, R: 15 and L: 14) had an opaque septum. In the present study, the cribriform septum was not observed.

Figure 2. a, b. The distal end of the humerus showing different types of the septum on dry bone and illustration (a: opaque septum, b: translucent septum)



DISCUSSION

Terminology

In the literature, this formation can be referred to as the supratrochlear foramen (1–40), septal aperture (1, 3, 5–7, 10–15, 17–25, 27, 29, 30, 32, 33, 38, 40–44), supratrochlear aperture (2, 5, 11, 36, 37, 39), intercondylar foramen (3, 5, 11, 15, 16, 18, 20, 21, 23, 27, 28, 32, 39), epitrochlear foramen (3, 5, 11, 15, 16, 18, 39), olecranon foramen (15, 21, 32, 39), or olecranon perforation (1). The reasons for these names are as follows:

- Supratrochlear foramen, supratrochlear aperture, and epitrochlear foramen are termed due to being located on the upper of the trochlea of the humerus.

- Septal aperture is termed due to the perforated septal part on the distal end of the humerus.
- Intercondylar foramen is termed due to being located between the medial and the lateral epicondyles.
- Olecranon foramen and olecranon perforation are termed due to being formed as a result of olecranon pressure.

Mathew et al. (15) stated that the reason for the use of many terms to express the same structure is the fact that the function and etiology of this formation have not yet been understood. There is no term for this formation in the Terminologia Anatomica (45). It is thought that using the term supratrochlear foramen is more appropriate, as this is the most commonly used term in the previous studies.

Many publications (3, 5, 6, 10, 16) reported that the supratrochlear foramen formation was first described by Meckel in 1825. Later on, the presence of the supratrochlear foramen in dogs, rats, cattle, hyenas, and other primates was investigated, especially by anthropologists (3, 6, 30, 46). Hirsh (8) stated that perforation is very commonly seen in primates other than humans. Erdogmus et al. (5) stated that the supratrochlear foramen attracts the great interest of anthropologists who believe that it is an important formation in establishing a relationship between lower animals and humans.

Causes

There is no precise information about the formation mechanism and incidence of this variation. In the literature, there are publications state that this variation might originate from interracial differences (5, 17, 30, 40). However, it is thought that this deduction cannot be made, as most of these studies were conducted on dry bones and it was hard to determine the race of these bones precisely. Moreover, some of these publications include inconsistencies. Singhal and Rao (30) stated that their study was conducted on a South Indian population. On the other hand, Das (47) expressed that the possibility that all bones belonged to a South Indian population is very low since Singhal and Rao (30) conducted their studies in a cosmopolitan city. Although Öztürk et al. (1) did not mention the racial origin of the bones used in their study conducted at Istanbul University, Istanbul Faculty of Medicine located in İstanbul (Turkey), Erdogmus et al. (5) referred to the study by Öztürk et al. (1) as a study performed on a Turkish population. Interestingly, Li et al. (17), Arunkumar et al. (25), Shivaleela et al. (39), Nayak et al. (29), Singhal and Rao (30), and Burute et al. (20) stated that Öztürk et al. (1) performed measurements on Egyptian bones. Owing to these conflicting studies, it is thought that it would not be appropriate to infer a relationship between the occurrence rate of the supratrochlear foramen and bone studies based on race. On the other hand, it is believed that this deduction may be made from studies conducted on living humans using radiologic imaging methods, as the age, gender, and ethnicity of these individuals are known.

It is not clearly known to what extent nutrition, work, and cultural factors affect the supratrochlear foramen and whether it has genetic factors (48, 49). There are publications reporting that the formation mechanism of the supratrochlear foramen can be due to genetic and/or environmental factors (1, 48, 49). Trotter (50) stated that the formation of the supratrochlear foramen may be associated with elbow hyperextension. Mays (42) and Papaloucas et al. (40) suggested that it may be associated with coronoid and/or olecranon process impingement. Myszka and Trzciński (51) reported that it may form as a result of osteoarthritis. Papaloucas et al. (40) stated that the formation may originate from osteoporosis. Hirsh (8) mentioned that the pressure of the olecranon may lead to form the septal aperture by reducing the blood supply.

Age of Occurrence

Akabori (43) reported that the supratrochlear foramen was not seen in embryonic and infantile humeri. Hirsh (8) stated that the septum exists until age 7 years and then becomes cribriform, and that lamellar atrophy begins, the intralamellar spaces enlarge, and absorption of the central part of the septum finally occurs. Trotter (50) reported that the incidence of the supratrochlear foramen was the highest in Caucasians aged 20–29 years and in Blacks aged 20–39 years. Koyun et al. (27) stated that the highest incidence of the supratrochlear foramen is seen in the second decade of life.

Incidence Rates, Diameters, Shapes, and Distance from the Epicondyles

The incidence rates of the supratrochlear foramen (Table 2) (1–3, 5–7, 9–12, 14–22, 25–32, 34, 36–39, 52, 53), the diameters of the supratrochlear foramen (Table 3) (1–3, 5, 6, 9, 10, 13–18, 20, 21, 25, 26, 28–31, 34, 36, 37, 39, 52–54), the shapes of the supratrochlear foramen (Table 4) (1–3, 5, 6, 9–16, 18–21, 25, 29–31, 34, 36, 37, 39, 52, 53), and the distance of the supratrochlear foramen from the epicondyles (Table 5) (5, 7, 13–15, 29, 31, 34) have been evaluated in various publications in the literature.

Evaluation of the Septum with Respect to Being Translucent or Opaque

In the previous studies, the septum has been evaluated into 2 groups consisting of translucent and opaque (Table 6) (5–7, 9, 10, 12, 14, 15, 22, 25, 29, 30, 36, 37, 39, 52, 53).

Clinical Significance

The existence of the supratrochlear foramen has been reported as clinically significant (5, 41). Sahajpal and Pichora (41) believed that the septal apertures in their otherwise healthy humeri probably act as stress risers from which these atypical fractures emerge following a low energy impact. The supratrochlear foramen located on the distal end of the humerus is associated with the intramedullary canal. The diameter of the intramedullary canal in the humeri that lack the supratrochlear foramen is approximately 6–8 mm, whereas this diameter is approximately 4 mm in cases that have the supratrochlear foramen (11, 33, 53). The incidence of the distal humerus intramedullary fixation has increased today due to traumatic injuries and pathologic fractures (33). Mahitha et al. (2) stated that the anatomical structure of the humerus may play an important role in the intramedullary fixation, thereby stressing the need for prior anatomical knowledge and preoperative planning in the presence of variations, such as the supratrochlear foramen in the distal end of the humerus. Radiologic imaging may be used to evaluate pathological lesions and abnormal cysts in the humerus (52). It is important not to

Table 2. The ratios of the supratrochlear foramen and comparison with the literature

	Right		Left		Total	
Study	**n**	**no. of STF (%)**	**n**	**no. of STF (%)**	**n**	**no. of STF (%)**
Arunkumar et al. (25)	188	37 (19.68)	167	39 (23.35)	355	76 (21.41)
Bhanu and Sankar (6)	49	13 (26.53)	72	24 (33.33)	121	37 (30.58)
Burute et al. (20)	58	12 (20.69)	55	18 (32.73)	113	30 (26.55)
Chagas et al. (26)	145	28 (19.31)	185	46 (24.86)	330	74 (22.42)
Dang et al. (52)	46	12 (26.09)	54	18 (33.33)	100	30 (30)
Diwan et al. (10)	905	183 (20.22)	871	245 (28.13)	1776	428 (24.10)
Erdogmus et al. (5)*	48	1 (2.09)	30	5 (16.67)	78	6 (7.69)
Erdogmus et al. (5)**	37	5 (13.51)	51	7 (13.73)	88	12 (13.64)
Jadhav and Zambare (12)	113	39 (34.51)	109	38 (34.86)	222	77 (34.68)
Joshi et al. (7)	85	20 (23.53)	85	41 (48.24)	170	61 (35.88)
Kaur and Zorasingh (38)	40	10 (25)	40	12 (30)	80	22 (27.5)
Krishnamurthy et al. (37)	84	(18)	96	(28)	180	42 (23.33)
Koyun et al. (27)*, ***					367	26 (7.1)
Koyun et al. (27)**, ***					342	35 (10.2)
Kumar et al. (3)	151	26 (17.22)	119	31 (26.05)	270	57 (21.11)
Kumarasamy et al. (14)	131	48 (36.64)	83	19 (22.89)	214	67 (31.31)
Li et al. (17)	137	9 (6.57)	125	18 (14.4)	262	27 (10.31)
Mathew et al. (15)	114	41 (35.96)	130	19 (14.62)	244	60 (24.59)
Mahitha et al. (2)	52	6 (11.54)	44	12 (27.27)	96	18 (18.75)
Mayuri et al. (18)		12		19	76	
Mahajan (22)*	36	6 (16.67)	36	9 (25)	72	15 (20.83)
Mahajan (22)**	14	5 (35.71)	14	6 (42.86)	28	11 (39.29)
Naqshi et al. (9)	40	10 (25)	40	12 (30)	80	22 (27.50)
Nayak et al. (29)	164	73 (44.51)	220	59 (26.82)	384	132 (34.38)
Ndou et al. (34)		87		140	453	227 (50.11)
Öztürk et al. (1)	54	4 (7.41)	60	5 (8.33)	114	9 (7.89)
Paraskevas et al. (32)					240	26 (10.83)
Patel et al. (36)	279	53 (19)	286	80 (27.97)	565	133 (23.54)
Ramamurthi (21)	82	22 (26.9)	78	16 (20.7)	160	38 (23.75)
Savitha and Dakshayani (11)	22	4 (18.18)	28	10 (35.71)	50	14 (28)
Singhal and Rao (30)	78	22 (28.21)	72	20 (27.78)	150	42 (28)
Shivaleela et al. (39)	72	16 (22.22)	70	22 (31.43)	142	38 (26.76)
Soni et al. (31)****		1				
Varalakshmi et al. (28)	41	9 (21.95)	44	13 (29.55)	85	22 (25.88)
Veerappan et al. (53)	35	5 (14.29)	39	9 (23.08)	74	14 (18.92)
This study	56	11 (19.64)	52	11 (21.15)	108	22 (20.37)

