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Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

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

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

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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Review

Drug Resistance in Parasitic Diseases

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ABSTRACT

Owing to the lack of or the ineffectiveness of vaccines for life-threatening parasitic diseases, chemotherapy is the current strategy to prevent parasitic diseases. Drug resistance disrupts chemotherapeutic options, thereby increasing the need for novel drugs in parasitological treatments. The most common resistance mechanisms are decreased drug uptake, export of drugs from parasites, genetic modifications, loss of drug activity, and alteration of the drug target. Drug resistance mechanisms should be well defined to develop new strategies to control parasitic diseases. This measure will ensure new effective treatment options for clinicians. In the recent years, isolation and characterization of resistance-related genes and proteins has considerably increased our knowledge. This review mostly focuses on new studies and common parasitic diseases.

Keywords: Chemotherapy, drug resistance, parasitic diseases

INTRODUCTION

Globally, parasitic diseases have immense health, social, and economic impacts, especially in tropical countries. Protozoan and helminthic diseases (mostly malaria and schistosomiasis) have resulted in almost 1.1 million deaths. Moreover, globally distributed protozoan parasites lead to high disability-adjusted life years (1). Owing to the lack of licensed vaccines and effective drugs, the burden of parasitic diseases rises regularly. Moreover, drug resistance is a threat in regions where appropriate medication is available. Accordingly, there exists an emerging need for novel drugs for the effective treatment of protozoal diseases, particularly for malaria, toxoplasmosis, and leishmaniasis (2).

Malaria

Malaria is one of the most common protozoan infections with a high morbidity and mortality rate. This human disease is caused by five *Plasmodium* species (*P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*). According to the World Health Organization report in 2018, approximately 3.2 billion people were under the risk of malaria, 198 million were infected, and 584.000 deaths were reported with malaria globally (3). Antimalarial drugs are mainly divided into three groups according to their mechanisms of action: quinolone, antifolates, and artemisinin derivatives.

Globally, antimalarial drug resistance to *P. falciparum, P. vivax,* and *P. malariae* have been reported; numerous researches have highlighted this topic. Chloroquine (CQ) resistance spread in Africa, thereby causing a 2-3 fold increase in malaria-related deaths in the 1980s. The resistance in *P. falciparum* was characterized by a mutation on CQ resistance carrier (Pfcrt) gene, localized in a 36 kb segment on chromosome 7 (4). After this dramatic increase, sulphadoxine/pyrimethamine (SP) became the first choice antimalarial drug instead of CQ treatment. At the beginning of 2000, the parasite improved SP resistance. Accordingly, combination therapy regimens were applied to increase drug efficacy and slow the development of drug resistance (5). Recently, artemisinin-based combination therapies are effectively used to treat malaria. However, artemisinin resistance has appeared in Southeast Asia, leading to a global risk for malaria treatment and control (6).

Antifolate drugs inhibit *P. falciparum* dihydropteroate synthase (Pfdhps) and dihydrofolate reductase-thymidylate synthase (Pf-dhfr-ts) enzymes, which are essential for folate biosynthesis. Biochemical and genetic studies on *P. falciparum* have claimed that the mutations in the aforementioned genes reduced the drug sensitivity of antifolates. Whole-genome sequencing of an artemisinin-resistant parasite revealed that mutation in artemisinin resistance was associated with kelch 13 (K13) protein in clinical and field isolates of *P. falciparum* (7). The *P. falciparum* multidrug resistance protein 1 gene (Pfmdr1) is located on chromosome 5, which has a single exon. This protein is similar to PfCRT protein; it is found in the digestive vacuole of the parasite and acts as a basis for adenosine triphosphate (ATP) binding. The N86Y, Y184F, S1034C, N1042D, and D1246Y mutations in Pfmdr1 gene help detect the drug sensitivity of a variety of drugs such as CQ, quinine, mefloquine (MQ), halofantrine, lumefantrine, and artemisinin. Among these, N86Y and N1042D mutations are associated with resistance. The K76T and A220S mutations in the Pfcrt gene and the N86Y mutations in the Pfmdr1 gene are associated with high resistance

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to CQ. In addition, the variation in the copy number of Pfmdr1 gene depends on the resistance levels of kinase, MQ, lumefantrine, halofantrine, and artemisinin (8). Another *P. falciparum* multidrug resistance-associated protein gene (Pfmrp) is located on chromosome 1 and has one exon; it belongs to the ATP-binding cassette carrier family and resembles the Pfmdr1 gene. This protein facilitates the transport of organic anionic substrates such as oxidized glutathione, glucuronate, sulfate conjugates as well as drug transport. Two mutations at positions Y191H and A437S in the Pfmrp gene were found to be associated with CQ and quinine resistance. On chromosome 13, *P. falciparum* has Pfnhe1 gene, which has two exons that encode sodium-hydrogen exchanger protein and is associated with resistance to quinine (9). *Plasmodium falciparum* bifunctional dihydrofolate reductase-thymidylate synthase gene (Pfdhfr-ts): Pyrimethamine resistance is mainly associated with the point mutation of the S108D codon in this gene; other mutations in the N51I, C59N, and 166L positions support resistance as well. *Plasmodium falciparum* dihydropteroate synthase gene (Pfdhps): Five mutations in the Pfdhps gene (S436A/F, A437G, L540E, A581G, and A613T/S) were reported to be associated with sulfadoxine resistance in *P. falciparum* (10).

Atovaquone, an antimalarial drug that binds to the ubiquinol binding site of cytochrome b (cytb), destroys the electrochemical potential of the mitochondrial membrane and is lethal to the parasite. The ubiquinol binding site is a highly conserved region; once mutated, it gives resistance to atovaquone. A single mutation in the Y268N/S/C codon in the cytb gene was associated with atovaquone resistance in *P. falciparum* isolates (11).

Increased chloroquine sensitivity in *P. vivax* is closely related to the Y976F mutation in the Pvmdr1 gene, a homologue of Pfmdr1 (12). Unlike *P. falciparum*, the Pvcrt gene, a homologue of the Pfcrt gene, is not associated with CQ resistance in *P. vivax*. The MQ resistance in *P. vivax* is associated with the amplification of the Pvmdr1 gene. In addition, in vitro studies have revealed that Y976F mutation in Pvmdr1 gene was associated with resistance to MQ and artesunate. However, further clinical trials are needed in this case. The point mutation in the codon F57L/ I, S58R, T61M, and S117T/N of the Pvdhfr gene has been reported to be associated with pyrimethamine resistance and treatment failure in *P. vivax* (13).

Toxoplasmosis

Toxoplasma gondii (*T. gondii*), the causative agent of toxoplasmosis, is an intracellular parasite infecting humans and a wide variety of vertebrates. Infection is usually asymptomatic; however, immunosuppression and congenital can lead to life-threatening outcomes in infants and congenitally infected fetuses in pregnancy. Three main clones of *T. gondii* have been identified: Type I (RH etc., highly virulent), Type II (ME-49 and PRU etc., avirulent), and Type III (NED etc., avirulent) (14). Sulfonamide and pyrimethamine are commonly used drugs to treat toxoplasmosis. They have synergistic effects in inhibiting *T. gondii* replication by sequentially inhibiting parasite dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr). These two enzymes prevent the synthesis of the folate compounds required for the survival and replication of parasite. However, numerous treatment failures have been reported in toxoplasmic encephalitis, chorioretinitis, and congenital infection. Some failures may be associated with drug intolerance, malabsorption, and/or drug resistance (15). In a study, common anti *T. gondii* drugs were tested in vitro on 17 different strains: sulfadiazine (SDZ), atovaquone, and pyrimethamine. Despite some differences, no resistance to pyrimethamine and atovaquone was detected; however, resistance to SDZ was detected in three strains (16). The amino acid mutations in dhps result in resistance to sulfonamides and sulfones. Antifolate resistance due to the point mutations in dhps and dhfr coding genes was reported in *P. falciparum*. Pyrimethamine resistance was linked to a mutation in the dhfr enzyme (Ser-108 Asn 108) and other mutations (N51I, C59R, I164L, and A16V). Resistance to sulfonamides and sulfones were due to the amino acid mutations in dhps at five positions (S436A/F, A437G, K540E, A581G, and A613/T) (17). Aspinall et al. (18) revealed six mutations at positions 407, 474, 560, 580, 597, and 627 in the dhps gene of *T. gondii*. In sulfonamide resistance, only one mutation (at 407) was reported to be equivalent to *Plasmodium* species (at 437). This mutation has also been detected in a sulfamethoxazole-resistant strain, which has been rendered resistant in the laboratory. In a study among five *T. gondii* isolates from congenital toxoplasmosis, dhps was compared between those and previous isolates. Four isolates have been reported to be resistant to SDZ. Nineteen polymorphisms were detected in the exon of the dhps gene, and four were detected for the first time in this study. However, no relationship exists between SDZ susceptibility and gene polymorphism (15). In a study conducted in 2017, a previously unidentified mitochondrial protein (TgPRELID) was identified in *T. gondii* and associated with multiple drug resistance. Furthermore, the study reported that the mechanism of resistance was necessary to investigate (19).

Main Points:

- Drug resistance in life-threating parasitic diseases is a major problem.
- The main mechanism of resistance are drug uptake, export of drugs from parasites, genetic modifications, loss of drug activity, and alteration of the drug target.
- New strategies to control parasitic diseases are necessary.
- The contribution of genetic studies to drug resistance is very precious.

Leishmaniasis

Leishmaniasis is a vector-borne infectious disease with a zoonotic/anthropic character, spreading worldwide except Antarctica. The disease has different forms: cutaneous (CL)/mucocutaneous leishmaniasis are relatively less important, non-lethal, and self-healing skin infections; visceral leishmaniasis (also known as Kala-azar) is a systemic infection that effect viscera and cause deaths of people in epidemics. Almost 12 million people in 98 countries have been infected with *Leishmania* species, and 350 million people live in risky regions (20). In Turkey, approximately 2000 leishmaniasis cases are reported annually.

Sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®) are used as the first choice in the treatment of leishmaniasis for more than 50 years. In recent years, resistance has appeared in South America, Europe, and the Middle

East, and India in particular. Treatment with pentavalent antimony compounds in Bihar, a leishmaniasis endemic region in India, has failed in 60% of the cases. Alternatively, few drugs that can be used include amphotericin B, pentamidine, and oral miltefosine. Reduced efficacy of miltefosine and resistant cases of meglumine antimoniate in the treatment of CL in the Middle East has been reported (21). The presence of drug-resistant leishmaniasis cases has also been reported in Turkey from Urfa, Hatay, Diyarbakir, and Aydin (unpublished data).

Resistance mechanisms in leishmaniasis have been better understood through molecular studies in recent years. The ubiquitin and amino acid permease (AAP3) have been reported to play a role in resistance in *L. tropica* isolates (22). In addition, another study found five genes that could play a role in resistance: aquaglyceroporin (AQP1) and ATP-binding cassette transporter (abc-3) that play a role in drug release; phosphoglycerate kinase (PGK) that plays a role in glycolysis metabolism; and mitogen-activated protein kinase (MAPK) and protein tyrosine phosphatase (PTP) responsible for the phosphorylation pathway. In resistant isolates, three of these genes (multidrug resistance protein A, PTP, and PGK) were reported to be upregulated and the other two (AQP1 and MAPK) were downregulated (23). MAPK1 (Ld-MAPK1) is associated with antimony resistance in *L. donovani*. Moreover, *L. major* MAP2 antimony resistance is regulated by phosphorylating influx pump AQ120. In another study, increased abc-3 and decreased AQP1 gene expression were shown in laboratory-derived Sb-resistant *L. panamanensis* isolates. However, it was not significant in clinical isolates in which abc-2 was significantly higher. Laboratory and clinical Sb-sensitive/resistant *L. panamanensis* isolates were significantly increased mt2a (xenobiotic scavenging) expression in Sb-sensitive isolates in different types of macrophages. Thus, gene expression was associated with drug transport, and metabolism in parasite-infected cells might also be important in resistance and susceptibility to *Leishmania spp*. (24). Antimony resistance mechanisms are usually studied experimentally in Leishmania because of the intracellular location of the parasite. Therefore, studying the effectiveness of drugs is difficult due to the release of the drug into the host cell and the interference of the drug in the cell compartments.

Giardiasis

Giardia intestinalis (*G. intestinalis, G.lambia*) is a microaerophilic protozoon found in the gastrointestinal tract of humans and an important cause of steatorrhea with an incidence of 200–300 million cases and an estimated prevalence of 1 billion (25). It is one of the most common intestinal parasites in our country. Depending on the genotype and drug resistance of the parasite, acute or chronic disease can develop. Symptoms include nausea, swelling, diarrhoea, vomiting, dehydration, malabsorption, and growth retardation. Treatment with different drugs have been used: Metronidazole (MTZ) (efficiency 73% to 100%), furazolidone, nitazoxanide, and benzimidazoles (albendazole and mebendazole) (26). Mutations in *G. intestinalis* ferredoxin oxidoreductase gene play a role in metronidazole resistance. In Iran, ferredoxin and GINR (*G. lamblia* nitroreductase) genes were investigated in 40 isolates from 38 symptomatic and 2 MTZ-resistant cases; accordingly, nitazoxanide could be used instead of MTZ due to the low mutations in these genes in symptomatic and resistant cases, and the resistance mechanisms were different as well. Therefore, a high ferredoxin mutation was detected in MTZ-resistant cases, and the number of resistant *G. intestinalis* isolates was increased (27).

Amoebiasis

Entamoeba histolytica (*E. histolytica*) is the causative agent of amoebiasis, affecting 500 million people annually. The parasite is transmitted by faecal-oral route and may invade other tissues, mainly liver. MTZ is the most common frequent drug choice for intestinal amoebiasis and amoebic liver abscesses. Although the mode of action is not fully understood, it inhibits DNA synthesis and damage to DNA, proteins, and other cell components by oxidation, as studied from other microorganisms (28). Pathogens develop different resistance mechanisms to MTZ; these mechanisms are associated with altered reduction efficiency, drug inactivation, decreased drug uptake, and increased DNA damage. Clinical MTZ-resistant *E. histolytica* isolates have been identified; however, in vitro resistant isolates have not yet been achieved. MTZ resistance in *E. histolytica* is linked with high iron-containing superoxide dismutase and peroxiredoxin as well as low expression of ferredoxin 1 and flavin reductase (29).

Trichomoniasis

Trichomoniasis is caused by *Trichomonas vaginalis* (*T. vaginalis*) and is the most common non-viral sexually transmitted infection in the world, with 276 million new cases per year. In Turkey, the frequency of *T. vaginalis* in different groups has been reported between 0.3% and 9% in recent studies. The first treatment choice of trichomoniasis is 5-nitroimidazole compounds; among these, MTZ and tinidazole are the most commonly recommended and used drugs. However, MTZ resistance has been reported in various countries since 1962. A study from Aydin presented the MTZ-resistant isolates for the first time in Turkey and reported 7.5% (3 out of 40) in vitro resistance among *T. vaginalis* isolates (30). Tinidazole, ornidazole, furazolidone, and topical pramoxine are currently available drugs used in MTZ-resistant cases. Nitazoxanide, a broad spectrum and low toxicity drug, was found to be effective in MTZ-resistant *T. vaginalis* in vitro and in clinically resistant cases (31). *T. vaginalis* trophozoites use low redox-potent electron-transporting proteins such as pyruvate ferredoxin oxidoreductase (PFOR) and ferredoxin. The reduced PFOR activity of the five-nitroimidazole resistance may be due to the changing structure of the hydrogenosome, the unexpected redox potential in ferredoxin, or intracellular ferredoxin reduction. In the recent studies, genetic markers of MTZ resistance are being investigated. Totally, 72 single nucleotide polymorphisms (SNPs) were related to MTZ resistance in clinical and laboratory isolates of *T. vaginalis*. Some of these identified SNPs were related to resistance (eg., Pfor gene) and drug activation (32).

Nematode infections

Parasitic helminth infections are common in developing countries; *Ascaris lumbricoides* (*A. lumbricoides*) infect 800 million people worldwide. In endemic countries, school children are treated with albendazole or mebendazole twice a year to prevent helminth infections. Benzimidazole (BZ) derivatives (albendazole,

fenbendazole, oxfendazole, mebendazole, and triclabendazole) are widely used to treat nematode diseases, which disrupt tubulin formation (33). Albendazole has been used for the treatment of helminth diseases for about 20 years; however, the presence of resistant isolates has been reported. Molecular tests have been applied in recent years to detect resistance in threadworm species. Different β-tubulin paralogs in the strongyloid and ascarid genomes cause misperception. BZ resistance is common among isotype 1; however, it is rare among isotype 2. In a previous study, no association was found between resistance to changes in the four separate β-tubulin genes in *A. lumbricoides*. The study reported that resistance may not be due to genetically resistant parasites but to other mechanisms such as drug metabolism (34). In whipworm, *Trichuris trichiura*, only one β-tubulin gene was found to be present in a specific isotype. The frequency was found to be increased in the treated cases, which was considered as a candidate for resistance development (35).

Ectoparasites

Pediculus humanus var. capitis (head louse), *P. humanus var. corporis* (body louse), and *Phthirus pubis* (pubic louse) are the louse species that parasitize on humans. These are permanent and obligate ectoparasites that feed with blood and cause pediculosis. The application of topical insecticides is the most effective method in pediculosis treatment. Today, for the treatment of lice, pediculicides such as natural pyrethrins (pyrethrum), synthetic "pyrethroid" (permethrin, phenothrin), organochlorine (indole), organophosphorus (malathion), and carbamate (carbaryl) are commonly used (36). Pyrethrin/pyrethroids and Dichlorodiphenyltrichloroethane target the domain in the voltage-sensitive sodium channel (VSSC) nervous system and increase the sodium flux. They result in neuromuscular paralysis and death by nerve depolarization and hyperexcitations. The widespread use of insecticides and the lack of appropriate replacements cause the development of resistance in pediculosis. One of the resistance mechanisms to pyrethrins or pyrethroids is the target region insensitivity of knockdown resistance (kdr), a heritable feature. Three-point mutations (M815I, T917I, and L920F) in the transmembrane segment were found, and these were present as haplotypes in the permethrin-resistant head lice populations (37). In a study from the United States, 908 bp VSSC α-subunit gene region was studied and the number of resistant lice was increased in comparison with that in the previous years (38).

Scabies is a dermal infection in humans caused by *Sarcoptes scabiei* (*S. scabiei*). It is still an important public health problem in the world, especially in developing countries. Permethrin (5% cream) is used for the first-step treatment in many countries; however, esdepalletrin is used in France instead of permethrin. Other common acaricides are benzyl benzoate 10% to 25%, crotamiton, or oral gum. The intensive use of pyrethroid compounds over the last 30 years has led to the development of resistance mechanisms in many arthropods (39). SNPs play an important role in resistance to pyrethroids in some arthropods. In vitro studies have revealed that the susceptibility of *S. scabiei* to permethrin is gradually reduced by repeated administration. In addition, an SNP at codon 733 in the VSSC gene is related with permethrin resistance in vivo and in vitro studies (40).

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Review

Immune Response and its Effects on the Host during Helminthic Infections

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ABSTRACT

Helminths are multicellular organisms causing chronic infections affecting nearly one-third of the global population. They are experts at immunomodulation, and pathologic outcomes are generally observed in patients with immunodeficiencies or with exaggerated levels of anti-helminth immune responses. Elimination of helminths is usually mediated by T-helper type-2 (Th2) immune responses, characterized by the induction of Immunoglobulin E (IgE) release, increase in eosinophil and mast cell levels, and elevation in the production levels of Th2 cytokines. However, the triggered mechanisms may also depend on the location of the parasite. This is because tissue invasion, an immune evasion strategy for parasites, was considered to activate more Thelper type 1 (Th1) cells in tissues. During chronic infections, immune response regulatory pathways become more influential, thereby reducing the levels of the peripheral T-cell-mediated responses against parasitic antigens. The resultant immune response is termed as "modified Th2 response" and is characterized by enhanced levels of anti-inflammatory cytokine production and regulatory immune cells as well as high IgG4/IgE ratios. Immunomodulation during chronic helminth infection is not limited to only parasite-specific responses. It can influence the efficiency of vaccination, host susceptibility to infections, and allergen or autoantigen responses. This review discusses anti-helminth immune responses. Moreover, it highlights current literature on the effects of chronic helminth infections on host health as well as their possible use as a treatment strategy against autoimmune, autoinflammatory, and allergic diseases.

Keywords: Co-infection, helminth, immune response, therapy, vaccine

INTRODUCTION

Helminths are parasitic multicellular organisms that include nematodes, cestodes, and trematodes (1). Although they are one of the most common infectious agents infecting nearly one-third of the global population today, they cause mostly asymptomatic infections (2,3). Pathologic outcomes can be observed in immunocompromised individuals and also in those with high levels of immune response triggered against low parasitic burden (3).

Helminths can exploit the host immune system for their own benefit and can survive within the host for weeks, months, or even years. They can utilize a wide range of immunomodulatory mechanisms, such as the secretion of molecules, that inhibit immune cell function and induce regulatory pathways (3). Since they are considered as experts in immunomodulation, helminths are currently being studied for their use in the treatment of allergic and autoimmune diseases. Autoimmune and abnormal T-helper type-2 (Th2 cells) cell-related disease (such as asthma or allergic rhinitis) levels in helminth-infected populations are relatively low (4). According to the "old friend" hypothesis, microorganisms, including helminths, evolved along with mammalian hosts over the ages and acted as triggers of immunomodulator mechanisms required for the development of a healthy immune system (1).

CLINICAL AND RESEARCH CONSEQUENCES

Effective Immune Response Triggered Against Helminths

Helminths are generally associated with host Th2 immune responses, which can be initiated to repair tissue damage as well as in disease states such as allergy and asthma (5). Because of this association, Th2 cells are thought to be triggered to improve resistance to helminths as well as to repair tissue damage caused by helminths colonizing tissues (3). The response is characterized by Immunoglobulin E (IgE) release, eosinophilia, mastocytosis, goblet cell differentiation, increased mucus production, and the production of Th2 cytokines such as interleukin-4 (IL-4) and IL-5 (6).

Although T-cells were initially thought to be the only source for Th2 cytokines, innate immune cells can also function as a reservoir

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for these cytokines. For example, previous studies have revealed that basophils, eosinophils, multipotent progenitor type 2 cells (MPPtype2), and type 2 innate lymphoid cells (ILC-2) are important sources for IL-4, IL-13, and IL-5 (7). Among those, ILC-2 was shown to be dependent on adaptive immune cells such that in the absence of B-cells and T-cells, they failed to facilitate worm expulsion from the host (8, 9). In addition to these cells, intestinal epithelial cells (IECs) were also shown to engage in the development of Th2 immune responses (10). Inability to develop Th2 immunity during helminth infections in mice has been associated with the disappearance of intestinal protective properties and subsequent fatal sepsis, resulting in intestinal bacterial infection (3).

While Th2 cells rarely kill parasites, they limit the infection, reduce the viability and reproductive properties of helminths, and physically remove them from the mucosal membranes (11). Nevertheless, the effector mechanisms may vary depending on the location of the parasite, whether in the duct lumen or in the tissue. Removal of parasites from lumenal regions depends on IgE-mediated mast cell degranulation and intestinal anaphylaxis, which is responsible for muscle contractility, fluid stimulation, vascular permeability enhancement, immune cell recruitment, and mucus secretion (11). Although the expulsion was triggered by Th2 cytokine release (6), it was mediated in the absence of adaptive immune system by IECs (8). Immunoglobin A (IgA) antibodies, another mediator, released during Th2 immunization are essential for the neutralization of metabolic enzymes as well as influence parasite nutrition (11).

Furthermore, tissue invasion, which is considered as a strategy for the parasite to evade from anti-helminth Th2 responses, activates T helper type 1 (Th1) immunity in these regions (11). Trematode strains such as *Schistosoma* spp. were suggested to require collaboration between Th2 and Th1 responses for effective protection (11). Th1 immunity mainly targets adult parasites, and the Th2 skewed response is initiated after parasite's eggs are produced (12). The absence of effective Th2 immunity at this stage may result in granulomatous inflammation mediated by Th1 and T helper type 17 (Th17) cells, thereby causing severe damage and death to the surrounding tissues (12). Effector mechanisms against helminths in tissues mainly include antibody-dependent cellular cytotoxicity, nitric oxide release by classically activated M1 macrophages, and granuloma formation (11).

Granuloma formation is frequently associated with Th1 immunity; however, it may also be detected during Th2 immune responses (11). Neutrophils and macrophages are among the first line of defence. They are responsible for the rapid development of a granulomatous build-up involving Th2 cells, eosinophils, and alternatively activated M2 macrophages (12). As time elapses after granuloma formation, fibrous extracellular matrix levels in granulomas increase. Controlled fibrogenesis are beneficial because of the limitation of granulomatous content, thereby preventing inflammation and damage caused by the spread of toxic egg products (13). Excessive fibrosis can be a serious complication, and stimulated tissue fibrosis may become pathologic (14). In addition, neutrophils may be invoked by helminths and may be effective against the parasites (15).

Main Points:

- While the elimination of helminths is mainly mediated by the induction of Th2-mediated immune response, tissue invasion can lead to activation of more Th1 cells.
- "Modified Th2 response", which is observed during chronic helminth infections, is characterized by enhanced levels of anti-inflammatory cytokine production and regulatory immune cells as well as high IgG4/IgE ratios.
- The immunomodulation during chronic helminth infection can influence the efficiency of vaccination, host susceptibility to infections, and allergen or autoantigen responses.

In contrast to classically activated M1 macrophages associated with high expression levels of pro-inflammatory cytokines and Th1 responses, M2 macrophages that are differentiated during the T_H2 immune responses participate in tissue remodelling and tumour progression as well as in anti-parasitic immunity (16). In addition to the contribution of control of parasitic tissue damage, M2 macrophages were also shown to be effective in providing protection against helminth infections by influencing the effects of Th2 cytokines, intestinal smooth muscle contractility, and worm expulsion (17).

The importance of the dendritic cells (DC) during the antigen presentation process in the initiation of anti-helminth immune responses is under debate (7). A recent study emphasized that basophils are involved in antigen presentation during *Trichuris muris* (*T. muris*) infection (18). Selective elimination of basophils from mice considerably reduced the levels of IL-4 mRNA expression and Th2 cytokine-dependent goblet cell hyperplasia in mice. In the same study, basophils were reported to induce CD4+ T cell proliferation in an MHC class II-dependent manner *in vitro* (18).

Regulatory Immune Response Triggered by Helminths

Peripheral T cells are known to be rendered insensitive to parasite antigens during chronic helminth infections (2). Helminthic parasites can directly act on the host immune cells to block their function and modify the immune response for their own survival within the host. The regulatory pathways triggered for this purpose causes the development of a host immune response known as the "modified Th2 response." This response, associated with anti-inflammatory cytokine production, such as IL-10 and Transforming Growth Factor (TGF)-β, and high IgG4/IgE ratios, besides regulatory immune cells, is likely involved in the Th2 immune reaction and play an active role in limitation of the overt symptoms frequently seen in helminth disease (4, 11).

Among the regulatory immune cells activated during the anti-helminth immune response, regulatory T-cells (Treg cells) comprise various subgroups. The cells in which Foxp3 transcription factor expression is initiated following the developmental stages in the thymus are called "natural" Treg (nTreg); cells that initiate Foxp3 expression in peripheral tissues are termed "induced" Treg cells. Treg cells such as type 1 regulatory (Tr1) cells do not possess any detectable level of Foxp3 expression. All these Treg populations can exert immunosuppressive effects by releasing IL-10

and TGF-β cytokines (19). Among those populations, in particular, Foxp3+ Treg cells have an important role in the development of self-tolerance, such that mutations affecting Foxp3 expression were shown to cause autoimmune diseases by influencing Treg cell expression and/or functional levels (19).