*: male (M); **: female (F); ***: bilaterally; ****: case report. All studies were conducted on dry bone except the study by Koyun et al. (27). The study by Koyun et al. (27) was conducted on radiogram and CT

STF: supratrochlear foramen; CT: computerized tomography

Table 3. Evaluation of the diameters of the supratrochlear foramen and comparison with the literature

			Transverse diameter (mm)						Vertical diameter (mm)					
	n		Right		Left		Total		Right		Left		Total	
Study	R	L	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Arunkumar et al. (25)	188	167	5.67± 1.71		5.39± 1.57				3.9± 1.32		3.84± 1.2			
Bhanu and Sankar (6)	49	72	6.68± 0.80		6.92± 2.00				5.75± 1.50		4.86± 1.20			
Blakely et al. (54)	115		3.79± 1.55		4.18± 1.78				4.78± 2.18		5.96± 2.76			
Burute et al. (20)	58	55					6.3						4.3	
Chagas et al. (26)	145	185	2.365± 1.396		2.332± 1.234				2.778± 2.197		2.780± 2.050			
Dang et al. (52)	46	54	5.14± 1.165		5.21± 2.13				3.79± 0.68		3.94± 1.40			
Diwan et al. (10)	905	871							****					
Erdogmus et al. (5)*	48	30	6.52± 0.0	6.7–6.7	6.7± 2.2	4.1–8.9			2.72± 0.0	2.7–2.7	4.26± 0.0	2.9–5.1		
Erdogmus et al. (5)**	37	51	5.34± 0.95	4.3–6.2	5.64± 1.66	2.7–7.9			4.59± 0.36	4.2–4.9	3.92± 0.93	2.1–5		
Joshi et al. (7)	85	85	5.5± 2.89		6.48± 2.47				3.75± 1.48		4.68± 1.43			
Kumarasamy et al. (14)	131	83	6.50± 2.26	2.20–10.04	5.82± 2.07	3.30–10.3			4.48± 1.86	2–8.10	3.98± 1.68	2.10–7.60		
Kumar et al. (3)	151	119	5.76± 2.22		6.36± 2.88				4.64± 2.45		4.76± 2.64			
Krishnamurthy et al. (37)	84	96	5.26± 2.47		6.50± 2.59				4.00± 1.52		4.70± 1.69			
Li et al. (17)	137	125	3.26± 1.15		4.47± 2.27				3.56± 1.30		5.07± 2.26			
Mahitha et al. (2)	52	44	4.6 (2–7)		6.2 (3–9)				3.4 (2–5)		4.2 (2–6)			
Mathew et al. (15)	114	130	5.12		4.9				3.48		3.27			
Mayuri et al. (18)	76							4–18						2.5–10
Nayak et al. (29)	164	220	5.99± 1.47	3.1–8.9	6.55± 2.47	2.3–10.3			3.81± 0.97	2.2–5.5	4.85± 1.64	2–7.5		
Naqshi et al. (9)	40	40	5.3± 2.37		6.6± 2.53				3.9± 1.32		4.6± 1.63			
Ndou et al. (34)*	164						6.2						4	
Ndou et al. (34)**	289						6.3						4.11	
Öztürk et al. (1)	54	60	6.51± 1.97		6.86± 2.07		6.70± 1.91	3.65–8.90	4.07± 0.99		4.95± 1.60		4.56± 1.37	2.85–6.95
Paraskevas et al. (13)***		1					7.81						5.09	
Patel et al. (36)	279	286	7.31± 1.77		7.03± 1.49				4.77± 1.15		4.90± 1.68			
Ramamurthi (21)	82	78	6.5		5.8				4.4		3.9			
Singhal and Rao (30)	78	72					6.92						4.64	
Soni et al. (31)***	1						6.22						4.64	
Varalakshmi et al. (28)	41	44	4.46		4.60				3.13		3.08			
Veerappan et al. (53)	35	39	8.30± 1.07		7.53± 1.28		7.94± 1.19		4.09± 1.13		5.35± 1.60		6.01± 1.49	
This study	56	52	6.55± 2.84		5.64± 1.96		6.09± 2.43		4.81± 1.38		4.82± 1.33		4.86± 1.32	

*: male; **: female; ***: case report; ****: the average vertical and transverse diameters of round-shaped STF were 0.28 mm on the right side and 0.23 mm on the left side, the vertical diameters of oval-shaped STF were 3.6 mm on the right side and 3.8 mm on the left side, the transverse diameters of oval-shaped STF were 5.5 mm on both sides, the height of triangular-shaped STF was 3.1 mm on the right side and 3.06 mm on the left side, and the length of triangular STF was 4.73 mm on the right side and 4.22 mm on the left side

R: right; L: left; STF: supratrochlear foramen

Table 4. Evaluation of the shapes of the supratrochlear foramen and comparison with the literature

	n		Oval		Round		Irregular		Triangular		Semilunar		Sieve		Reniform		Kidney	
Study	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**
Mahitha et al. (2)	52	44	2	6	2	4	2	2										
Kumar et al. (3)	151	119	19	26	5	5			1		1							
Bhanu and Sankar (6)	49	72	9	17	4	7												
Joshi et al. (7)	85	85	8	32	2	0												
Diwan et al. (10)	905	871	152	201	28	37			3	7								
Dang et al. (52)	46	54	4	8	8	10												
Savitha and Dakshayani (11)	22	28	1	3	1	4			1	1			1	2				
Jadhav and Zambare (12)	113	109	27	25	6	7	6	6										
Paraskevas et al. (13)***		1		1														
Mathew et al. (15)	114	130	8	23	5	8			2	1			1	4	1	6		
Öztürk et al. (1)	54	60	4	5														
Singhal and Rao (30)	78	72	20		2	19				1								
Soni et al. (31)***	1		1															
Erdogmus et al. (5)*	48	30	1	4						1								
Erdogmus et al. (5)**	37	51	2	6	1	1				1				1				
Shivaleela et al. (39)	72	70	6	10	8	10	2	2										
Ndou et al. (34)	453		136		77		34		9									
Veerappan et al. (53)	35	39	6		5				2				1					
Burute et al. (20)	58	55	****		5		8											
Naqshi et al. (9)	40	40	20		2													
Kumarasamy et al. (14)	131	83	55		12													
Mayuri et al. (18)	76		15		13				2				1					
Veerappan et al. (16)	35	39	6		5				2				1					
Patel et al. (36)	279	286	125		6				2									
Krishnamurthy et al. (37)	84	96	*****															
Arunkumar et al. (25)	188	167	71		2				3									
Nayak et al. (29)	164	220	123		7				2									
Ramamurthi (21)	82	78	55		12													
This study	56	52	3	6	5	5											3	

*: male; **: female; ***: case report; ****: transversely: 16 and vertically: 1; *****: oval–shaped STF was more common than vertical–shaped STF
R: right; L: left; n: total humerus

evaluate the supratrochlear foramen as an osteolytic lesion. In addition, it is significant to be aware of the existence of the supratrochlear foramen for an accurate radiologic diagnosis in individuals who have this variation (17). The supratrochlear foramen is also very important to the work of radiologists, anatomists, anthropologists, and orthopedic surgeons.

CONCLUSION

It is apparent that the supratrochlear foramen has been evaluated on dry bones generally in the literature, and that there are differences in incidence rates. It is thought that studying on a wider population of living individuals using radiologic imaging methods will contribute to the literature owing to the clinical

Table 5. Evaluation of the distance of the supratrochlear foramen from the epicondyles and comparison with the literature

	n			STF-medial epicondyle (mm)		STF-lateral epicondyle (mm)	
Study	**R**	**L**	**T**	**R**	**L**	**R**	**L**
Joshi et al. (7)	85	85	170	24.7±3.3	25.2±3.2	24.7±1.9	25.7±2.7
Paraskevas et al. (13)***		1			21.59		
Kumarasamy et al. (14)	131	83	214	24.4±2.89	24.5±2.50		
Mathew et al. (15)	114	130	244	24.91±2.93	24.39±3.15	27.2±2.95	26.92±2.46
Nayak et al. (29)	164	220	384	28	26.1		
Soni et al. (31)***	1			24		29	
Erdogmus et al. (5)*	48	30	78	30.56	28.97±1.59	29.54	28.68±1.23
Erdogmus et al. (5)**	37	51	88	24.70±1.95	23.93±2.65	26.65±0.68	26.92±1.28
Ndou et al. (34)*	228	225	453	27.6		28.1	
Ndou et al. (34)**				24.0		25.1	
This study	56	52	108	25.00±3.07	24.73±3.04	26.19±2.64	26.91±1.97

*: male; **: female; ***: case report
R: right; L: left; STF: supratrochlear foramen