Microfilaremic filariasis (Mf) patients were previously reported to have higher Foxp3+ Treg cell levels than the control group patients (20). In addition, an *in vitro* study revealed an elevation of Th2 in Mf-positive patients when Treg cells were depleted (21). Although an increase in nTreg marker expression levels was observed during asymptomatic infection, such an increase was not observed in patients with filarial lymphedema (22). Therefore, the pathogenesis of lymphatic pathology during filarial infections is associated with the strengthening of pro-inflammatory responses and lowering of anti-inflammatory cell subset levels (22).

Tr1 cells have also been implicated in immunosuppression during infection with *Onchocerca volvulus* (*O. volvulus*), another parasitic filarial nematode worm (23). Peripheral blood mononuclear cells from individuals suffering from general oncocytosis produce higher levels of IL-10, and the observed decline in T-cell proliferative levels in this group can be reversed by anti-IL-10 and anti-TGF-β antibodies (24). In addition to these cases, Treg cells have also been associated with the pathogenesis of *Schistosoma mansoni* (*S. mansoni*), *S. Haematobium*, and hookworm infections (2).

There are other adaptive immune cells called regulatory B-cells (Breg) that are effective in the modulation of the immune responses. Like Treg cells, Breg cells were shown to be influential in the suppression of autoimmune diseases (25). Similar to Treg cells, Breg cells are thought to suppress pathogenic T cells and autoreactive B cells via cellular contact and release of cytokines such as IL-10 and TGF-β (25). Besides, they are also known to suppress immune responses by inducing Treg differentiation, suppressing the DC antigen-presentation function and releasing anti-inflammatory antibody isotypes such as IgG4 (26). In mouse experiments, the induction of Breg cells has been previously documented during infections with *S. mansoni* and *Heligosomoides polygyrus* (2).

DCs play an important role in immune responses induced by helminths. Accordingly, mice injected with DCs treated with helminth extracts *in vitro* have been reported to induce Th2 immunologic responses (27). In contrast, DCs have also been reported to trigger mechanisms responsible for the regulation of the immune responses (28). Currently, the mechanisms that induce Th2 or Treg differentiation are not yet fully clarified. However. the maturation levels in tolerance-inducing DCs are low, and helminth products were shown to inhibit IL-12 secretion by DC (2). Furthermore, some helminth products have been shown to interfere with DC function by blocking host antigen processing or inducing mRNA degradation (2).

Additionally, M2 macrophages, another innate immune cell, have also been reported to suppress T-cell responses (29). The human patent filariasis infection pathogenesis was shown to be related with the induction of M2 macrophages (30, 31). Monocytes from non-endemic donors directly inhibit CD4+ T cell proliferation and cytokine production in IL-10 or PD-1-mediated manner when stimulated with Mf-lysates (32).

Effect of Chronic Helminth Infection on Host Bystander Responses

Immunosuppression during helminth infection is not limited to only parasite-specific responses, and chronic helminth infections have also been reported to impact the efficiency of vaccination, host susceptibility to infections, and allergen or autoantigen responses. The effects of helminths on the immune responses can be classified into two distinct categories: inhibitors of Th1, Th2, and Th17 immunoreactivity through regulatory mechanisms (eg., Treg cells) and inhibitors of Th1 and Th17 mediated responses by activating the Th2 responses (33).

Efficiency of Vaccination

Levels of Th1 cytokines released in response to oral cholera vaccination drop in the presence of parasitic infection, and anti-parasitic worm therapy before challenge partially reverses this reduction (34). The same effect was also observed in the immune responses induced by the tetanus vaccine, and the level of Th1 response triggered was found to be lower in patients with schistosomiasis, onchocerciasis, and lymphatic filariasis (35-37). In addition, low Bacillus Calmette–Guérin vaccine immunogenicity level was observed in helminth-infected individuals (38).

Co-Infection

Due to the above-mentioned effects on the immune system, helminths are believed to provide resistance to Th2-responsive pathogens and increase host sensitivity to pathogens that induce Th1 immune responses (11). Accordingly, an increase in the prevalence of bacterial infections such as malaria, HIV/AIDS, and tuberculosis was detected in areas where helminth infection was endemic (39). Patients suffering from helminth infection have lower levels of immune responses against malaria, HIV, and tuberculosis than the control group (40-43).

Allergic, Autoinflammation, and Autoimmune Diseases

According to the "hygiene hypothesis," decreased exposure to childhood infections due to increased hygienic conditions in the Western and developing countries reduces the possibility of cross infection and prevents the development of a healthy immune system, increasing the chances of autoimmune and allergic diseases in later ages (1). In accordance with this view, many studies have observed relatively low levels of autoimmune and abnormal Th2-related disease (such as asthma or allergic rhinitis) cases in helminth-infected populations (4).

Epidemiological and immunological evidence has led to the use of helminth parasites to carry out many clinical trials for the treatment of diseases such as allergy, autoimmunity, and autoinflammation. Studies with the administration of *Trichuris suis* (*T. suis*) eggs (TSO) for the treatment of inflammatory bowel diseases, Crohn's disease, multiple sclerosis, and colitis reported a reduction in disease severity (44-47). Besides, a study using human hookworms also reported a reduction in the levels of pathologi-

cal Th1/Th17 immune responses responsible for celiac disease by the induction of Th2 and IL-10 pathways (48). Another study also revealed that Necator americanus may be effective against food allergies via induction of Treg cells (49).

On the other hand, there are also studies in the literature showing that using helminths for treatment has no effect on the patients monitored. These conflicting findings can be explained by the helminth genus used (1). Studies focusing on the therapeutic use of helminth or helminth derivative products still continue today (50).

CONCLUSION

Helminths can manipulate the host immune system for their own benefit and survive in the host for a long time. Due to these excellent immunomodulatory properties, chronic helminth infections can influence vaccine efficacy as well as susceptibility to pathogens in the environment and is held responsible for the increased prevalence of allergic and autoimmune diseases observed, especially in developed countries. Therefore, helminths and immunomodulator products they express are probably future anti-inflammatory molecules to be used against autoinflammation, autoimmune diseases, and allergies. Since the effects of helminths on the host immune system cannot be generalized among species, future work on the immune responses induced by candidate therapeutic agents will be a unique contribution to this area.

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Original Research

Providing Graft Tension in ACL Reconstruction with Preservation of the Hamstring Tibial Attachment Site: A Report on the Technique and Clinical Results

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ABSTRACT

Objective: Hamstring autograft is the most commonly used graft in the surgical technique for anterior cruciate ligament reconstruction. Different femoral fixation materials can be used in this surgery. This study aimed to share the surgical technique for anatomic single-band anterior cruciate ligament reconstruction, preserving hamstring tibial attachment site and the clinical results.

Methods: Total 42 consecutive patients who were operated for anterior cruciate ligament rupture were included in the study. Anatomic single-band anterior cruciate ligament reconstruction was performed for patients without disjointing the hamstring distal attachment site. Patients were evaluated in terms of age, sex, four-arm tendon length, total tunnel length from the hamstring attachment site, femoral tunnel length, length of the graft in the femoral tunnel, and the average tendon length calculated as per the tunnel length. The mean follow-up duration was 17 months. The patients were evaluated clinically using the Tegner activity score, Lysholm score, and International Knee Documentation Scale (IKDC). The anterior translation of the tibia was evaluated with a KT 1000 device.

Results: The preoperative and postoperative mean Tegner, IKDC, and Lysholm scores were improved significantly.

Conclusion: The surgical method for anatomic single-band anterior cruciate ligament reconstruction with preservation of the hamstring attachment site is a useful technique. Moreover, this technique is cost-effective and did not increase patient morbidity.

Keywords: ACL reconstruction, graft tension, hamstring tendon preserving

INTRODUCTION

Hamstring autograft is the most commonly used graft in the surgical technique for anterior cruciate ligament reconstruction. Different femoral fixation materials can be used in this surgery (1, 2). In the anterior cruciate ligament reconstruction technique with Hamstring autograft, the graft is first removed from the attachment site. Thereafter, the graft is inserted through the tibial and femoral tunnel drilled with appropriate diamater and fixed on the femoral tunnel. Then, upon stretching from the tibial side, it is fixed in the tibial tunnel. Stretching is performed on the tibial side, and the fixation is ensured here. In this technique, graft rupture and pull out are possible early complications (3). It is known that the hamstring graft undergoes necrosis in 4 weeks during its ligamentization, after which it is revascularized and ligamentized (3, 4). During this period, the graft is weak, and there is a possibility of rupture (3). On the tibial side, there is a risk of pulling out of the graft before the graft tunnel is completely healed (3). In our study, we harvested the hamstring autograft, preserving the tibial attachment site, and have shared our results of the anterior cruciate ligament reconstruction surgery. We used the ToggleLoc Fixation Device with ZipLoopTechnology by Biomet Orthopedics 56 East Bell Drive P.O. Box 587 Warsaw, Indiana 46581 USA for femoral fixation. Owing to the use of this device and the measurements we performed during the surgery, we ensured graft tension and completed the surgery. The clinical results of this surgery are shared in this report.

METHODS

This was a retrospective study. All the procedures performed in studies involving human participants were as per the ethical

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standards of the institutional and national research committees and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the subjects enrolled in the study.

Total 42 adult patients who underwent single-band anatomic anterior cruciate ligament reconstruction with a 4-arm hamstring autograft at the Orthopedics and Traumatology Clinic between January 2016 and December 2017 were included in this study retrospectively. For the dependent groups, t-test was used. Mean and standard deviation values were calculated as descriptive statistics, and a $p<0.05$ was considered to indicate statistical significance.

Surgical treatment was indicated for patients with positive (+) Lachman test result in the physical examination, a complaint of instability, and anterior cruciate ligament rupture in the MRI. All the patients were operated by the same surgeon. The patients who had undergone anterior cruciate ligament reconstruction surgery previously were excluded. The patients were evaluated in terms of age, sex, four-arm tendon length, total tunnel length from the hamstring attachment site, femoral tunnel length, length of the graft in the femoral tunnel, and the average tendon length calculated as per the tunnel length. The mean follow-up period was 17.86±5.266 months. The patients were clinically evaluated before the operation and at the last visit after the operation with the IKDC subjective evaluation score, Lysholm score, and Tegner activity score. The anterior translation of the tibia was measured with a KT 1000 device.

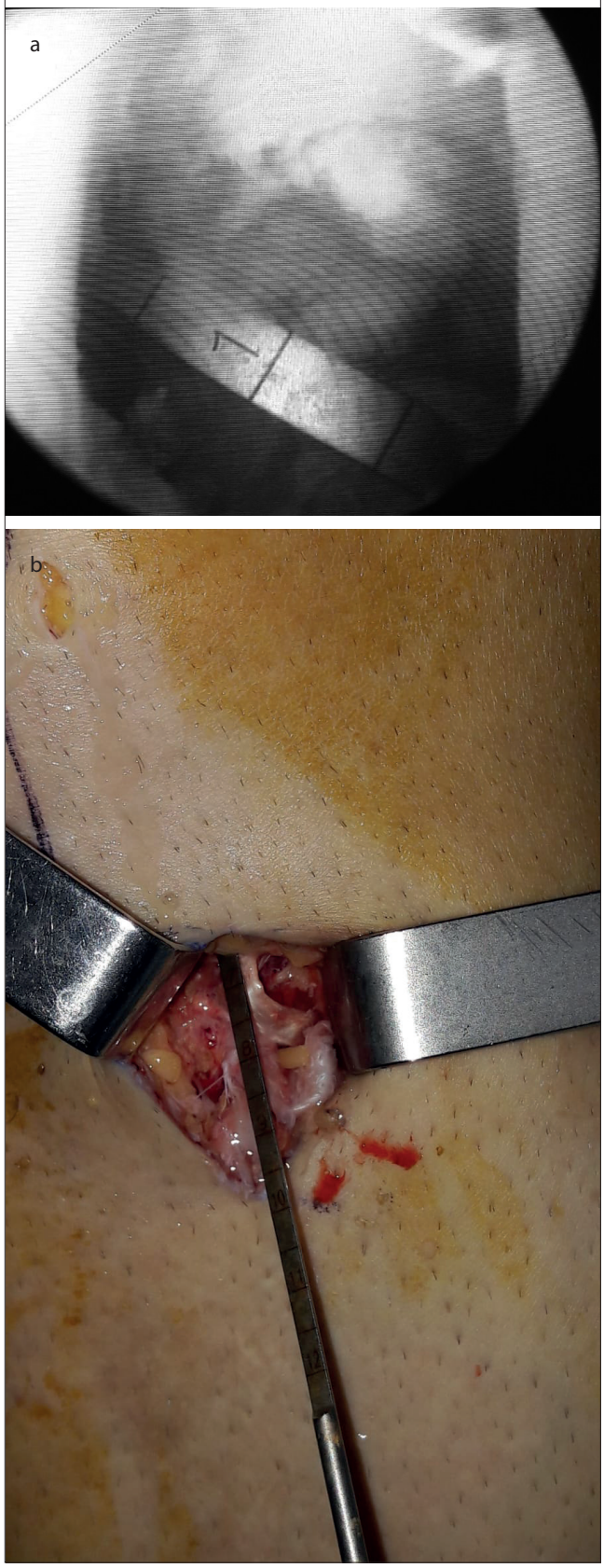
Surgical Technique

Diagnostic arthroscopy was performed from the inferolateral and inferomedial portals opened after sterile preparation of the patient's knee. Anterior cruciate ligament rupture was detected, and grafting was initiated. The hamstring tendons were reached by an approximately 5 cm longitudinal incision opened from the distal to the tibial tuberosity over the hamstring tendons. The fascia was opened, and the tendons (gracilis and semitendinosus)

Main Points:

- The most important advantage of the technique we describe in our study is to leave a distance of 1 centimeter to ensure the tension of the hamstring autograft to be placed thanks to the calculation of the distance from the hamstring tendon sticking point to the button apparatus used for femoral fixation with completely mathematical calculations. Thus, the failure of ACL reconstruction technique with preserving tendon attachment site to maintain the graft tension is eliminated by our technique.
- The fact that no graft rupture occurs as a result of the anterior cruciate ligament reconstruction technique that we have described supports that the preservation of the tendon adhesion site does not disrupt the feeding of the graft and increases the graft incorporation.
- Another advantage of the described technique is that it is cost effective since fixation material is not required in the tibial tunnel.

Figure 1. a, b. Measuring the distance from origin of the hamstring adhesion site to beginning of the femoral tunnel.



were removed with an open-ended tendon scraper without disjointing the tibial attachment site. Tendons were removed from the muscle tissue and folded over themselves to obtain a four-arm tendon graft. Graft thickness and length were measured. Then, the second inferomedial portal was opened, and the camera was moved to this portal. The knee was flexed 90°. Using the freehand technique, the guide wire was advanced to the femoral tunnel from approximately 2 mm anterior of the stump of the anteromedial band. The wire was removed from the lateral of the thigh. The tunnel required for the fixation button of the ZipTight Fixation device (Biomet, Warsaw, IN) to pass was opened with a 5-mm drill. The length of the tunnel was measured, and the femoral tunnel was opened where the graft would be placed in a way that it was 5 mm shorter than the length of the tunnel. The guide wire was

Figure 2. View of prepared 4-arm Hamstring graft

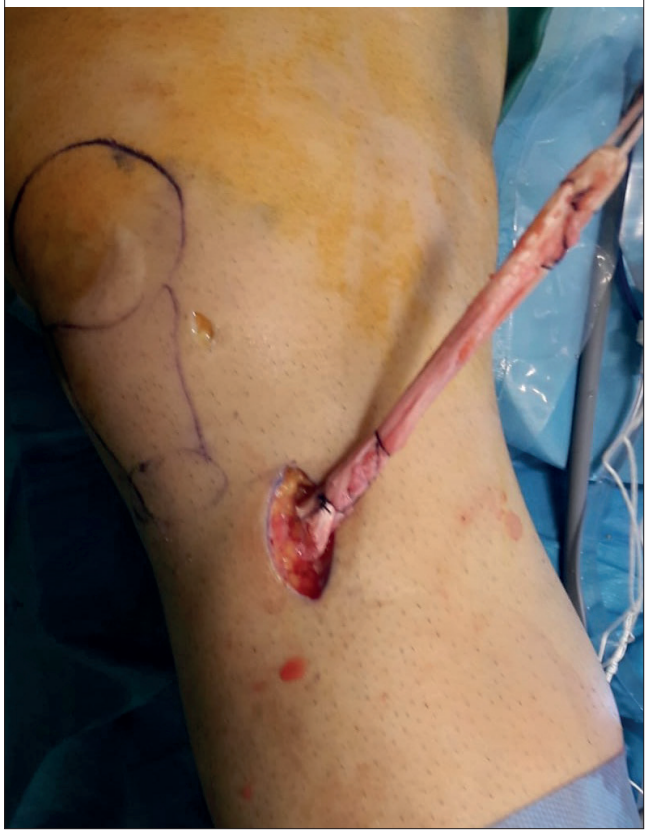


Figure 3. Control of graft tension at the end of surgery

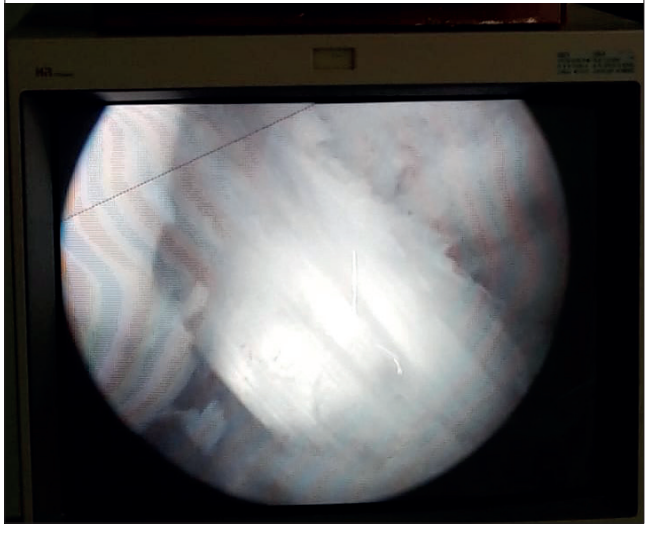
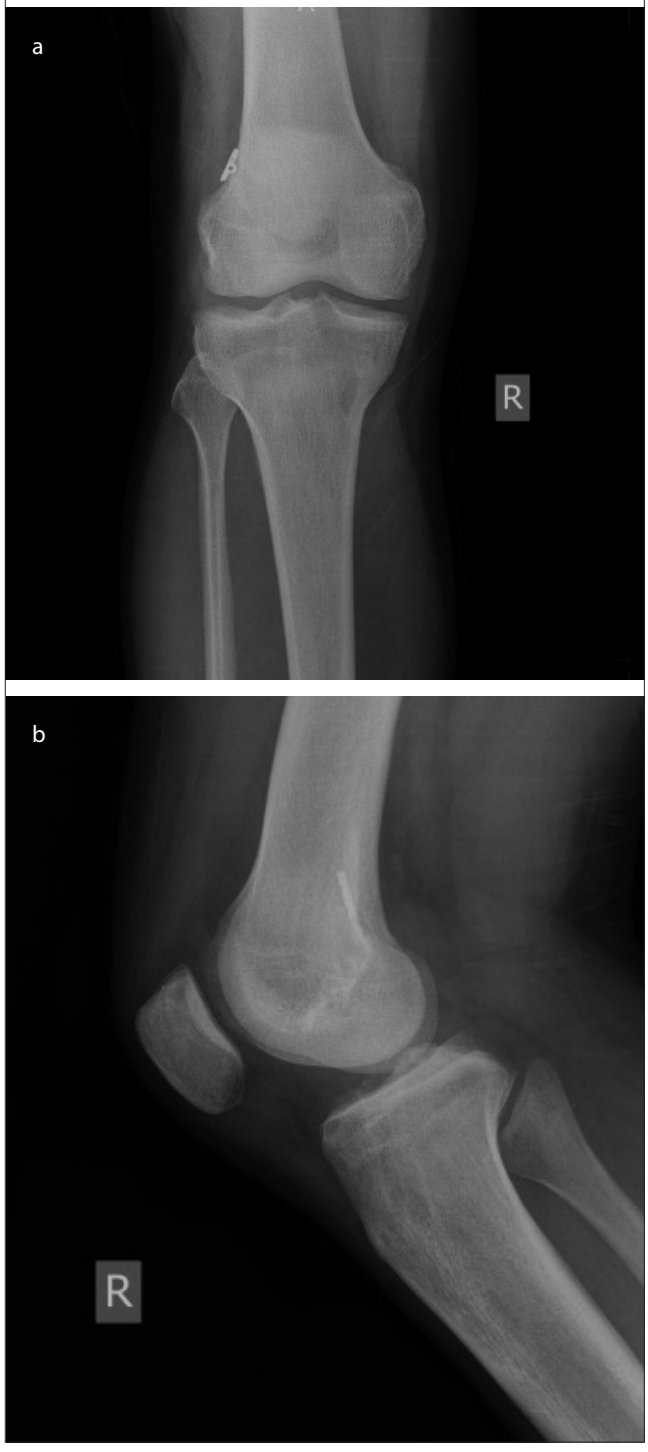


Figure 4. a, b. Postoperative anteroposterior and lateral knee radiographs



withdrawn from the lateral thigh so that the other end of the wire was at the intraarticular origin of the femoral tunnel. The knee was again flexed 90°. The tibial tunnel was opened using a 55-degree tibial tunnel guide based on footprint, and the distance from the hamstring attachment site to the femoral tunnel was measured (Figure 1a, b). The femoral tunnel length was added to this measurement, and the average length was determined. The length of the graft was prepared to be 1.5 cm shorter than this measured value (5 mm healthy bone tissue length left at the end of the femoral tunnel and 1 cm length required to stretch the tendon). The 4-fold hamstring tendon was first suspended in the rope system of the ZiptTight Fixation device (Biomet, Warsaw, IN) graft stretching was performed with a sterile ruler. The ruler was leaned against the hamstring attachment site and held parallel to the graft, and the graft was sutured using absorbable sutures from the distal in a way that its length was 1.5 cm shorter than the measured tunnel length (Figure 2). A marker suture was placed on the graft length point within the femoral tunnel, proximal to the graft. The ropes of the ZipTight Fixation device (Biomet, Warsaw, IN) that would allow the graft to pass through the tunnels and ensure fixation in the femoral tunnel, were sent through the tibial tunnel into the joint with the help of a grasper; the ropes were moved out using a discharge cannula placed in the anteromedial portal. The knee was flexed to 120°, and the guide wire in the femoral tunnel was pushed out of the discharge cannula. The ropes of the ZipTight Fixation device (Biomet, Warsaw, IN) were threaded through this end of the wire that had a hole, and the ropes were pulled from the end on the lateral of the thigh and taken to the lateral of the thigh. The knee was flexed 90°, and the button of the ZipTight Fixation device (Biomet, Warsaw, IN) was passed through the cortex in the femur lateral and rolled over. After the position of the button was monitored with a single-dose fluoroscope, the ropes of ZipTight Fixation device (Biomet, Warsaw, IN) to shift the graft into the femoral tunnel were taken from the anteromedial portal. The ropes suitable for the axis of the femoral tunnel were retracted, and the graft was placed in the tibial and femoral tunnel. With the optical camera, the marker suture on the graft was observed, and the graft was completely placed in the femoral tunnel. We checked the stretching of the graft. Then, the knee was flexed 30°, and the ropes in the medial port were retracted. We checked the tension of the graft again (Figure 3). At this stage, it was determined that the marker suture was lost in the tunnel in all patients. Intraarticular washing was performed, and hemovac drains were placed in the graft-harvesting site and the joint. The portals and the wound were closed (Figure 4, 5). No kneepad was used, they were given partial weight bearing with crutches the following day, and quadriceps exercises were described. Knee Range Of Motion was planned to be complete after 3 weeks and full weight bearing was planned after 3 weeks. The sutures were taken at 2 weeks. Routine control follow-up was performed at 4, 6, 8, and 12 weeks, and at 3 and 6 months. The subjects were allowed to climb up the stairs after 8 weeks, flat race after 3 months, and participate in pivot sports after 6 months. At the end of the study, the patients were called for a final follow-up.

Clinical evaluation was performed, using the Tegner activity score, Lysholm score, and IKDC subjective evaluation score. Anterior tibial translation objective measurement was performed using a KT 1000 device. Clinical evaluation tests and anterior tibial translation measurement with KT 1000 device were performed preoperatively and postoperatively at the last follow-up. T-test was used for the statistical analyses.

RESULTS

The mean age of the patients was 29.33±8.714 years. There were 39 male and 3 female patients. Meniscus injury was detected in 19 patients; 7 of these patients had bucket handle medial meniscal tear, and these tears were repaired using the inside-out suture technique. The remaining 12 patients underwent partial meniscectomy. The mean duration of the tourniquet application was determined as 57.52±5.190 minutes. The mean graft thickness was 7.774±0.8968 mm, the mean four-arm hamstring tendon length was 130.40±7.626 mm, the mean tunnel length was 137±5.548 mm, the mean femoral tunnel length was 40.76±2.335 mm, the length of the graft in the femoral tunnel was 25.76±2.335 mm, and the mean graft length calculated according to the tunnel length was 122.12±6.122 mm. The mean follow-up duration was 17.86±5.266 months. While the preoperative mean Tegner activity score was 3.79±0.725, that at the last follow-up was 5.81±0.64; the preoperative IKDC subjective evaluation score was 55.39±8.418 and that at the last follow-up was 84.57±6.421; the preoperative Lysholm score was 46.29±8.819 and that at the last follow-up was 95.92±2.421. The preoperative anterior translation of the tibia using the KT 1000 device was 11.05±1.607 mm and the value at the last follow-up was 4.01±1.041 mm. The change in all the scores was statistically significant. No avulsion in the hamstring tibial attachment site occurred; further, no fracture and relaxation or loosening in the cortical area of the preoperative femur were detected in any patient. Infection, surgical site infection, graft re-rupture, and pull out were not observed in the patients.

DISCUSSION

In the literature, there are studies of anterior cruciate ligament reconstruction with hamstring autograft applied with preservation of the hamstring tibial attachment site (1-3, 5-9). In these studies, interference screw, staple, and a combination of interference screw and staple were used for tibial fixation. In 2 studies, no fixation was used on the tibial side (3, 9). For the femoral fixation, endobutton (8) and interference screw (3, 9) were used. In one study, the inside-out femoral tunnel was opened (with flip cutter), and the second-generation cortical suspensory device (7) was used. In our study, no fixation was used on the tibial side, and the ZipTight Fixation device (Biomet, Warsaw, IN) was used for the femoral side. This system allows fixation on the femoral lateral cortex with a button and is a system where the ropes are pulled into the femoral tunnel and self-tied in the tunnel.

An important problem in the studies of anterior cruciate ligament reconstruction that preserve the hamstring tibial attachment site seems to be the inability to ensure graft stretching. In the study performed by Sinha et al. (3), graft stretching was achieved by manual stretching of the graft from the femoral side, and femoral fixation was performed with an interference screw so that it would be outside in. In this technique of preserving the hamstring tendon attachment site, the femoral tunnel was

opened outside in and the grafts were passed from both the tunnels without calculating the tunnel and graft lengths. Graft tension was obtained manually from the femoral side, and the graft was fixed outside in. In this technique, it is difficult to find a solution to problems, such as shortness of the graft because the length of the graft and tunnels is not calculated. If the graft length is short, fixation and graft tension will be difficult (4). In the study by Ali et al. (8), in cases where the stretching was insufficient, stretching was achieved with stitch tapes passed from the hamstring attachment site to the tendons, and fixation was achieved with a staple on the tibial side. An important attribute of our study is that the tunnel length was calculated, and the graft was prepared to be 1 cm shorter than the tunnel length; further, a distance of 1 cm was left in the tunnel for graft stretching. The stretching of the graft was checked manually using the manual probe at the hamstring tendon attachment site after the completion of the surgery. There might be a question regarding the reason for leaving a distance of 1 cm for graft stretching. The study by Kim et al. on intraoperative graft isometry was used as a reference for this length (10). In this study, when the graft was retracted with 30 Ibs power in 30° of flexion, the change in the length of the graft at the exit of the tibial tunnel was investigated, and a graft length change between 0.4 and 0.6 mm was observed. Therefore, in our study, a 1-cm stretching share was found appropriate for the graft in the femoral tunnel. Moreover, in our study, a tunnel with a diameter equal to that of the graft was opened, and the graft was fitted to the tunnel.