Table 6. Evaluation of the septum and comparison with the literature

	R			L			T		
Study	**N**	**Translucent (%)**	**Opaque (%)**	**N**	**Translucent (%)**	**Opaque (%)**	**N**	**Translucent (%)**	**Opaque (%)**
Bhanu and Sankar (6)	49	27 (55.10)	6 (12.24)	72	42 (58.33)	9 (12.50)	121	69 (57.02)	15 (12.40)
Joshi et al. (7)	85	35 (41.18)	30 (35.29)	85	27 (31.76)	17 (20)	170	62 (36.47)	47 (27.65)
Naqshi et al. (9)	40	22 (55)	8 (20)	40	19 (47.50)	9 (22.5)	80	41 (51.25)	17 (21.25)
Diwan et al. (10)	905	658 (72.70)	64 (7.07)	871	497 (57.06)	129 (14.81)	1776	1155 (65.03)	193 (10.87)
Dang et al. (52)	46	18 (39.13)	16 (34.78)	54	12 (22.22)	24 (44.44)	100	30 (30)	40 (40)
Jadhav and Zambare (12)	113	9 (7.96)	42 (37.17)	109	6 (5.50)	48 (44.03)	222	15 (6.76)	90 (40.54)
Kumarasamy et al. (14)	131	41 (31.30)	42 (32.06)	83	23 (27.71)	41 (49.40)	214	64 (29.91)	83 (38.79)
Mathew et al. (15)	114	70 (61.40)	25 (21.93)	130	69 (53.08)	20 (15.38)	244	139 (56.97)	45 (18.44)
Mahajan (22)*	36	27 (75)	3 (8.33)	36	21 (58.33)	6 (16.67)	72	48 (66.67)	9 (12.50)
Mahajan (22)**	14	8 (57.14)	1 (7.14)	14	6 (42.86)	2 (14.29)	28	14 (50)	3 (10.71)
Arunkumar et al. (25)	188	106 (56.38)	45 (23.94)	167	76 (45.51)	52 (31.14)	355	182 (51.27)	97 (27.32)
Nayak et al. (29)	164	54 (32.93)	37 (22.56)	220	89 (40.45)	52 (23.64)	384	143 (37.24)	89 (23.18)
Singhal and Rao (30)	78	51 (65.38)	5 (6.41)	72	48 (66.67)	4 (5.56)	150	99 (66)	9 (6)
Erdogmus et al. (5)*	48	10 (22.22)	37 (77.08)	30	7 (23.33)	18 (60)	78	17 (21.79)	55 (70.51)
Erdogmus et al. (5)**	37	7 (18.92)	25 (67.57)	51	10 (19.61)	34 (66.67)	88	17 (19.31)	59 (67.05)
Ramamurthi (21)	82	41 (50)		78	23 (29.49)		160	64 (40)	
Patel et al. (36)	279	126 (45.16)	100 (35.84)	286	101 (35.31)	105 (36.71)	565	227 (40.18)	205 (36.28)
Krishnamurthy et al. (37)	84	37 (44.05)		96	55 (57.29)		180	92 (51.11)	
Shivaleela et al. (39)	72	36 (50)	20 (27.78)	70	32 (45.71)	16 (22.86)	142	68 (47.89)	36 (25.36)
Veerappan et al. (53)	35			39			74	30 (40.54)	30 (40.54)
This study	56	30 (53.57)	15 (26.79)	52	27 (51.92)	14 (26.92)	108	57 (52.78)	29 (26.86)

*: male; **: female. All studies were conducted on dry bone
R: right; L: left; T: total

significance of this formation. In addition, although there are different terms used to express this formation in the literature, it is thought that adopting the name, which is commonly used as supratrochlear foramen, is most appropriate.

Ethical statement: Author declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

Informed Consent: Informed consent was not required because research was performed on anatomic specimens.

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Original Research

The Relationship between Admission HbA1c Level and Infarct–Related Artery Patency in ST Elevation Myocardial Infarction Patients

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ABSTRACT

Objective: Patency of infarct-related artery (IRA) in patients with ST segment elevation myocardial infarction (STEMI) before primary percutaneous coronary intervention (pPCI) is associated with lower mortality and better clinical outcome. This study aimed to investigate the relationship between admission hemoglobin A1c (HbA1c) and IRA patency before mechanical reperfusion in patients with STEMI.

Methods: A total of 140 consecutive patients with STEMI undergoing pPCI within 12 h from symptom onset were retrospectively evaluated. The IRA patency was assessed by the thrombolysis in myocardial infarction (TIMI) flow grade. Patients were initially divided into two groups based on the TIMI flow grade. Impaired flow was defined as TIMI grades 0 and 1, and normal flow or patency was defined as TIMI 2 and 3. Patients were divided into three groups based on admission HbA1c levels as group I (HbA1c ≤ 5.6%), group II (HbA1c 5.6%–6.5%), and group III (HbA1c ≥ 6.5%).

Results: Among 140 patients, 46 (32.8%) had pre-pPCI TIMI 2 and 3 flow in IRA. The IRA patency was found to be similar in all three HbA1c groups (p=0.269). Admission HbA1c levels were similar in both IRA groups (p=0.314). In multivariate regression analysis, only MPV (OR:0.589, 95%CI:0.365–0.951, p=0.030) was significantly and independently associated with IRA patency.

Conclusion: HbA1c is not an independent predictor of the IRA patency in patients with STEMI treated with pPCI. However, MPV is a simple, low-cost, and easily accessible parameter and might be used as a predictor of IRA patency.

Keywords: HbA1c, infarct-related artery patency, mean platelet volume, primary percutaneous coronary intervention, ST elevation myocardial infarction

INTRODUCTION

Coronary heart disease (CHD) and its subgroup acute myocardial infarction (AMI) are the leading causes of mortality in our country and world wide (1, 2). AMI, also known as ST segment elevation myocardial infarction (STEMI), has high mortality rates with poor prognosis after survival (3). In STEMI, primary percutaneous coronary intervention (pPCI) is accepted as the reference therapeutic strategy (4).

Diabetes mellitus (DM) is a systemic metabolic disease associated with hyperglycemia, dyslipidemia, glycosuria, and accompanying clinical symptoms and biochemical findings. DM is regarded as a CHD risk equivalent. CHD is the most common cardiovascular (CV) complication that leads to morbidity and mortality in patients with DM (5). A patient with diabetes has 2-4 fold increased risk of CV mortality as compared to patients not suffering from diabetes. In this patient group, the CV diseases are responsible for 70%-80% of mortality. In patients with diabetes, blood glucose levels and exposure time to hyperglycemia are major risk factors for development of microvascular complications (6). However, there is no evidence that proves the same relation between blood glucose levels and exposure time to hyperglycemia and macrovascular complications (7, 8).

Hemoglobin A1c (HbA1c) is routinely used as a marker of long-term glycemic control and is unaffected by acute perturbations. The HbA1c levels could give us a better insight to understand the relation between long-term glycemic control and complications of hyperglycemia. On the other hand, studies investigating the relation between HbA1c and mortality have failed to demon-

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strate a direct relation (9, 10). Many studies have proved that in patients with diabetes, DM with poor glycemic control is related with CHD severity (11). But these previous studies generally focused on long-term mortality and CV events instead of acute incidences.

Infarct-related artery (IRA) is defined as the stenotic coronary artery with atheroma or thrombus that is responsible for acute coronary syndrome (ACS) (12). Early IRA patency is associated with better clinical outcomes in patients with STEMI (13-16). Huge data are present in the literature regarding factors affecting IRA patency .Previous studies have proved that some proinflammatory and prothrombotic biomarkers such as NLR (neutrophil to lymphocyte ratio), PLR (platelet to lymphocyte ratio), and RDW (red blood cell distribution width) are related with pre-pPCI IRA patency (17-19). However, no study in the literature investigates the direct relationship between HbA1c levels and pre-PCI IRA patency. Therefore, the relation between admission HbA1c levels and pre-PCI IRA patency in patients with STEMI was investigated in this study.

METHODS

Subjects

This is a retrospective study performed on patients admitted to our hospital with the diagnosis of STEMI between January 2016 and December 2016. A total of 140 patients without previously known CHD and chronic kidney disease who were diagnosed with STEMI in the first 12 hof chest pain and received pPCI were included in this study. The patients were divided into three groups according to baseline HbA1c levels in accordance with ADA criteria (20). As a result, 35 patients with HbA1c levels <5.6%, 65 patients with HbA1c levels between 5.6% and 6.5%, and 40 patients with HbA1c levels >6.5%were investigated. Seven patients in the HbA1c >6.5% group did not have DM diagnosis and diagnosed as new DM according to our findings. Patients deceased during the pPCI procedure, those who presented after 12 h of chest pain, and those who refused the pPCI procedure were excluded from the study.

Ankara Numune Training and Research Hospital ethics committee approved the study (date: 22.02.2017, no: E-17-1248), and all subjects provided informed consent to participate in the study. All patients were treated in accordance with European Society of Cardiology guidelines (21).

Angiographic Evaluation

Selective left and right coronary angiography (CAG) was performed in all cases using the Judkins technique with Siemens Axiom Artis Zee and Shimadzu IVR master systems. Experienced (>75 cases/year) interventional cardiologists performed the CAG and pPCI procedures. Coronary arteries were visualized in right and left oblique positions with cranial, caudal, and antero-posterior views. Angiographic images were evaluated with calibration techniques for the degree of stenosis. Critical coronary artery stenosis was accepted as >50% stenosis for left main coronary artery and >70% for other epicardial arteries. The IRA patency was defined as TIMI 2-3 flow in the distal vascular segment, while TIMI 0-1 flow was evaluated as non-patent artery (22).

Biochemical Evaluation

Hemoglobin A1c levels were evaluated by immunoturbidimetric method with Cobas 6000 device.

Statistical Analysis

All data were analyzed by using Statistical Package for the Social Sciences 20.0 statistical software package (SPSS IBM Corp.; Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. The normally distributed variables were presented as average ± standard deviation. For all other numerical variables, the reported values were corresponded to the medians. The categorical variables were given with their frequencies and the associated percentages. When analyzing the differences among the parametric variables, the one-way analysis of variance test was used for multiple-group comparisons, and the post-hoc Scheffe test was used for group-by-group comparisons. For non-parametric variables, Kruskal-Wallis test was used for multiple-group comparisons, and the Mann-Whitney U test was used for group-by-group comparisons. Categorical variables were compared with χ^2 and Fisher's exact tests. The predictors for IRA dilation patency were determined using multivariate logistic regression with backward elimination. All base-demographic features, CV characteristics, and laboratory parameters that led to p-values smaller than 0.10 in univariate analyses were included in the multivariate analyses. A p value <0.05 was considered statistically significant.

RESULTS

Patients' Characteristics

Patient groups according to baseline characteristic features are summarized in Table 1. A total of 140 patients were divided into three groups as group 1 (HbA1c ≤5.6%, n=35), group 2 (HbA1c 5.6%-6.5%, n=65), and group 3 (HbA1c ≥6.5%, n=40). According to post-hoc analyses, patients in group 3 were significantly older than those in group 1 (p=0.002). The number of male patients in group 3 was significantly lower compared to the other groups (p=0.003). There were more patients with hypertension in groups 2 and 3 compared to those in group 1 (group 1-2, p=0.046; group 1-3, p=0.001). On the other hand, there was no statistical significance for hypertension prevalence between groups 2 and 3 (p=0.103). As expected, DM prevalence was higher in group 3 (p<0.001), whereas it was similar between groups 1 and 2 (p=0.155). Among 40 patients in group 3, 33 patients have already had DM diagnosis and 7 patients got new diagnosis according to the current guidelines. Smoking rates were significantly lower in group 3 than groups 1 and 2 (p=0.035). There were no statistically significant differences between the three groups in terms of hyperlipidemia incidence, family history for CHD, left ventricular ejection fraction, and chest pain to CAG time.

Laboratory Findings

The laboratory findings of study population are listed in Table 2. According to these findings, blood glucose levels at the time of admission were significantly higher in patients with HbA1c levels >6.5% (p<0.001). On the other hand, there was no significant difference in blood glucose levels between groups 1 and 2. In addition, hemoglobin levels were significantly higher inpatients in group 1 than those in group 3 (p=0.033). However, neutrophil,

Table 1. Demographic features of the patients according to HbA1c levels

Variables	HbA1c≤5.6 (n=35) Group 1	HbA1c 5.6-6.5 (n=65) Group 2	HbA1c≥6.5 (n=40) Group 3	p
Age, years	49±9	54±9	57±11	0.002
Male, n (%)	34 (97)	59 (91)	29 (73)	0.003
DM, n (%)	1 (3)	8 (12)	33 (83)	<0.001
HT, n (%)	9 (26)	30 (46)	25 (63)	0.006
HL, n (%)	5 (14)	6 (9)	9 (23)	0.169
Smoking, n (%)	27 (77)	50 (77)	22 (55)	0.035
Family history, n (%)	12 (34)	31 (48)	13 (33)	0.221
LVEF, %	47 (40-53)	50 (45-56)	45 (39-55)	0.207
Pain duration,min	130 (90-300)	180 (120-270)	180 (120-300)	0.379

Mean values (standard deviation) and % (n) were reported for continuous and categorical variables, respectively
DM: diabetes mellitus; HL: hyperlipidemia; HT: hypertension; LVEF: left ventricular ejection fraction

Table 2. Laboratory findings of the patients according to the HbA1c level

Variables	HbA1c≤5.6 (n=35) Group 1	HbA1c 5.6-6.5 (n=65) Group 2	HbA1c≥6.5 (n=40) Group 3	p
Glucose, mg/dL	118 (106-138)	122 (110-148)	252 (170-308)	<0.001
Creatinine, mg/dL	0.93 (0.83-0.9)	0.90 (0.86-1.04)	1.00 (0.84-1.10)	0.738
Uric acid, mg/dL	5.1 (4.3-6.2)	5.4 (4.8-6.0)	5.6 (4.4-6.5)	0.392
Amylase, U/L	65±28	62±21	57±25	0.289
TC, mg/dL	184 (164-204)	183 (156-196)	177 (152-205)	0.872
LDL-C, mg/dL	114 (102-142)	116 (89-134)	111 (73-138)	0.811
HDL-C, mg/dL	38.4±8.1	36.3±7.1	37.2±8.0	0.479
Triglyceride, mg/dL	112 (63-171)	135 (94-186)	128 (85-170)	0.287
WBC ($\times 10^9$/L)	11.1 (9.0-15.5)	11.8 (9.8-14.5)	11.3 (9.3-13.3)	0.986
Neutrophil ($\times 10^9$/L)	7.9 (6.3-12.9)	9.7 (7.2-11.2)	9.4 (6.0-10.5)	0.801
Lymphocyte ($\times 10^9$/L)	1.7 (1.3-2.6)	1.9 (1.2-2.8)	1.5 (1.1-2.4)	0.630
Hemoglobin, g/dL	14.5±1.4	14.2±1.6	13.9±2.1	0.033
Hematocrit, %	42.8±8.4	42.1±5.7	41.6±6.0	0.710
MPV, Fl	8.6 (7.7-9.1)	8.5 (7.9-9.1)	8.6 (7.8-9.4)	0.864
Platelet ($\times 10^9$/L)	238±64	243±78	251±61	0.709
RDW, %	13.0 (12.6-13.6)	13.1 (12.6-13.9)	13.5 (12.7-14.5)	0.237
HbA1c, %	5.5 (5.4-5.6)	5.9 (5.7-6.1)	7.9 (7.0-10.1)	<0.001

Mean values (standard deviation) and % (n) were reported for continuous and categorical variables, respectively
HbA1c: hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MPV: mean platelet volume; RDW: red cell distribution width; TC: total cholesterol; WBC: white blood cell

lymphocyte and platelet counts, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and creatinine levels were similar between all groups.

Angiographic Data

Angiographic data of the study population are presented in Table 3. Localization of infarct-related lesion (left anterior descend-

Table 3. Coronary angiography results of the patients according to the HbA1c level

Variables	HbA1c≤5.6 (n=35)	HbA1c 5.6-6.5 (n=65)	HbA1c≥6.5 (n=40)	p
The number of vessels with critical (>70%) stenosis				
Single vessel, n (%)	24 (68)	40 (61)	22 (55)	0.243
Two vessels, n (%)	10 (29)	14 (22)	10 (25)	
Three vessels, n (%)	1 (3)	11 (17)	8 (20)	
Infarct-related artery				
LAD, n (%)	20 (56)	32 (48)	21 (52)	0.751
RCA, n (%)	8 (22)	23 (35)	14 (35)	0.397
Cx, n (%)	7 (19)	9 (14)	5 (13)	0.622
LMCA, n (%)	1 (3)	2 (3)	0	0.540
TIMI blood flow				
TIMI 0, n (%)	25 (71)	38 (58)	20 (50)	0.343
TIMI 1, n (%)	1 (3)	7 (11)	3 (8)	
TIMI 2, n (%)	6 (17)	13 (20)	8 (20)	
TIMI 3, n (%)	3 (9)	7 (11)	9 (22)	
Patent IRA, n (%)	9 (26)	20 (31)	17 (43)	0.269

Cx: circumflex coronary artery; IRA: infarct-related artery; LAD: left anterior descending artery; LMCA: left main coronary artery; RCA: right coronary artery; TIMI: thrombolysis in myocardial infarction

Table 4. Coronary angiography results of diabetic patients according to the HbA1c level

Variables	HbA1c<6.5% (n=9)	HbA1c≥6.5% (n=33)	p
The number of vessels with critical (>70%) stenosis			
Single vessel, n (%)	7 (77.8)	18 (54.5)	0.301
Two vessels, n (%)	1 (11.1)	9 (27.3)	
Three vessels, n (%)	1 (11.1)	6 (18.2)	
Infarct-related artery			
LAD, n (%)	7 (77.8)	17 (51.5)	0.166
RCA, n (%)	1 (11.1)	12 (36.4)	0.154
Cx, n (%)	1 (11.1)	4 (12.1)	0.936
LMCA, n (%)	0 (0)	0 (0)	0.000
TIMI blood flow			
TIMI 0, n (%)	9 (100)	18 (54.5)	0.016
TIMI 1, n (%)	0 (0)	1 (3)	
TIMI 2, n (%)	0 (0)	8 (24.2)	
TIMI 3, n (%)	0 (0)	6 (18.2)	
Patent IRA, n (%)	0 (0)	14 (42.4)	0.016

Cx: circumflex coronary artery; IRA: Infarct-related artery; LAD: left anterior descending artery; LMCA: left main coronary artery; RCA: right coronary artery; TIMI: thrombolysis in myocardial infarction

ing artery, circumflex artery, right coronary artery) was similar between all the three groups. Number of coronary arteries with significant stenosis, TIMI flow grade, and IRA patency rates were also similar between the three groups. When patients were divided into the IRA patent and non-patent groups, in the IRA non-patent patient group, smoking rates were determined to be significantly higher (p=0.029); duration of chest pain was found to be significantly shorter (p=0.028); and LDL cholesterol levels were significantly higher (p=0.032). The MPV levels were significantly lower in the IRA patent group (p=0.014). There was no statistically significant difference between the three groups for the relation between IRA patency and HbA1c levels (p=0.260).