The hamstring graft is revascularized in 6–12 weeks (3, 11, 12). There is a possibility of early rupture or pullout of the graft due to problems in graft tunnel healing (13). Separation of the tibial attachment site facilitates the biological healing of the tendon in the tunnel (14). Preservation of the tibial attachment site in the hamstring increases the tendon feeding by not disturbing the feeding of the tendon from the inferior geniculate artery (15). The hamstring tendon has longitudinal blood vessels that are located at the junction of the osteotendinous and the proximal musculotendinous. The proximal musculotendinous part is detached while harvesting the graft with this technique; therefore, the avascular necrosis is expected to occur only in the proximal detachment part (3, 15). In the animal experiment by Papachristion, it was observed that the necrosis was bypassed with this technique and feeding was provided, thus increasing the graft viability (16). Ruffili et al. (7) investigated the contribution of anterior cruciate ligament reconstruction with preservation or detachment of the hamstring tibial attachment site to the graft ligamentization. With a magnetic resonance imaging examination performed at the postoperative 6th month, the graft ligamentization was compared and it was argued that the preservation of the hamstring tibial attachment site increased intraarticular ligamentization. The need for better-designed studies was also reported (8). Therefore, it can be concluded that the risk of early graft rupture was lower with this technique; this result was also observed in our study wherein there were no cases of graft rupture.

Moreover, studies suggest that anterior cruciate ligament reconstruction surgery with preservation of the tibial attachment site is applicable and provides clinically superior results (3, 7-9). The point we observed in these studies is the uncertainty in providing graft stretching. The graft length was achieved using mathematical measurements in our technique, and stretching was achieved by applying a manual force, such as anterior cruciate ligament reconstruction performed with classical hamstring autograft, enabled by the femoral fixation system. In this technique, the graft was fit to the tunnel because a tunnel diameter that was equal to the thickness of the graft was opened.

CONCLUSION

The clinical result of the anatomic single-band anterior cruciate ligament reconstruction with preservation of hamstring tibial attachment site is a successful surgical procedure. The disadvantage is the difficulty in performing the grafting technique and the long surgical duration because arthroscopic treatment cannot be continued during the tunnel distance measurements and graft preparation. The advantages include those reported in the literature, such as superior graft feeding and viability, ability to achieve graft stretching without any additional incisions, and cost-effectiveness (given that there is no additional fixation on the tibial side). Research shows that the disadvantage of the surgical technique is the difficulty in ensuring graft tension; the ZipTight Fixation device (Biomet, Warsaw, IN) used in this study successfully achieved tensioning the graft without increasing patient morbidity.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of SANKO University (Date 03.10.2019, Decision number: 02).

Informed Consent: Informed consent was obtained from the subjects enrolled in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.C.; Design – G.B.S.; Supervision – G.B.S.; Resources – G.B.S.; Materials – G.B.S.; Data Collection and/or Processing – G.B.S.; Analysis and/or Interpretation – G.B.S.; Literature Search – G.B.S.; Writing Manuscript – G.B.S.; Critical Review – G.B.S.; Other – C.C.

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

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Original Research

A Single Center Anesthesia Experience in Children Posted for Cleft Lip and Palate Repair: A Retrospective Analysis from a Post-Anesthesia Care Unit

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ABSTRACT

Objective: Cleft lip and palate (CLP) is one of the most commonly seen craniofacial abnormalities in children. Anesthesia management for these surgeries is challenging due to the emergence of airway problems and perioperative complications. In this study, we aimed to evaluate airway difficulties and perioperative anesthetic complications in children suffering from CLP.

Methods: After obtaining approval from the institutional review board, this retrospective study was conducted on 29 children that underwent CLP repair from January 2014 to December 2016 at a single center. Demographic parameters, patients with CLP, patients having micrognathia, associated syndromes, associated congenital abnormalities, difficult mask ventilation, difficult intubation, duration of anesthesia, number of intubated patients to be transferred to the post-anesthesia care unit (PACU), airway-associated complications, and intraoperative and postoperative complications were recorded.

Results: Data from a total of 29 patients with cleft palate were included. Out of the 29 patients, 15 patients had a cleft lip, 17 patients had micrognathia, and 10 patients had both cleft lip and micrognathia. Three patients had difficult mask ventilation, while seven had difficult intubation. Intubation failure was seen in three patients in whom a fiber optic laryngoscope was successfully utilized. Airway-associated complications were seen in six patients. Only three patients had postoperative complications. There were no mortalities.

Conclusion: CLP deformities in children with associated abnormalities are predisposed to difficult airway-associated and postoperative complications. Specialized perioperative care is necessary.

Keywords: Anesthesia, cleft lip and palate repair, difficult airway, postoperative complications

INTRODUCTION

Cleft lip with or without the palate involvement is a congenital malformation that has a worldwide incidence of 1 in 700 live births (1). Children, especially infants with cleft lip and palate (CLP), have a higher incidence of airway-related complications. Due to their corrupted airways and anatomical defects, they are prone to difficult mask ventilation, laryngoscopy, and endotracheal intubation, as well as other airway complications (2). In a study conducted on airway management, it was reported that airway complications occurred in 7.8% children under anesthesia. Airway complications varied with the type of airway device used, with laryngeal mask airway (LMA) having the highest incidence of 10.2%, followed by endotracheal tube (7.4%) and facemask (4.7%) (3).

There is an increased risk of intraoperative airway and respiratory complications for patients undergoing cleft repair. Recurrent infections of the respiratory tract as a result of continuous irritation and aspiration increase airway reactivity and may result in laryngeal and/or bronchospasm. In another study, Takemura et al. (4) defined perioperative respiratory symptoms as laryngospasm or bronchospasm occurring at induction, increased airway secretions and desaturation (<90%) during maintenance, and respiratory symptoms immediately observed after extubation. In addition to craniofacial abnormalities, congenital cardiac disease, central nervous system abnormalities, mental retardation, and seizures are the most common abnormalities that worsen the physical status and complicate the management of anesthesia.

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METHODS

After obtaining approval from the institutional review board, the records of patients who underwent cleft lip and palate (CLP) deformities from January 2014 to December 2016 were reviewed by evaluating them on the basis of anesthesia records in the post-anesthesia care unit's (PACU's) case sheets at a single center. Patients' written informed consents were obtained from their parents in strict accordance with the principles set by the Declaration of Helsinki.

A total of 31 patients were operated in the abovementioned duration, but we were only able to review 29 cases in the final evaluation due to 2 missing case reports.

The recorded data include demographic parameters, patients having cleft lip, patients having micrognathia, associated syndromes, associated congenital abnormalities, difficult mask ventilation, difficult intubation, anesthesia duration, number of intubated patients in the PACU, airway complications, and intraoperative and postoperative complications.

This was a hospital-based study where trained anesthetists and surgeons were available. Anesthetic management and surgeries were performed by the same team with experience of over 10 years.

Anesthetic Management

Preoperative fasting was 2 h for milk and 4 h for solid food. All the children were premedicated with 0.05 mg/kg midazolam. Baseline vital parameters of the heart rate, noninvasive blood pressure, and pulse oximetry were recorded in the operating room. Propofol (2 mg/kg) was used for the induction of anesthesia. After ensuring mask ventilation, intubation was carried out with rocuronium at a dose 0.5 mg/kg. After confirming bilateral equal air entry, the tube was fixed in the center of the lips. Anesthesia was maintained with 50% air in oxygen with sevoflurane. Vital parameters were monitored throughout the procedure. Ringer lactate was intraoperatively infused at 10 mL/kg/h. The reversal of anesthesia was achieved with 0.05 mg/kg neostigmine with 0.01 mg/kg atropine. Children were shifted to the PACU for observation and vital monitoring for one day; if the hemodynamics were stable, they were transferred to the pediatric ward. Any adverse event during anesthesia was recorded. Desaturation was considered to be a fall in oxygen saturation of <90%, and laryngospasm, a partial or complete airway obstruction with a fall in oxygen of <90%; the presence of a wheeze was defined as bronchospasm. Bradycardia was defined as the situation when the heart rate was <20% of the baseline value and tachycardia, if the heart rate was >30% of the baseline value. A fiber optic laryngoscope was kept ready in the case of a failure of intubation after three attempts.

Main Points:

- Cleft lip and palate (CLP) is one of the most commonly seen craniofacial abnormalities in children.
- Anesthesia management for these surgeries is challenging due to the emergence of airway problems and perioperative complications.
- Anesthetic management needs detailed monitoring and postoperative care with skilled personnel to minimize perioperative complications in a multidisciplinary setting with a team approach.

Statistical Analysis

The differences between the two groups for categorical variables were tested with the Mann–Whitney U test. The chi-squared test was used for determining the differences between two categorical variables. The Fisher's exact test was used for differences between the small groups with categorical variables. All the analyses were performed using Statistical Package for Social Sciences (SPSS Inc.; Chicago, IL, USA) version 17.0 for Windows with a 95% confidence interval level.

RESULTS

A total of 29 patients with the cleft palate were included. Out of these patients, 16 were male and 13 were female. Out of the 29 patients, 15 had cleft lip, 17 had micrognathia, and 10 had both cleft lip and micrognathia. Sixteen patients had syndromes and congenital abnormalities. Three patients had difficult mask ventilation, while seven of them had difficult intubation. Intubation failure was seen in three patients for whom the fiber optic laryngoscope was successfully used. Laryngospasm occurred in two patients, and bronchospasm developed in one patient at induction and was recorded as an intraoperative complication. Airway-associated complications were seen in six patients. Only three of the patients had postoperative complications. Desaturation was seen in three patients, with bradycardia in one patient. No complications related to surgery were observed. There were no mortalities.

A comparison of the patients with and without cleft lip is shown in Table 1.

The demographic parameters, duration of PACU stay, presence of micrognathia, operation duration, intraoperative complications, and airway-associated and postoperative complications did not differ between the patients with and without cleft lip. Systemic diseases and congenital abnormalities were found to be statistically significant in patients with cleft lip (p=0.042). Difficult intubation and intubated patients to be transferred to the PACU included six patients with cleft lip. This finding was statistically significant when compared to patients without cleft lip (p=0.031).

A comparison of the variables between the patients with and without micrognathia is shown in Table 2.

Difficult mask, difficult intubation, intubated patients transferred to the PACU, intraoperative complications, and airway-related and postoperative complications were reported in patients with micrognathia, but they were not reported in the patients without micrognathia.

A comparison of the variables between the patients with and without congenital anomalies is shown in Table 3.

The presence of a cleft lip was seen as statistically significant in patients with a congenital anomaly (p<0.042) (Figure 1).

Table 1. A comparison of the variables between the patients with and without cleft lip

	Without cleft lip (n=14)	With Cleft Lip (n=15)	p
Gender, n (%)			
Male	6 (42.9)	10 (66.7)	0.198[a]
Female	8 (57.1)	5 (33.3)	
Age, mean (SD)	1.71±0.82	1.53±0.52	0.715[b]
Duration of PACU stay, mean (SD)	1.14±0.53	1.13±0.52	0.983[b]
Weight, mean (SD)	10.00±2.15	9.40±1.55	0.591[b]
Presence of Micrognathia, n (%)	7 (50.0)	10 (66.7)	0.362[a]
Syndromes, n (%)	5 (35.7)	11 (73.3)	0.042[a]
Congenital anomaly, n (%)	5 (35.7)	11 (73.3)	0.042[a]
Difficult mask, n (%)	–	3 (20.0)	N/A
Difficult intubation, n (%)	1 (7.1)	6 (40.0)	0.031[c]
Duration of the operation, mean (SD)	151.43±46.22	151.33±52.49	0.813[b]
Intubated in PACU, n (%)	1 (7.1)	6 (40.0)	0.031[c]
Intraoperative complications, n (%)	1 (7.1)	3 (20.0)	0.305[c]
Airway complications, n (%)	1 (7.1)	5 (33.3)	0.099[c]
Postoperative complications, n (%)	1 (7.1)	2 (13.3)	0.527[c]

[a]Chi-squared test, [b]Mann-Whitney U test, [c]Fisher's exact test
PACU: post-anesthesia care unit

Table 2. A comparison of the variables between the patients with and without micrognathia

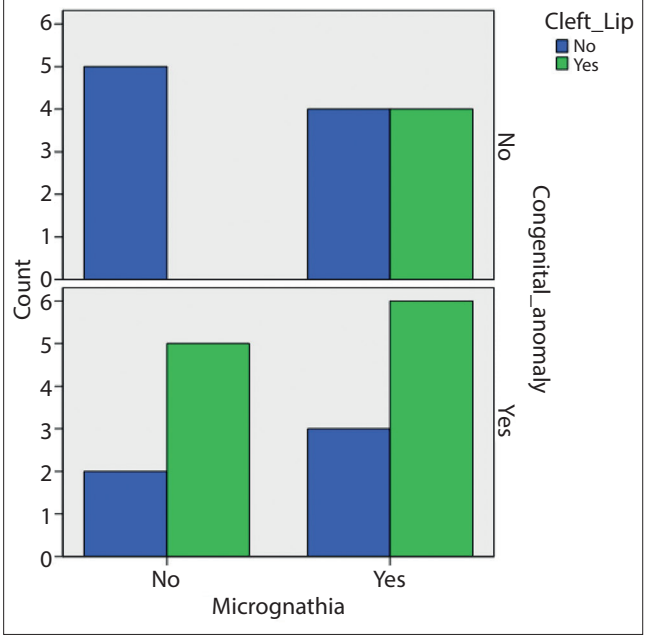
	Without Micrognathia (n=12)	Without Micrognathia (n=17)	p
Gender, n (%)			
Male	8 (66.7)	8 (47.1)	0.296[a]
Female	4 (33.3)	9 (52.9)	
Age, mean/year/(SD)	1.50±0.67	1.71±0.69	0.444[b]
Duration of PACU/day/mean (SD)	1.00±0.01	1.24±0.66	0.616[b]
Weight, mean/kg (SD)	9.67±1.83	9.71±1.93	0.948[b]
Cleft lip, n (%)	5 (41.7)	10 (58.8)	0.362[a]
Syndromes, n (%)	7 (58.3)	9 (52.9)	0.774[a]
Congenital anomaly, n (%)	7 (58.3)	9 (52.9)	0.774[a]
Difficult mask, n (%)	–	3 (17.6)	N/A
Difficult intubation, n (%)	–	7 (41.2)	N/A
Duration of operation/min/ mean (SD)	161.67±51.32	144.12±46.91	0.325[b]
Intubated in PACU, n (%)	–	7 (41.2)	N/A
Intraoperative complications, n (%)	–	4 (23.5)	N/A
Airway complications, n (%)	–	6 (35.3)	N/A
Postoperative complications, n (%)	–	3 (17.6)	N/A

[a]Chi-squared test, [b]Mann-Whitney U test, PACU: post-anesthesia care unit

For patients with a congenital anomaly, the rate of the presence of micrognathia was found to be higher in patients with a cleft lip, whereas for patients without a congenital anomaly, the presence of micrognathia rates were similar for patients with or without a cleft lip (Figure 2).