The patients with diabetes were also divided into two groups according to the HbA1c levels as HbA1c <6.5% (optimal blood glucose control) and HbA1c ≥6.5% (suboptimal blood glucose control) groups. Angiographic data of the diabetic patients according to their HbA1c level are showed in Table 4. According to these results, the number of vessels with critical stenosis (>70%) and the distribution of IRA were similar between both the groups. However, the rate of IRA patency was significantly higher in the suboptimal blood glucose control group compared to that in the optimal blood glucose control group (p=0.016).

The baseline demographic and laboratory findings according to the patency of IRA are demonstrated in Table 5. There were no significant differences in terms of age, incidence of hyperlipidemia, HT, DM, family history of CHD, glucose, creatinine, total cholesterol, HDL cholesterol, and triglyceride levels between the groups. In the occluded IRA group, the incidence of smoking was significantly higher compared to the patent group (p=0.029). The

Table 5. The baseline demographic and laboratory findings according to the patency of infarct-related artery

Variables	Occluded IRA (n=94)	Patent IRA (n=46)	p
Age, years	53±10	55±10	0.125
Hyperlipidemia, n (%)	15 (16)	5 (11)	0.419
Hypertension, n (%)	40 (43)	24 (52)	0.283
Diabetes mellitus, n (%)	28 (30)	14 (30)	0.937
Smoking, n (%)	72 (77)	27 (59)	0.029
Family history, n (%)	40 (43)	16 (35)	0.378
Glucose, mg/dL[Θ]	130 (112-174)	132 (110-224)	0.824
Creatinine, mg/dL	0.95±0.17	0.96±0.15	0.919
Total cholesterol, mg/dL	187±50	171±40	0.079
LDL cholesterol, mg/dL	121±44	104±32	0.032
HDL cholesterol, mg/dL	37.6±8.2	36.0±6.3	0.223
Triglyceride, mg/dL[Θ]	118 (79-162)	136 (98-188)	0.313
Uric acid, mg/dL	5.5±1.2	5.8±1.5	0.166
Amylase,U/L	64±25	56±21	0.070
HbA1c, %[Θ]	6 (5.6-6.5)	5.9 (5.7-7.3)	0.314
WBC ($\times 10^9$/L)[Θ]	12.01 (10.1-14.3)	11.05 (8.6-13.4)	0.121
Neutrophil ($\times 10^9$/L)	9.2±3.7	8.8±3.2	0.526
Lymphocyte ($\times 10^9$/L)	2.3±1.5	1.8±0.9	0.076
Hemoglobin, g/dL	14.4±1.7	14.1±1.8	0.248
Hematocrit, %	42.7±5.6	40.9±8.0	0.121
Platelet count ($\times 10^9$/L)	238±64	257±79	0.129
RDW, %[Θ]	13.1 (12.6-13.9)	13.3 (12.7-14.4)	0.109
MPV, fL	8.7±0.9	8.3±1.0	0.014
LVEF, %	47±9	49±10	0.207
Pain duration, min[Θ]	150 (90-270)	240 (120-360)	0.028

Data are expressed as mean±standard deviation
[Θ]Parameters without normal distribution
HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LVEF: left ventricular ejection fraction; MPV: mean platelet volume; RDW: red cell distribution width; WBC: white blood cell

LDL cholesterol level was also significantly higher in the occluded IRA group than the patent IRA group (121±44 mg/dL vs 104±32 mg/dL, respectively, p=0.032). In addition, MPV was significantly higher in patients within the occluded IRA group than that in the patent IRA group (8.7±0.9 fL vs 8.3±1.0 fL, respectively, p=0.014).

Table 6. Multivariate regression analysis of variables related with the patency of infarct-related artery

Variables	Possibility rate	95.0% confidence interval	p
Smoking	1.276	0.783-2.092	0.324
Mean platelet volume	0.589	0.365-0.951	0.030
Lymphocyte count	0.582	0.335-1.012	0.055
Total cholesterol	1.008	0.986-1.030	0.482
LDL cholesterol	0.978	0.952-1.004	0.101
Amylase	0.991	0.972-1.011	0.379
Pain duration	1.001	0.998-1.003	0.603

LDL: low-density lipoprotein

Multivariate regression analysis of variables related with the IRA patency is presented in Table 6. According to these results, the MPV level was detected to be an independent predictor for IRA patency (p=0.03). However, smoking (p=0.320), duration of chest pain (p=0.603), and LDL cholesterol level (p=0.101) were not independent predictors for IRA patency.

DISCUSSION

This study investigated the relation between admission HbA1c levels and IRA patency in patients with STEMI. It was found that HbA1c levels are not an independent predictor for IRA patency in these patients. Previous studies in the literature proved that coronary patency is an independent predictor for adverse events in patients with STEMI (23). Patients with patent IRA before pPCI have higher TIMI 3 flow rates after pPCI (24). In Euromax trial (25), it was proved that patients with TIMI 3 flow in IRA have better outcomes than patients with TIMI 0-2 flow in one-month follow-up. Rakowski et al. (26) showed that early IRA patency in patients with STEMI that are planned to undergo pPCI is related with better revascularization outcomes, lower mortality rates, fewer stent thrombosis, and better clinical outcomes in one-year of follow-up. Patients with TIMI flow 0 or 1 before pPCI have higher in-hospital mortality rates and major CV events (27). Patients with TIMI 0 flow before pPCI have higher short and long-term mortality rates even if TIMI 3 flow have been accomplished after pPCI (28). Previous studies showed that age, left ventricular ejection fraction, glomerular filtration rate, and Killip classes are long-term predictors for CV mortality in patients with STEMI (29). A wide range of studies have proved that HbA1c levels were related with in-hospital, long-term and short-term CV mortality, and major adverse cardiac events in patients with STEMI (30-34). Elevated levels of HbA1c are related with basic characteristics for increased CV risk. This relationship is also responsible for increased short- and long-term mortality rates. It is also known that not only patients with DM but also clinical conditions such as impaired fasting glucose, impaired glucose tolerance, and other insulin resistance conditions have very high risk for coronary artery disease development (35, 36). INTERHEART study included patients of various ethnic origin and proved that there is a strong correlation between HbA1c level and AMI incidence

independently from previously diagnosed DM (37). Lee et al. (38) conducted a study on 183 patients with DM and assessed the impact of glycemic control on occurrence of no-reflow in these patients undergoing pPCI for myocardial infarction. As a result, they found that optimal glycemic control had similar rates of no-reflow compared to the suboptimal control group. However, in this study, the rate of no-reflow was significantly higher in patients with diabetes with optimal glycemic control (HbA1c <6.5%) than those with the suboptimal group (HbA1c ≥6.5%).This result may be due to the very low number (n=9) of patients with diabetes with optimal glycemic control. Besides, elevated HbA1c levels are related with increased mortality and CV disease incidence in general population including patients with prediabetes (39, 40).

Coronary plaque rupture or erosion is considered the major cause of development of STEMI. Thrombocyte aggregation results in thrombus formation that ceases myocardial blood supply. This process causes myocardial damage. However, the actual triggering mechanism of this pathophysiologic phenomenon is unknown. Inflammation is proved to have a role in both initiation and progression of this process (41). In addition, there is a significant interaction between inflammation and atherothrombosis (42). Platelets, leukocytes, and endothelium actively participate in this process by interacting with each other. These interactions initiate autocrine and paracrine activation that leads to leukocyte migration to the vascular wall. This interaction between inflammation and thrombosis during the whole pathophysiologic process may affect IRA patency (42, 43). Previous studies have proved that proinflammatory and prothrombotic biomarkers (PLR, NLR, RDW, MPV) are directly related with IRA patency (17-19). In this study, among these biomakers including HbA1c levels, only MPV was found to be related with IRA patency in patients with STEMI before pPCI. Absence of relation between these biomarkers (except MPV) and IRA patency before pPCI may have a couple of explanations. These are different characteristics of the study population, limited number of patients, and presence of unpredictable pathophysiologic mechanisms. Brener et al. (44) showed that IRA patency before pPCI affects procedural success, in-hospital mortality, and preservation of ventricular performance. In our study, procedural success and in-hospital mortality were not evaluated. On the other hand, LVEF was measured to appraise ventricular performance. Contrary to Brener et al. (44), we found that LVEF was similar between pre-pPCI IRA patent and non-patent groups (p=0.20). Different characteristics of the study population, limited number of patients, and most importantly short chest pain-balloon time in our study may be responsible for this finding. Previous studies about patients with STEMI proved that various prothrombotic and proinflammatory biomarkers such as NLR, PLR, platelet count and reactivity, MPV, RDW, and epicardial adipose tissue thickness are related with IRA patency (17, 19, 45-49). On the scope of previous studies, we considered to evaluate the relation between HbA1c values and pre-pPCI IRA patency rates in patients with STEMI. As a result, we found similar pre-pPCI IRA patency rates in all the three HbA1c groups. Also, there was no statistically significant difference regarding HbA1c values between the IRA patent and non-patent groups. Similar to our findings, a number of studies in the literature investigating the relation between mortality and HbA1c levels in patients with AMI found no relation between IRA patency and HbA1clevels as well (32, 33). In a previous study, 374 patients with STEMI who had undergone successful pPCI were divided into three groups according to their HbA1c levels. Pre-PCITIMI flow rates were similar between groups as in our study (32). Timmer et al. (33) investigated the effect of HbA1c levels of patients with STEMI without previous DM diagnosis on short- and long-term mortality. They found that patients with higher initial blood glucose levels were associated with lesser pre-PCI TIMI 3 flow (p=0.01), while they were unable to find a difference between patients with high HbA1c levels and the control group (p=0.15) (33). In our study, we also could not find any difference regarding pre-PCI TIMI3 flow rates between patients with high HbA1c levels and the control groups.