For patients without a congenital anomaly, difficult mask ventilation was seen only in patients with a cleft lip (Figure 2).

Figure 1. Distribution of patients with and without cleft lip with respect to congenital anomaly and micrognathia



Difficult intubation was seen in patients having a congenital anomaly and having a cleft lip. The rates of difficult intubation were seen more frequently in patients with a cleft lip. Difficult intubation was not seen in patients having a congenital anomaly but without a cleft lip (Figure 3).

Difficult mask ventilation was observed in difficult intubation cases, whereas difficult mask ventilation was not observed in cases that were easily intubated (Figure 4).

Figure 2. Distribution of difficult mask

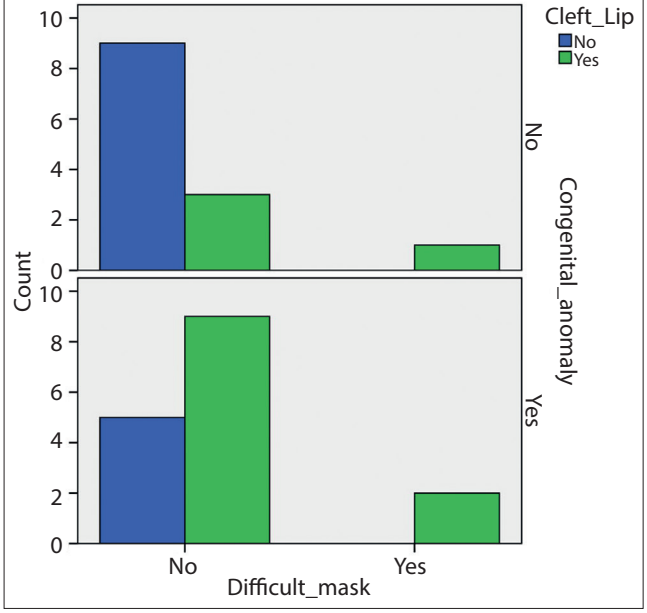


Table 3. A comparison between the patients with and without congenital anomalies

	Congenital anomaly (n=13)	Without congenital anomaly (n=16)	p
Gender, n (%)			
Male	7 (53.8)	9 (56.3)	0.897[a]
Female	6 (46.2)	7 (43.8)	
Age, mean (SD)	1.38±0.65	1.81±0.65	0.101[b]
Duration of PACU stay, mean (SD)	1.00±0.01	1.25±0.68	0.589[b]
Weight, mean (SD)	9.23±1.69	10.06±1.95	0.249[b]
Cleft lip, n (%)	4 (30.8)	11 (68.8)	0.042[a]
Micrognathia, n (%)	8 (61.5)	9 (56.3)	0.774[a]
Difficult mask, n (%)	1 (7.7)	2 (12.5)	0.580[c]
Difficult intubation, n (%)	3 (23.1)	4 (25.0)	0.626[c]
Duration of the operation/min/ mean (SD)	159.23±46.63	145.00±50.86	0.423[b]
Intubated transferred patients, n (%)	3 (23.1)	4 (25.0)	0.626[c]
Intraoperative complications, n (%)	1 (7.7)	3 (18.8)	0.383[c]
Airway complications, n (%)	2 (15.4)	4 (25.0)	0.435[c]
Postoperative complications, n (%)	2 (15.4)	1 (6.3)	0.420[c]

[a]Chi-squared test, [b]Mann-Whitney U test, [c]Fisher's exact test, PACU: post-anesthesia care unit

Figure 3. Distribution of difficult intubation

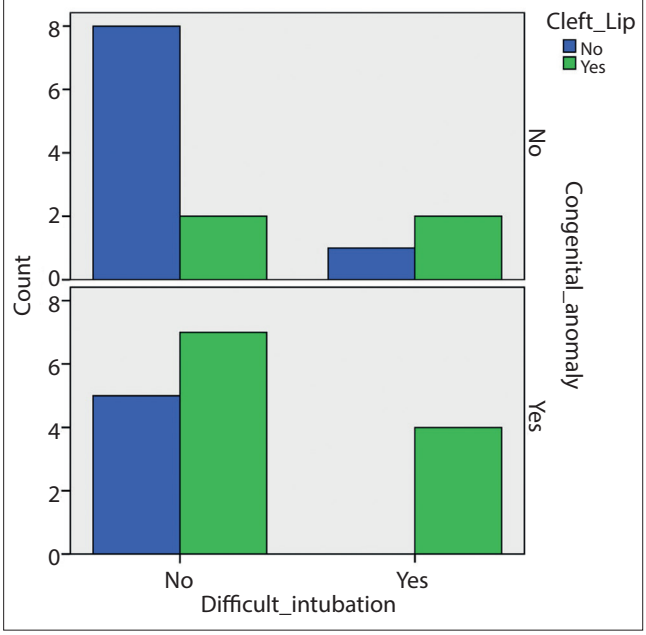
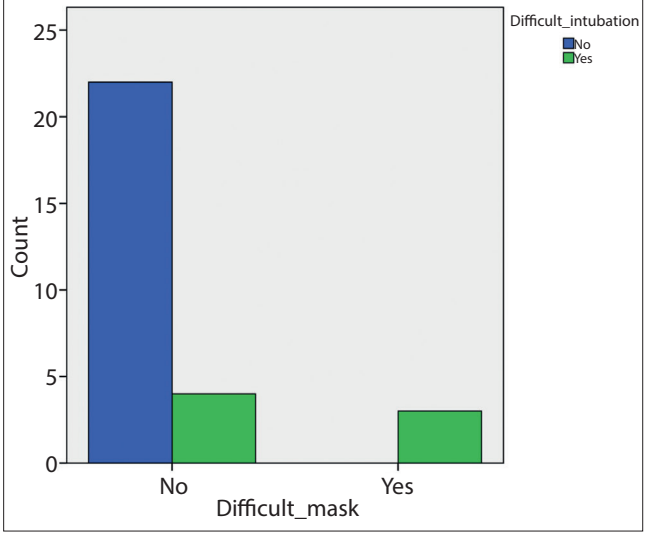


Figure 4. Difficult intubation score



DISCUSSION

Cleft lip and palate deformity repair is a complicated surgery that requires a team approach. Children with a wide cleft palate have an increased risk of prolapse of the tongue into the nasopharynx, causing a serious problem during the induction of anesthesia; at this stage, the assessment of the degree of the airway difficulty is not always possible (5).

In a study, Tiret et al. (6) reported that the anesthesia-related complication rate was 0.5/1000 in children and 4.3/1000 in infants, while Cohen et al. (7) reported higher morbidity rates in children (35%) in comparison to adults (17%). Jindal et al. (8) reported the incidence of failed intubation as 0.16% due to the anesthesiologist's anticipation of a potentially difficult intubation for every patient. Kulkarni et al. (2) reported intubation-related problems in 2.4% cases in cleft repair and 8.7% cases in palate repair. In their study, intubation failure occurred in three patients that suffered from the Pierre Robin syndrome.

In our cases, intubation was successful since a straight-bladed laryngoscope was used after the confirmation of the lungs ventilating bilaterally. Intubation failure was seen only in 3 (10.34%) of the patients who had the Pierre Robin syndrome with micrognathia. The use of a fiber optic laryngoscope facilitated intubation in these patients.

Similar to the studies in the literature, 16 out of the 29 children in our study had Down syndrome (8/29), Pierre Robin syndrome (5/29), Rubinstein–Taybi syndrome (2/29), and Treacher Collins syndrome (1/29); the most commonly observed syndrome accompanying congenital abnormalities was micrognathia, which occurred in 9 children. Encephalocele and meningomyelocele were the other accompanying abnormalities that were recorded in our study.

Fillies et al. (9) reported major complications such as laryngospasm, arrhythmias, and excessive bleeding in 45.2% cases of lip repairs. McQueen et al. (10) reported 31% overall complications in the data reviewed in a two-year period. A majority of the reported complications were difficult intubation, bronchospasm, and airway obstructions. The overall intraoperative complication rate was 10.34% in our study; laryngospasm occurred in 2 patients, and bronchospasm developed in 1 patient at induction. This could be due to the fact that a team of surgeons and anesthetists was working together for more than 10 years on cleft surgeries, and the experience gained over the years would have been contributory.

The postoperative period is very important after lip and palate repair surgeries. Patients with syndromes presented with airway problems, such as mucosal edema of the oropharynx or larynx, because of the prolonged pressure caused by the extension of head and also changes in the oral/nasal airway dynamics (11, 12). Post-operative respiratory complications could occur following the closure of the cleft palate or due to the hypoplasia of the mandible or hematoma. The aspiration of the collected blood or secretions in the nasopharynx is a very important step to avoid complications and to regain reflexes (13, 14). Children should be maintained in the lateral position for air movement and to avoid aspiration. The arms should be restrained to keep them away from the surgical site. Vital signs should be monitored in a well-equipped PACU (15-17).

In our study, we observed postoperative complications only in three children due to transient mucosal edema and oxygen desaturation, which is in accordance with the studies in the literature. All the children were postoperatively monitored in the PACU for a day according to the institution's regimen. Once the vitals were stable with no evidence of bleeding, the children were transferred to a well-equipped ward.

The limitation of our study was that it was based on retrospective assessment. The results may vary among different institutions. Prospective randomized future trials are needed.

CONCLUSION

Surgical repair of CLP in children is challenging for anesthesiologists due to the peculiar variety of anomalies and accompanying perioperative complications. We conclude that anesthetic management needs detailed monitoring and postoperative care with skilled personnel to minimize perioperative complications in a multidisciplinary setting with a team approach.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep MMT Hospital (Decision date: 25.07.2019, Decision no: 2019/638).

Informed Consent: Written informed consent was obtained from patients.

Peer-review: Externally peer-reviewed.

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

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Original Research

Comparative Evaluation of Thoracoscopic Pericardial Drainage and Subxiphoid Tube Insertion in Patients with Prior Cardiac Surgery

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ABSTRACT

Objective: Clinically symptomatic pericardial effusion (PE) develops in 0.8-5% of patients after open-heart surgery, and delayed effusion is related to morbidity. Comparative evaluation of the outcomes of thoracoscopic pericardial drainage and subxiphoid tube pericardiostomy, which is the standard surgical procedure, has been scantily reported.

Methods: We conducted a longitudinal observation of delayed PEs treated with thoracoscopic pericardial drainage (TPD group; 48 patients) and subxiphoid pericardiostomy (SX group; 91 patients) between May 2012 and June 2017. Changes in the hemodynamic parameters, functional status of patients, and procedure outcomes were compared between the two procedures.

Results: The TPD group had a significantly greater size of effusion (3.9±0.6 cm vs. 3.1±0.5 cm; p<0.01), higher pulmonary artery pressure (41.2±9.8 mmHg vs. 36.4±5.6 mmHg; p<0.01), and less time interval to emerge syptoms [6 weeks (3–15 weeks) vs. 8 weeks (3–21 weeks); p<0.01). Even though the mean operation time was shorter in the SX group (44.6±12.2 min vs. 69.2±22.3 min; p<0.01), the same amount of fluid was drained (637.9±182.9 mL vs. 661.3±168.4 mL; p=0.45). Improvements in postoperative hemodynamic variables and functional status following both procedures were similar, but symptomatic and echocardiographic recurrence of effusion was significantly more in the SX group (19 patients; 20.9% vs. 2 patients, 4.2%; p<0.01) within approximately 2 years of follow-up.

Conclusion: The post-pericardiotomy effusion is a chronic inflammatory process, and the SX drainage provides temporary resolution. TPD may provide equally favorable surgical outcomes; however, it is generally performed to treat more complicated PEs.

Keywords: Cardiac surgery, pericardial effusion, thoracoscopy

INTRODUCTION

Clinically significant pericardial effusion (PE) may develop following open-heart surgery in less than 5% of patients (1, 2). Valve surgery, coagulation disorders, excessive mediastinal drainage, anticoagulant use, autoimmune reactions, and post-pericardiotomy syndrome predispose to the development of effusion (3). Although rare, delayed PE after cardiac surgery may lead to significant morbidity (4-6).