In this study, we also found that MPV and IRA patency were independently and significantly associated with each other. Elbasan et al. (19) investigated the relation between pre-PCI IRA patency and MPV levels in 840 patients with STEMI and found that patients with MPV >9.9 fL have statistically significant lower pre-PCI TIMI 3 flow rates compared with those with MPV between 8.3 and 9.9 and MPV<8.3 fL groups. In our study, relation between MPV levels and post-pPCI TIMI flow rates was not evaluated as we assumed that the pre-pPCI TIMI flow rates could project post-pPCI rates as it is known that procedural success rates are better in pre-pPCI IRA patent patients (44). In another study conducted by Celik et al. (46), relation between MPV and post-pPCI TIMI flow rates were investigated in 306 patients with STEMI, and MPV levels were found to be lower in patients with post-pPCI TIMI 3 flow (10.9 fL vs 10.1 fL, respectively, p<0.001). However, relation between MPV and pre-pPCI IRA patency we declared in this study is consistent with other studies in the literature (19, 45, 46). Huczek et al. (50) investigated the prognostic value of MPV for angiographic reperfusion and they found that MPV is a strong, independent predictor of impaired angiographic reperfusion. In a study conducted by Kurtul et al. (51), a cut-off value of MPV >8.65 was found as a predictor of angiographic no-reflow in patients with STEMI. Moreover, in another study, Kurtul et al. (52) assessed PLR in predicting angiographic reflow in patients with STEMI and determined higher PLR in no-reflow patients compared to the normal reflow group.

There are various limitations in our study. First, retrospective single-center design may cause bias. The second most important limitation is relatively small study population. In addition to that, HbA1c levels may be affected by patients with hemoglobinopathy. Conditions such as hemolytic anemia and iron deficiency anemia may also affect test results. We did not consider these conditions.

CONCLUSION

This study showed that there is no relation between admission HbA1c levels and pre-pPCI myocardial perfusion in patients with STEMI. Besides that, data regarding increased mortality rate due to higher HbA1c levels in CHD should be reevaluated and more detailed studies are needed. Data from these studies may lead to higher threshold levels for HbA1c in patients with CHD with implementation of less aggressive treatment strategies in these patient population.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara Numune Training and Research Hospital (date: 22.02.2017, no: E-17-1248).

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

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Case Report

Cutaneous Vasculitis after Radiotherapy

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ABSTRACT

Cutaneous vasculitis is a large heterogeneous group of diseases, where blood vessels are targeted by immunological and inflammatory reactions, which are the primary causes of this condition. Infections, medications, systemic collagenosis, chronic diseases, and malignancies are the secondary factors that cause cutaneous vasculitis. Hemangiomas are the most common primary benign tumors of the spinal cordand are rarely symptomatic. The most commonly manifestedsymptom is pain, but in rare cases, cutaneous vasculitis may lead to paraparesis and paralysis. Radiotherapy (RT) is a safe and effective treatment for symptomatic spinal cord hemangiomas. A 44-year-old male patient was admitted to our dermatology polyclinic with a complaint of a bilateral rash on both legs that had lasted for 1 week. The medical history of the patient included no disease other than a sacral hemangioma with symptomatic pain, for which the patient had been treated with 4500 cGy curative radiotherapy 1 month previously. In our case, it was thought that cutaneous vasculitis was caused by the radiotherapy without any other triggering factor. A skin biopsy was taken to arrive at a definite diagnosis, and in the histopathological examination, abundant amounts of extra-red blood cells and lymphocytes were observed, along with endothelial profiling in superficial vessels; all of which are findings consistent with vasculitis. The patient was diagnosed with cutaneous vasculitis, both clinically and histopathologically. To the best of our knowledge, radiotherapy as a cause of vasculitis has been the subject of very few studies in the literature to date. In this regard, the present report describes a case of cutaneous vasculitis as a possible immune-related side effect of RT.

Keywords: Cutaneous vasculitis, hemangioma, radioimmunology, radiotherapy

INTRODUCTION

Vasculitis refers to the inflammation of blood vessels and may affect any part of the body (1). Deep small and medium sized blood vessels may be affected by either primary or secondary inflammation (1). Infections, medications, systemic collagenosis, chronic diseases, and malignancies are secondary causes of cutaneous vasculitis (1).

Hemangiomas are the most common primary benign tumors of the spinal cord, and are rarely symptomatic, with the most common symptom being pain, although in rare cases it may lead to paraparesis and paralysis (2). Radiotherapy is considered a safe and effective treatment method for symptomatic spinal cord hemangiomas (2).

To the best of our knowledge, cutaneous vasculitis following radiotherapy is an under-researched subject in literature. We present here a case of cutaneous vasculitis that describes the effects of radiotherapy administered for a sacral hemangioma.

CASE PRESENTATION

A 44-year-old male patient was admitted to the dermatology polyclinic with a complaint of a bilateral rash on both legs that had emerged 1 week earlier. A dermatologic examination revealed palpable purpura on both legs (Figure 1). The medical history of the patient included no disease other than a sacral hemangioma. The S1 and S2 sacral vertebrae were identified with expansive hemangiomas in an MRI (Figure 2). The patient had no history of drug use other than the occasional paracetamol for back pain, and the subject's family history was not characteristic for this condition.

The patient had been treated with 4500 cGy curative radiotherapy in 25 fractions, with 180 cGy per day, for a sacral hemangioma with symptomatic pain 1 month previously. In a laboratory review, the following measures were recorded; C3: 1.06 g/dL, C4: 0.234 g/dL, ANA: negative, anticardiolipin antibodies: negative. Other laboratory tests were found to be normal, and no autoimmune pathogenesis was detected in the patient. It was thought that cutaneous vasculitis was the cause of radiotherapy in our

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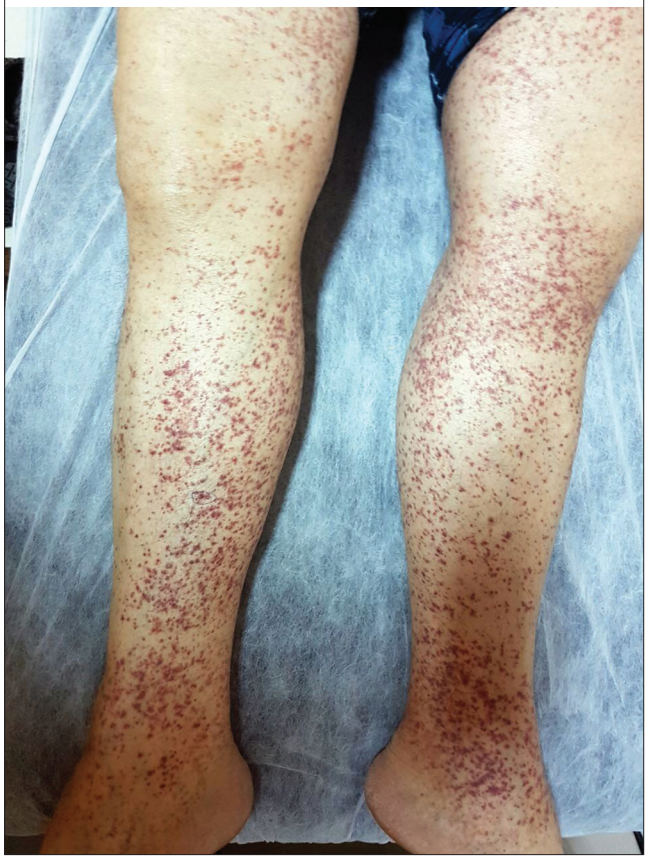
case without any other triggering factor. A skin biopsy was taken to make a definite diagnosis.

In the histopathological examination, abundant quantities of extra-red blood cells and lymphocytes were observed, along with endothelial profiling in superficial vessels, which was consistent with vasculitis (Figure 3). The direct immunofluorescence assay was negative, and the patient was diagnosed with cutaneous vasculitis, both clinically and histopathologically.

Figure 1. Palpable bilateral purpura on both legs



Treatment with 48 mg of oral prednisolone was begun and was gradually reduced in 2 months. The lesions of the affected lesion decreased in 2 months and there was no recurrence of skin lesions in the medical follow-up.

Informed consent was obtained from the patient for the publication of this case report and the associated images.

DISCUSSION

Cutaneous vasculitis can, in rare cases, develop through secondary malignancies (3), and is known as paraneoplastic vasculitis.

Figure 2. T1 MRI showing expansive hemangiomas in the S1 and S2 vertebrae

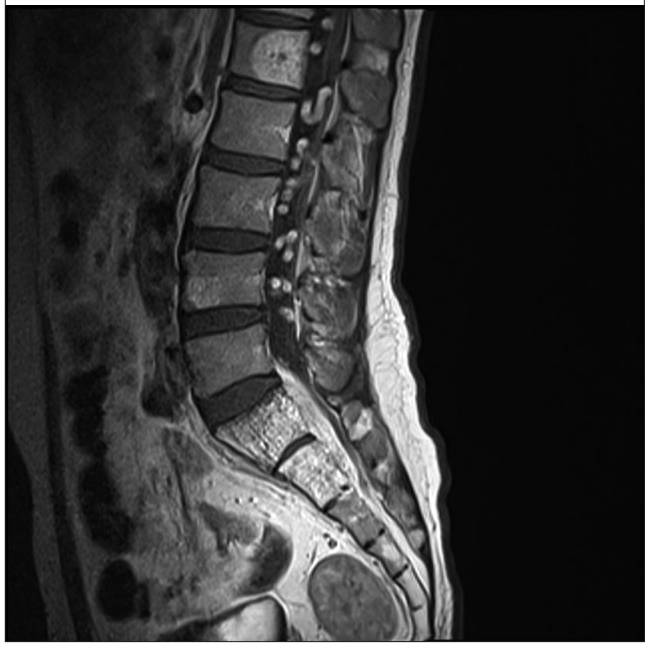
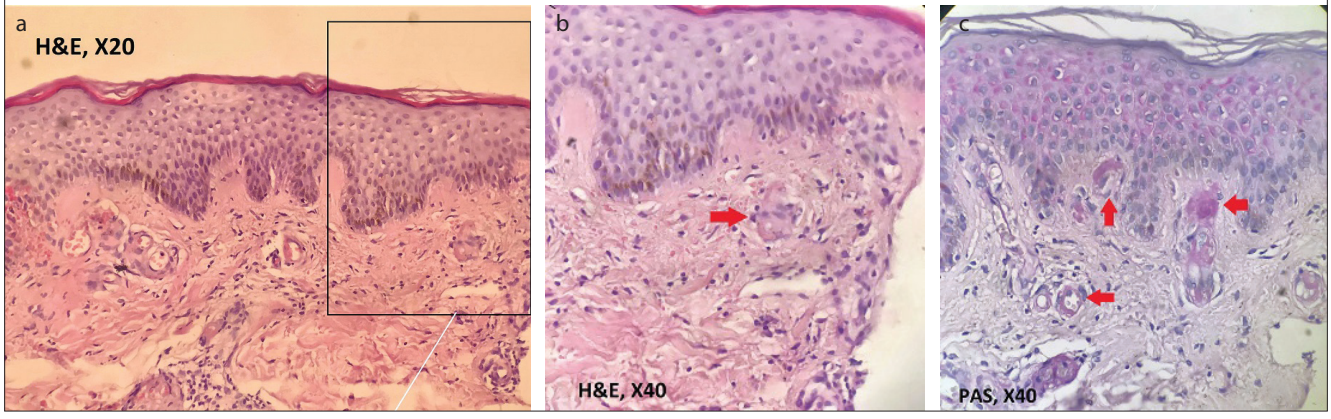


Figure 3. a-c. Histopathological examination showing abundant amounts of extra-red blood cells and lymphocytes, along with endothelial profiling in superficial vessels, all of which are consistent with vasculitis



The mechanism behind the development of paraneoplastic vasculitis is unknown, although it may cause tumor cells to act directly or indirectly as sensitizing agents, or to induce vascular damage to the cytokines that are secreted by the tumor (3). Although vasculitis is sometimes associated with malignancy, it is thought to appear incidentally in most cases (3). In our case, paraneoplastic vasculitis was not considered as the sacral hemangioma was a benign tumor.

Radiotherapy is used for a variety of oncologic conditions and radiation-induced changes to the skin are significant adverse effects of such therapies. Advances in technology and changes in therapeutic regimens have reduced the burden of the cutaneous side effects of radiotherapy (4).

Vertebral hemangiomas are indicated for treatment if the pain is severe and if there is a neurological deficit. In the previous literature, pain palliation was achieved in 82% of patients who underwent 36-40 Gy radiotherapy for a sacral hemangioma. Radiotherapy is a noninvasive, safe, and effective treatment option, although it is not known exactly which mechanism is effective in vertebral hemangiomas. Radiotherapy is thought to cause ischemic changes in segmental capillaries, and may, at the same time, affect the microvascular network. It also leads to a reduction in pain as a result of its anti-inflammatory effects (5). In the present case, radiotherapy was chosen as the treatment method for reasons of safety.

Radiotherapy may cause harm to Langerhans cells, basal cells, and vascular endothelium (6), and these cells have also been found to start an inflammatory cascade and ischemia-reperfusion damage (7). The inflammatory answer to radiation arises mainly from a proinflammatory cytokine cascade (IL-1, IL-3, IL-5, IL-6, TNF-a), chemokines (IL-8, eotaxin, CCR receptor), receptor tyrosine kinase, and adhesions molecules (ICAM-1, VCAM, E-selectin). These factors compose a reaction of eosinophils and neutrophils, leading to self-perpetuating tissue injury and a loss of preventive factors (8).

Until now, the use of radiotherapy in the treatment of cancer has been based on its high potential to induce tumor cell death and to halt the survival of clonogenic tumor cells. In recent years, the increasingly frequent emphasis has been placed on the immunological effects of radiotherapy, which has been well detailed by Vatner et al. (9) in the field of radioimmunotherapy. Although the effects of irradiation on the human immune system are clear, the diversity of the organ and cellular components of the immune system, their complex interactions, and the patterns of cellular migration have made the exact characterization of these abnormalities difficult (9). Vascular injury due to radiation is an uncommon side effect of radiation therapy, although it has been identified in many major vessels, including the aortic, renal, iliac, carotid, and subclavian-axillary arteries (10). To date, however, cutaneous vasculitis associated with radiation therapy has never been mentioned in literature. In our opinion, radiotherapy may bring on vasculitis by bringing about ischemic changes and immunity in capillary vessels.

CONCLUSION

To the best of our knowledge, there has been no research investigating vasculitis as a result of radiotherapy in the literature. Aiming to fill this gap, this research draws attention to cutaneous vasculitis as a possible immune-related side effect of radiotherapy.

Informed Consent: Written informed consent was obtained from the patient for the publication of this case report and the associated images.

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

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Case Report

Two Cases of Menkes Disease Diagnosed with Hair Findings and Novel Mutation

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ABSTRACT

Menkes disease is a rare neurodegenerative disorder. Its clinical signs and symptoms appear due to a defect in copper metabolism. Its clinical manifestation is marked by pili torti and trichorrhexis nodosa, which are the disease-specific hair findings. Additionally, neurological signs may occur, such as hypotonia and convulsions. Detection of a mutation affecting the P-type ATPase gene is highly specific. The lack of an effective treatment modality has increased the importance of prenatal diagnosis and genetic counseling. Here, we report two patients who were diagnosed with Menkes disease by virtue of hair findings and genetic studies while being tested for hypotonia. One of the patients was detected to have a novel mutation.

Keywords: Genetic, hair, hypotonia, Menkes disease

INTRODUCTION

Menkes disease (Menkes kinky hair syndrome) is an X-linked recessive neurodegenerative disorder that starts to manifest clinical findings in early infancy (1). In 1967, Menkes (2) first defined the disease in five male children from the same family who presented with neuromotor delay, growth retardation, white hair, and cerebral-cerebellar degeneration. The gene responsible for the disorder is found in Xq13.3 and causes deficiency of P-type ATPase (ATP7A) that is responsible for copper absorption and transport. The classical form of the disorder is characterized by severe neurological signs and has a fatal course. On the other hand, the more rarely encountered milder Menkes disease and the occipital horn syndrome are the milder forms. Serum copper and ceruloplasmin levels are low (3, 4). The disorder is typically seen in males, but it also rarely affects females. It has an estimated incidence of 1:35,000-1:250,000 (5). In this case report, the two classical cases of Menkes disease presented were reported to emphasize the disorder's genetic-based diagnosis and the importance of hair findings for its recognition.

CASE PRESENTATIONS

Case 1

A 12-month-old male infant was admitted to the pediatric neurology outpatient clinic for hypotonia and reduced attention to his environment. He had no notable feature regarding his prenatal history. He had been born on term spontaneously via normal vaginal route. His birth weight was 3250 g. His parents were first-degree cousins. On physical examination, he was conscious, restless, and hypoactive. He had a body temperature (armpit) of 36.6°C, a cardiac apex beat rate of 108/min, a respiratory rate of 24/min, blood pressure of 78/42 mmHg, body weight of 10 kg (50%), height of 76 cm (50%), head circumference of 43 cm (10%-25%), an anterior fontanel of 1×1 cm with normal curvature, a dry skin, a fair complexion, a dysmorphic face, brown eyes, and a wide auricula. No organomegaly or cardiac, respiratory, or gastrointestinal abnormality was noted. On neurological examination, hypotonia was revealed. He could not establish eye contact. His fundoscopic examination was normal. His deep tendon reflexes were hyperactive. On laboratory tests, complete blood count, routine biochemistry, including kidney and liver function tests, ammonia, lactate, coagulation tests, urinary organic acid, congenital metabolic screening, serum quantitative amino acid levels, vitamin B12 level, and biotinidase level were all normal. There was no abnormality regarding intrauterine infectious antibody (TORCH) titers. His cranial magnetic resonance (MR) and diffusion cranial MR imaging (MRI) tests showed cerebral atrophy (Figure 1). His cardiac examination, echocardiography, and abdominal ultrasonography (USG) were all normal. As he had light hair, his hair was examined microscopically, which showed pili torti and trichorrhexis nodosa (Figure 2). Serum copper level was <10 µg/dL (normal level: 85-190 µg/dL), and ceruloplasmin level was 0.06 g/L (normal level: 0.15-0.48 g/L). His whole gene analysis (Miseq-Illumina, Illumina Way, San Diego, CA/USA) revealed deletions in ATP7A gene's 15th, 16th, and 17th exons. The patient's family was offered genetic counseling. He was consulted by the

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Figure 1. Marked atrophy of the brain parenchyma in the frontotemporal region in Case 1