Various modalities have been used for treatmen of the PE, ranging from observation, anti-inflammatory therapy, pericardiocentesis, and eventually open surgery (7). The subxiphoid (SX) drainage is the standard surgical treatment of a PE if the pericardial fluid has a connection to the inferior or anterior pericardial space. This method is also associated with a higher recurrence rate (2, 8). Inferior or anterior adhesions are observed in patients with a prior pericardial intervention (Figure 1) (9-11). Therefore, thoracotomy or thoracoscopic pericardial drainage (TPD) are the best alternatives if the effusion is localized posteriorly or laterally instead of traumatic re-sternotomy in such patients during the recovery period. Thoracotomy is not minimally invasive and often result in pulmonary complications and prolonged postoperative hospitalization (8). However, because the thoracoscopic procedure has low morbidity and mortality rates, it has evolved as the preferred mode of PE treatment considering the high procedural risk of subxiphoid access (12). The morbidity and mortality rates, as well as the efficacy of the procedures in preventing recurrence, should be the basis to determine the most suitable method of surgical management of PE.

The aim of this prospective, observational and longitudinally designed study was to compare the efficacy of TPD and SX for the treatment of localized effusion secondary to cardiac surgery. Notably, the prospective data collection regarding TPD after cardiac surgery has been scantily reported.

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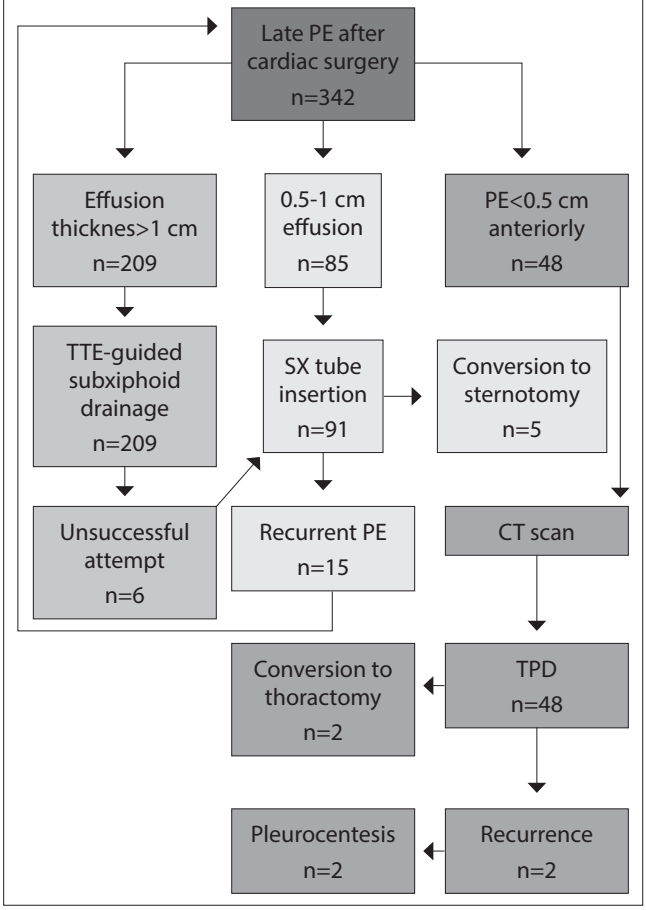
METHODS

Patient Selection

After approval of the prospective observational study protocol by the institutional ethics committee of Kavaklıdere Umut Hospital, Ankara on 3 May 2012, we performed 4731 adult open-heart surgeries in the cardiovascular surgery department between May 2012 and June 2017. Overall, 342 consecutive patients were treated for delayed symptomatic PEs according to the following protocol set as:

Figure 1. The CT image of a symptomatic patient 6 weeks after the mitral valve ring annuloplasty. Most of the pericardial fluid (white arrow) is located to the left lateral side of the left ventricle causing diastolic collapse



Main Points:

- Subxiphoid pericardial drainage serves as an ideal treatment in most symptomatic pericardial effusions requiring surgical interventions.
- Pericardial adhesions late after cardiac surgery may result in an unusual localization of pericardial effusion especially in the posterior and lateral pericardial sacs that are not easily accessible through a subxiphoid incision.
- Thoracoscopic pericardial drainage instead of thoracotomy serves the same successful results comparable with subxiphoid pericardial drainage in this patient subset.

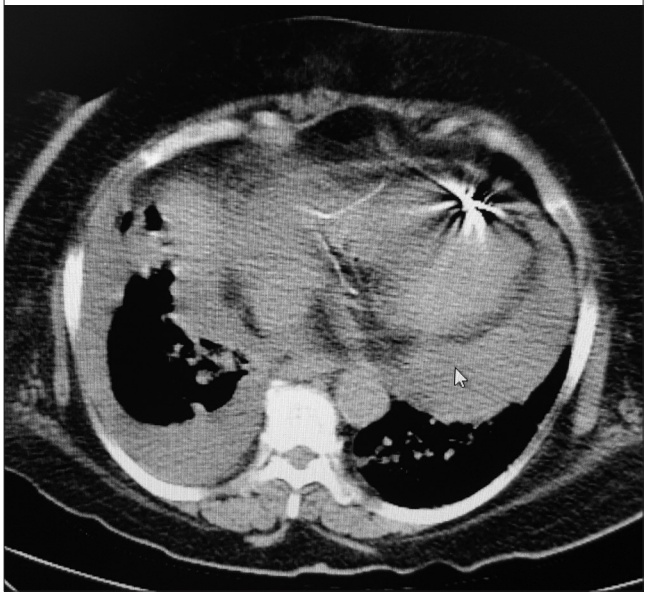
- Massive PEs (203 patients, 59.4%) with >1 cm effusion thickness beneath the posterior border of the sternum were drained using transthoracic echocardiography (TTE)-guided subxiphoid puncture. If this procedure was unsuccessful, these patients were referred for subxiphoid tube insertion.
- Symptomatic massive PE (91 patients, 26.6%), 0.5–1 cm in size, at the anteroinferior pericardial reflection were drained using subxiphoid tube insertion (SX group).
- PEs localized primarily to the lateral or posterior pericardium (Figure 1) with or without <0.5 cm anteroinferior connection (48 patients, 14%) were selected for TPD (TPD group). A computerized tomography (CT) scan was performed on each patient of this group.

The subxiphoid surgery, which is the standard surgical procedure, and the TPD subsets were followed up to compare the results (Figure 2). Our primary endpoints were the success of the procedure in relieving symptoms and recurrence of PE. Informed consent was obtained from each patient.

Diagnostic Tests and Evaluation of PE

Delayed pericardial effusion was described as any effusion in the pericardium developing after discharge from the hospital and the effusion that was not related to possible active surgical bleeding associated with anticoagulation. Notably, the propriety of the percutaneous procedure and the need for surgical drainage was evaluated by the cardiologist based on clinical symptoms, as well as the TTE and CT findings. Echocardiographic evaluation (General Electric Vivid S5, California, USA) was used to measure the size of the echo-free space between the pericardial layers, and to determine cardiac tamponade in case inferior vena cava plethora, right atrial compression, and right ventricular diastolic collapse were present. The symptomatic patients with an anterior echo-free space smaller than 5 mm were further evaluated using the conventional CT scanning (Figure 1). Any collection in

Figure 2. Demonstration of the flow of patients among the procedures



the pericardial space observed on the CT attenuation of less than 10 Hounsfield units was accepted as transudate. The CT images were also used to identify the primary location and extent of PE, adhesions in the pleural space, and functional bypass grafts.

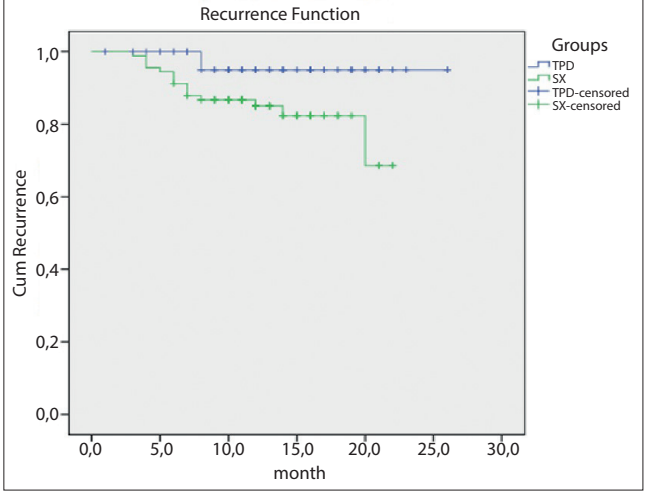
Furthermore, the largest diameter of PE, the changes in hemodynamic parameters [central venous pressure (CVP) measured from central venous line, echocardiographic assessment of pulmonary artery pressure (PAP), and the heart rate], medical management (diuretic use, inotrope infusion, and anti-inflammatory drugs), and the New York Heart Association (NYHA) functional class were recorded to assess the efficacy of both procedures.

Operation Techniques

Except for the clinically unstable patients who required general anesthesia (13 patients; 11 patients with hemodynamic collapse because of severe tamponade and 2 with congestive heart failure with moderate PE, 14.3%), all others underwent subxiphoid tube insertion under local anesthesia. A 5–7 cm skin incision was performed, extending from the linea alba to the xiphoid process. Either fingertip dissection to reach the inferior pericardial margin or blunt surgical dissection was performed to separate the retrosternal tissue. A puncture of the inferior pericardial sac was accomplished to drain the pericardial fluid. A chest tube was inserted after ensuring maximum pericardial drainage.

The TPD patients were operated under general anesthesia and single lung ventilation by using a double-lumen endotracheal tube. Patients were placed supine and their related arms fixed to the operating table, with the shoulder posteriorly extended to approximately 45° and the elbow joint semi-flexed. Three 1 cm trocars were introduced for the passage of a 30° camera and surgical instruments. The camera was introduced first, and adhesions were dissected, if present, between the thoracic interior wall and the anterior surface of the lung. The phrenic nerve was visualized and used as the cornerstone for the pericardial dissection. The pericardial dissection was performed 1.5–2 cm anterior to the phrenic nerve, as much was needed to drain the pericardial fluid. A part of the pericardium was resected to create a window to the pleural space (Figure 3). Any existing PE was removed at the same time by using a surgical suction. Sometimes a blunt dissection was performed over the infero-posterior pericardium or through the posterior of the intrapericardial inferior vena cava with a blunt-ended suction tube to drain the other side of the heart. A chest tube was inserted into the pericardial cavity or to the pleural space in every case.

Figure 3. The thoracoscopic view of the large pericardial window performed in the patient with previous upper J-sternotomy



Clinically stable patients were extubated on the operating table immediately after the operation. The chest tube was removed when the daily drainage had decreased below 50 mL over the previous 12 hours.

Follow-up

Data were recorded in terms of risk factors necessitating the pericardial drainage, operation time, amount of effusion removed, treatment results, as well as the complications. Patients were followed up with TTE and chest radiogram in the first, second, sixth, and twelfth months after the surgery. Changes in the clinical symptoms before and after TPD were assessed with a physical examination. Any effusion detected on postoperative TTE that required further treatment was defined as recurrence.

Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences version 22 (SPSS IBM Corp.; Armonk, NY, USA). Descriptive statistics were presented as frequencies (%), mean and standard deviation for parametric variables, and median (minimum-maximum values) for nonparametric variables. Independent samples *t*-test was performed to assess the statistical significance of differences in parametric variables. Nonparametric variables were compared using the chi-square test, Mann-Whitney U test, and Fisher's exact test, whenever appropriate. Changes in hemodynamic variables after procedures were evaluated using the repeated measures analysis of variance (ANOVA). The Kaplan-Meier analysis was used to estimate the recurrence rate during follow-up. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The clinical data of 139 patients are listed in Table 1. Notably, all patients presented with symptoms, such as fatigue, exertional dyspnea, and edema. Both groups had similar preoperative demographic characteristics. However, the TPD group had a significantly greater PE size (3.6±0.6 cm vs. 3.1±0.5 cm; p<0.01), higher PAP (41.2±9.8 mmHg vs. 36.4±5.6 mmHg; p<0.01), and less time interval of symptom emergence [6 weeks (3–15 weeks) vs. 8 weeks (3–21 weeks); p<0.01] at the time of the index procedure. At least one successful percutaneous pericardiocentesis was performed in 22.9% of TPD patients compared with 41.8% of the SX group in the interval between the cardiac surgery and pericardial drainage procedure (p=0.03). Regarding the additional risk factors, the TPD group had more patients with malignancy, chronic renal failure (CRF), upper abdominal surgery, and morbid obesity, but univariate analysis revealed a nonsignificant difference in frequencies of these comorbidities.