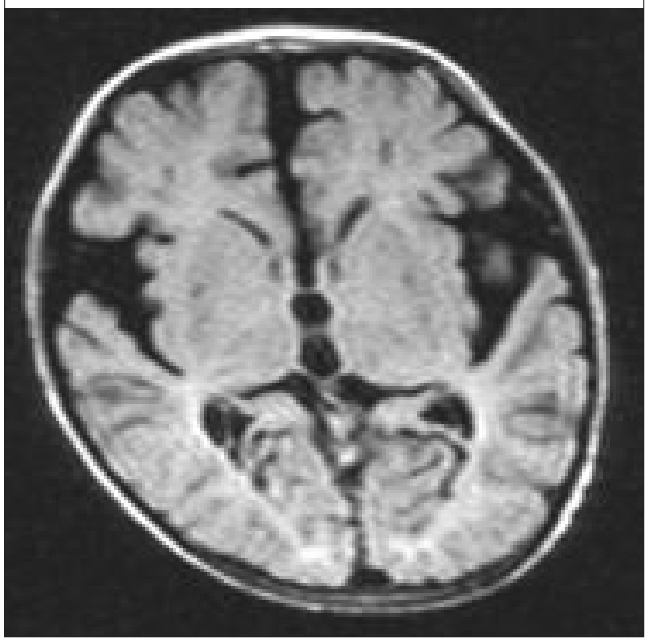
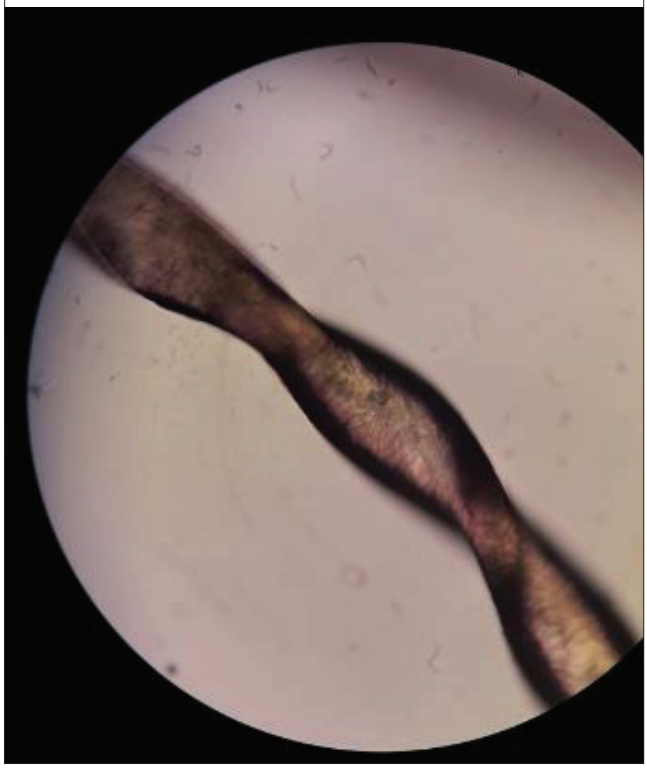
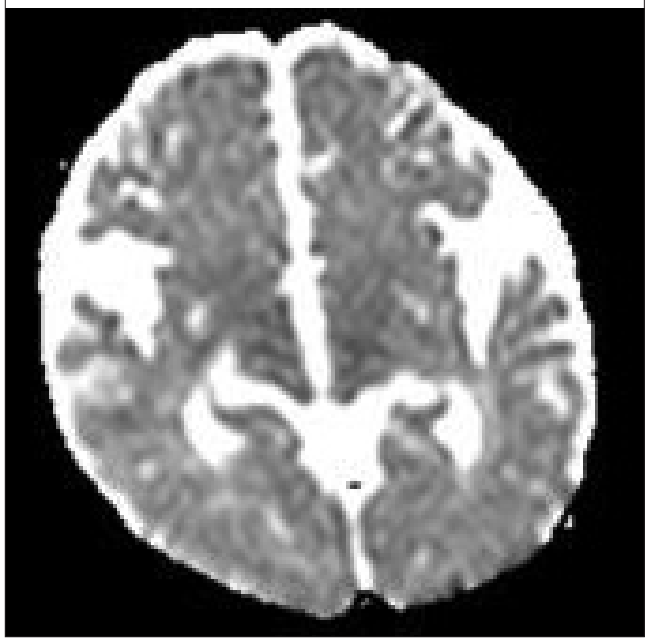


Figure 2. The pili torti sign in the microscopic examination of the hair in Case 1



physical therapy and rehabilitation clinic and started an exercise program. Written informed consent was obtained from the patient's parents.

Figure 3. Brain magnetic resonance imaging shows frontotemporal cortical atrophy in Case 2



Case 2

A 7-month-old male infant was admitted with weak head control and inability to sit unassisted. His prenatal history was unremarkable. He had been born on term via normal spontaneous vaginal route. His birth weight was 3080 g. His parents were second-degree relatives. On physical examination, he was conscious, restless, and hypoactive. He had a body temperature (armpit) of 37.2°C, a cardiac apex beat rate of 118/min, a respiratory rate of 27/min, blood pressure of 80/50 mmHg, body weight of 7.5 kg (25%-50%), height of 68 cm (25%-50%), an anterior fontanel of 3×2 cm with normal curvature, a dry skin, and a light complexion. He did not have organomegaly and cardiac, respiratory, or gastrointestinal abnormality. On neurological examination, he was hypotonic and made weak eye contact. His fundoscopic examination was normal. His deep tendon reflexes were active. On laboratory tests, complete blood count, routine biochemistry, including renal and hepatic function tests, ammonia, lactate, coagulation tests, urinary organic acid, congenital metabolic screening, blood amino acid levels, vitamin B12 level, and biotinidase level were all normal. There was no abnormality regarding intrauterine infectious serology (e.g., TORCH IgM and IgG). Cranial MR and diffusion cranial MR imaging showed cerebral parenchymal atrophy (Figure 3). On cardiac examination, a 2/6 murmur was revealed. On echocardiography, he had patent foramen oval and pulmonary stenosis. His abdominal USG was normal. As he had fair hair, a microscopic hair examination was performed, which revealed pili torti and trichorrhexis nodosa. His serum copper level was 36 µg/dL (normal level: 85-190 µg/dL), and ceruloplasmin level was 0.11 g/L (normal level: 0.15-0.48 g/L). A whole gene sequence analysis by in silico examination (Miseq-Illumina, Illumina Way, San Diego, CA/USA) showed a novel, previously unreported IV58

homozygous mutation in the ATP7A gene. Written informed consent was obtained from the patient's parents.

DISCUSSION

Copper is a trace element that is essential as a cofactor of many enzymes. ATP7A is the protein that is responsible for copper release from the intracellular to extracellular space, as well as copper transport in the intracellular space. The deficiency of this protein causes copper to accumulate inside the cell, making copper-dependent enzymes dysfunctional (1). In Menkes disease, the clinical signs appear due to dysfunctional copper-dependent enzymes, such as tyrosinase, cytochrome c oxidase, dopamine beta-hydroxylase, and lysyl oxidase (3). Its signs and symptoms include hypotonia, difficulty nourishing, convulsions, dysmorphic face, and cognitive and motor retardation that typically start in the first months of life (6). These signs may be confused with many chronic neurometabolic disorders. The available findings suggested neurometabolic disorders in both of our patients. As cerebral parenchymal atrophy was detected by cranial MRI, metabolic causes were sought. Our tests revealed no finding suggestive of congenital metabolic, infectious, or cardiac disorder. His neurological signs progressed during follow-up. His history was remarkable for consanguineous marriage, dysmorphic signs, and a progressive neurological course, which altogether suggested a neurodegenerative disorder. However, no specific finding was found in this regard. Our patient had no hair in the early infancy stage, and he was suspected to have Menkes disease only after he grew hair.

Hair signs are the major sign that supports the diagnosis of Menkes disease (4). Although rare, patients may have normal hair at birth, which later evolve into light-colored, short, brittle, wooly hair. The microscopic examination of the hair may reveal pili torti and trichorrhexis nodosa (1-5). Both of our patients were detected to have pili torti and trichorrhexis nodosa by the pathology examination of their light-colored, brittle, wooly hair. Light hair and pili torti may also be present in some other metabolic, hereditary disorders, such as phenylketonuria, trichorrhexis nodosa, or biotin deficiency (4, 5). The above differential diagnoses were excluded by family history of our patients, as well as their clinical, imaging, and laboratory findings.

Menkes disease is diagnosed based on reduced serum copper and ceruloplasmin levels (1, 3, 4). Our patients' low serum copper and ceruloplasmin levels supported the diagnosis. The definitive diagnosis of the disorder is made by genetic analysis (1-3). Prenatal diagnosis is now possible (1-5). The blood samples of our patients were studied for genetic etiology, and the mutations were revealed in both patients. The families were recommended genetic counseling, but they could not be reached thereafter.

Currently, there is no effective treatment for Menkes disease. The mean life expectancy for Menkes disease is 3 years. Starting treatment with subcutaneous copper histidine when the diagnosis is made in the early postnatal days has been reported to improve prognosis (6). The first patient was diagnosed at age approximately 12 months, and the second patient was diagnosed at age 7 months. Physical treatment and symptomatic treatment are applied to the patients for severe neurological complications.

CONCLUSION

In addition to progressive neurological signs, it should be kept in mind that typical hair signs may cause Menkes disease in the infancy period, and they have a prominent role for the differential diagnosis of chronic neurological disorders. The presence of a possible genetic diagnosis and genetic counseling opportunities increases the importance of the disorder.

Informed Consent: Written informed consent was obtained from patient's parents.

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