Table 1. Preoperative demographic and clinical parameters of both groups

	TPD Group (N:48)				SX Group (N:91)				
Preoperative Variables	**N**	**%**	**M±SD**	**M(min-max)**	**N**	**%**	**M±SD**	**M(min-max)**	**p**
Age, y			56.6±11.7				54.3±11.5		0.27
Sex									0.40
Male	32	66.7			54	59.3			
Female	16	33.3			37	40.7			
BMI, kg/m^2			26.0±4.0				25.0±3.3		0.14
Prior surgery									
Coronary	12	25			26	28.6			0.65
Valve	31	64.9			47	51.6			0.14
Combined	4	8.3			11	12.1			0.49
Other	1	2.1			7	7.7			0.26
Prior pericardiocentesis	11	22.9			38	41.8			0.03
Preoperative intubation	4	8.3			10	11			0.77
Time to PE symptoms, week				6 (3-15)				8 (3-21)	<0.01
Heart rate, bpm			109.5±23.3				104.7±13.5		0.13
Size of PE, cm			3.6±0.6				3.1±0.5		<0.01
CVP, mmHg			12.4±2.8				11.4±2.6		0.42
PAP, mmHg			41.2±9.8				36.4±5.6		<0.01
Ejection Fraction, %			46.6±9.1				45.57±9.1		0.53
Other Risks									
COPD	3	6.2			5	5.5			1
CRF	6	12.5			6	6.6			0.34
Malignancy	5	10.4			4	4.4			0.27
UAS	2	4.2			0	0			0.12
Morbid obesity	2	4.2			1	1.1			0.27
Concomitant pleural effusion									
Unilateral	5	10.4			15	16.5			0.33
Bilateral	8	16.7			8	8.8			0.17
NYHA Functional Class									0.10
II	5	10.4			20	22			
III	26	54.2			34	37.4			
IV	17	35.4			37	40.7			
Ant-inflammatory drugs									0.77
Ibuprofen	22	45.8			41	45.1			
Colchicum	17	35.4			27	29.7			
Other	3	6.2			9	9.9			
Inotrope use									0.95
Dopamine infusion	16	33.4			37	40.7			
Dobutamin infusion	8	16.7			25	27.5			
Diuretic	33	68.6			75	82.4			0.66

TPD: thoracoscopic pericardial drainage; SX: Subxiphoid; PE: pericardial effusion; BMI: body mass index; bpm: beat per minute; COPD: chronic obstructive pulmonary disease; CRF: chronic renal failure; CVP: central venous pressure; EF: ejection fraction; M±SD: mean±standard deviation; M (min-max): median (minimum-maximum); NYHA: New York Heart Association; PAP: pulmonary arterial pressure; UAS: upper abdominal surgery

Skin-to-skin operation time was shorter in the SX group than the TPD group (44.6±12.2 min vs. 69.2±22.3 min; p<0.01); however, almost the same amount of fluid was drained during both procedures (637.9±182.9 mL vs. 661.3±168.4 mL; p=0.45). Cardiac trauma and bleeding were the reasons for conversion to sternotomy in five patients of the SX group, and two patients with severe pleural adhesions were converted to thoracotomy in the TPD group. Pneumothorax persisting more than 12 hours was more frequently observed in patients who underwent TPD. However, considering all complications, no significant variations in frequencies were observed between both groups (Table 2).

Postoperative hemodynamic variables and functional status were similar for both procedures (Table 3). Symptomatic and

Table 2. Procedural data and procedure-related complications

	TPD Group (N:48)				SX Group (N:91)				
Operative Variables	**N**	**%**	**M±SD**	**M(min-max)**	**N**	**%**	**M±SD**	**M(min-max)**	**p**
Operation, min			69.2±22.3				44.6±12.1		<0.01
Conversion to open surgery	2	4.2			5	5.5			1
Drainage, mL			637.9±182.9				661.3±168.4		0.45
Blood Products, pacs				0 (0-4)				0 (0-5)	0.04
Complications									
Pneumothorax	6	12.5			3	3.3			0.06
Cardiac trauma	0	0			5	5.5			0.16
Phrenic nerve injury	1	2.1			0	0			0.37
Bleeding	4	8.3			2	2.2			0.18
Infection	2	4.2			2	2.2			0.61
CVA	2	4.2			1	1.1			0.27

M±SD: mean±standard deviation; M (min-max): median (minimum-maximum); CVA: cerebrovascular accident

Table 3. Hemodynamic parameters after drainage of the pleural effusion and follow-up data

	TPD Group (N:48)				SX Group (N:91)				
Follow-up Variables	**N**	**%**	**M±SD**	**M(min-max)**	**N**	**%**	**M±SD**	**M(min-max)**	**p**
CVP, mmHg			4.5±2.2				4.6±2.2		0.37
PAP, mmHg			27.5±5.7				27.7±5.0		0.82
EF, %			48.3±7.9				47.0±8.2		0.36
Heart rate, bpm			90.0±10.9				89.5±9.9		0.77
Drainage at ward, mL			246.3±109.3				261.0±109.9		0.46
NYHA Functional Class									0.67
I	25	52.1			51	56			
II	17	35.4			29	31.9			
III	5	10.4			11	12.1			
IV	1	2.1			0	0			
Recurrent PE	2	4.2			15	16.5			0.03
Pleural effusion	14	29.2			9	9.9			<0.01
Pleurocentesis	7	14.6			2	2.2			<0.01
Hospital stay, day				4(2-13)				3(2-20)	<0.01
Mortality	4	8.3			11	12.1			0.50
Follow-up, month			13.2±6.0				12.2±4.4		0.26

CVP: central venous pressure; PAP: pulmonary artery pressureEF: ejection fraction; NYHA: Newyork Heart Associatin; M±SD: mean±standard deviation; M (min-max): median (minimum-maximum); PAP: pulmonary arterial pressure; PE: pleural effusion

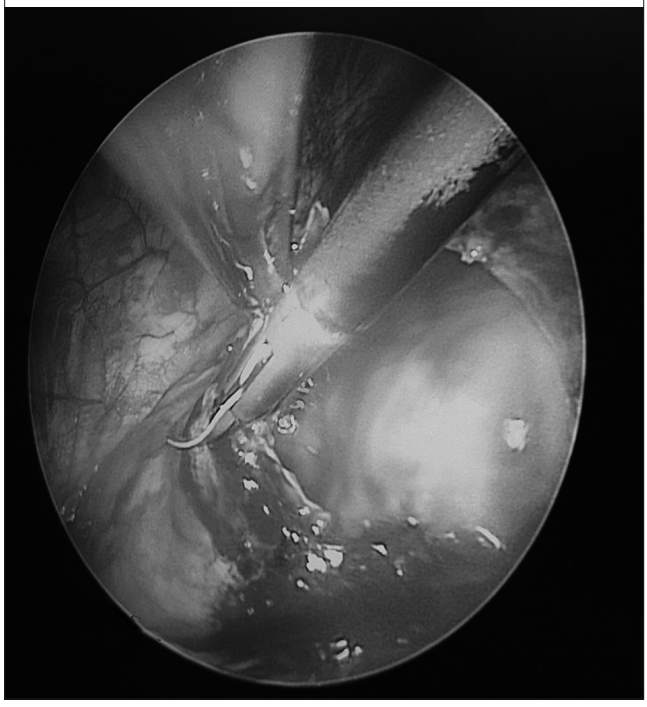
echocardiographic PE recurrence was encountered more in the SX group (14 patients; 15.4% vs. 2 patients, 4.2%; p=0.03). Two patients with recurrent PE (>2 cm on TEE) in the TPD group had concomitant massive pleural effusion at the same site of thoracoscopy, which resolved completely after pleurocentesis. Fifteen patients in the SX group with recurrent PE required re-intervention with a percutaneous puncture (five patients), repeat subxiphoid surgery (six patients), and TPD (four patients). During the follow-up period, patients of the TPD group required significantly more frequent pleurocentesis (14.6% vs. 2.2%; p<0.01) for pleural effusion (29.2% vs. 9.9%; p<0.01) compared with the SX group.

Table 4. Comparison of hemodynamic changes between and within the groups with repeated measures ANOVA

Hemodynamic Parameters	Repeated Measures ANOVA
CVP	
Within the groups	p<0.01
Between the groups	p=0.18
PAP	
Within the groups	p<0.01
Between the groups	p<0.01
Ejection Fraction	
Within the groups	p<0.01
Between the groups	p=0.46
Heart Rate	
Within the groups	p<0.01
Between the groups	p=0.09

CVP: central venous pressure; PAP: pulmonary artery pressure

Figure 4. Kaplan-Meier survival analysis demonstrating recurrence-free survival of both groups



Efficacy of the pericardial drainage was evaluated by comparing the postoperative changes in CVP, PAP, and ejection fraction (EF). All hemodynamic parameters together with NYHA functional status improved early after both procedures (Table 4). Overall 4 patients in the TPD group, including the 2 of whom were converted to thoracotomy, and 11 patients in the SX group were lost during the follow-up period (p=0.5). The Kaplan-Meier analysis estimated a significantly better recurrence-free survival in the TPD group (mean recurrence time of 25.1±0.6 months with 95% CI: 23.8–26.3 than the SX group (19.2±0.7 months with 95% CI: 17.9–20.5; p=0.03) (Figure 4).

DISCUSSION

Post-cardiac injury syndrome refers to a group of disorders comprising postmyocardial infarction pericarditis (Dressler syndrome), post-pericardiotomy syndrome, and post-traumatic pericarditis (13). Even though the etiopathogenesis of this syndrome is incompletely understood, it is presumed to have an autoimmune reaction triggered by the initial damage of pericardial mesothelial cells, with the possibility of recurrence (13). Notably, no standardized criteria exist for diagnosis, but it is crucial to identify prior pericardial injury (14). Delayed PE after cardiac surgery is considered a post-cardiotomy syndrome, with the reported incidence ranging between 1% and 15% (2, 10, 13). The detected incidence of symptomatic PE in the present study was 7.2%, and more than half of the cases had been treated with percutaneous subxiphoid drainage. The relatively high incidence might be due to the routine echocardiographic follow-up resulting in early detection of the PE even in patients taking aggressive diuretics and anti-inflammatory drugs which may obscure symptoms like fever and oliguria.

A failed medical treatment warrants an interventional decompression to be performed (15). The choice between surgical intervention and pericardiocentesis is based on the location and character of the effusion (10). Subxiphoid puncture and tube insertion are typically sufficient for drainage of the effusion. Nonetheless, they do not provide complete resolution and carry a significant risk of recurrence since both are temporary tunneling (16, 17). When an operation is required for PE management and the subxiphoid drainage is risky or not feasible, the two surgical options have been considered reasonable: thoracotomy and TPD. Notably, TPD is technically and therapeutically advantageous. It is less traumatic to the patient during the recovery period than anterior thoracotomy and provides a more extensive pericardial resection. Localized effusions, even those located posteriorly that cannot be reached without extensive thoracotomy, can be easily drained and look better cosmetically.

A clear indication of an absolute advantage of one technique over the other in terms of mortality, morbidity, recurrence of effusion, and cost, can help the surgeon to make an informed decision when choosing between the two procedures. Nevertheless, from an ethical point of view, a randomized comparison between traumatic thoracotomy procedures and TPD may lead to numerous

debates. Therefore, we designed this study to focus on comparing the outcomes of TPD and standard subxiphoid surgery.

In this study, patients who underwent TPD had effusions that were moderate and greater in size and all were associated with an echocardiographic appearance suggestive of tamponade physiology. All patients tolerated general anesthesia and single lung ventilation well. In some series, clinically unstable patients often needed percutaneous intervention with echocardiography-guided needle puncture before any surgical procedure to avoid instability during the induction of anesthesia (15). In the present study, 23% of the TPD group and 42% of the SX group had already experienced percutaneous decompression, albeit electively, before the procedure. Hence, we believe that the presence of moderate and greater tamponade in a clinically stable patient does not hinder TPD.

Previous studies have reported changes in the EF and cardiothoracic ratio (18). Both groups in this study had a significant decrease in CVP and PAP, accompanied by an approximately 5% increase in EF. Despite the evidence of a more complicated location of PE in the TPD group, both groups had similar hemodynamic improvements, concordant with their clinical status. Nonetheless, a disparity emerged during the follow-up period. The two of seven patients with recurrent pleural effusion in the TPD group had concomitant moderate PE on echocardiographic and radiographic controls, which was resolved by pleurocentesis. Despite the same patient profiles with the same etiology, pericardial window creation makes a significant decrease in re-accumulation rates. A 16.5% recurrence rate after subxiphoid pericardial tube drainage was similar to those reported for comparison of open versus percutaneous interventions (9, 11). Therefore, the SX group requires comparatively higher re-intervention for recurrent PE. The rationale could be that the extensive pericardiotomy during the TPD procedure through the potentially risky area prevents intrapericardial re-accumulation. The pleural effusions, -pericardial in origin, drained to thorax through the window which remained open for a considerable time and they required simple needle aspiration in the seven symptomatic patients. Although most of it was absorbed through pleura (15, 19) the pleural effusion after TPD should be considered as recurrence, and it reflects the chronicity of the disease process in the pericardial cavity. The possible absorption by the pleura together with the distribution of fluid to a broader thoracic cavity might cause latency of symptoms to emerge, and this seems to be one of the major advantages over subxiphoid drainage wherein the recurrence rate is about 10.2%–32% (16, 17). Concordant with our results, Piehler et al. (20) proposed that there is a direct relationship between the extent of pericardiectomy and the incidence of recurrence. Therefore, complete pericardiectomy is recommended instead of subxiphoid resection.

Some studies suggest that posterior localization of post-pericardiotomy delayed effusion ranges between 41%–86.1% (10, 21, 22). Removal of the anterior pericardial layers followed by the retrosternal adhesions precludes fluid collections to the retrosternal space where the tube or catheter is inserted during the subxiphoid approach. Notably, these adhesions have the potential to adduct the heart and bypass grafts to the sternum, making them susceptible to injury during blunt dissection. Moreover, anterior pericardial adhesions that jeopardize the subxiphoid access could be avoided with the TPD procedure.

Although some studies in the literature covering the PEs without prohibitive factors for a subxiphoid approach reported no complications with thoracoscopic drainage (4, 20). However, we observed that complications were not rare in our series and could be unique to the drainage of PE after open-heart surgery. Notably, pleural tears that cause pneumothorax and bleeding were seen in 12.5% and 8.3%, respectively. Post-procedural bleeding (between 100 and 200 mL for more than 3 consecutive hours) was managed medically without conversion to thoracotomy, and we sutured the large tears on the visceral pleura in two patients during TPD. We witnessed- cerebral events as a complication of carbon dioxide insufflation. These patients recovered without any sequelae within 36 hours and were extubated in the intensive care unit. Furthermore, conversion to thoracotomy might occur in approximately 5% of cases. Nevertheless, we believe that cardiac trauma, which could be observed during both percutaneous or subxiphoid drainage, is unlikely in TPD because all maneuvers are performed with the guidance of the thoracoscopic camera.

The 10 TPDs were performed from the left side of the thoracic cage. As a procedural note, we recognized that the heart becomes closer to the left thoracic wall in patients with cardiomegaly and obesity, making these patients vulnerable to cardiac injury during the insertion of trocars and surgical instruments. Therefore, we recommend using short trocars without cutting bladeand the camera must be placed first.

We think that the opening of the pericardial space to the left pleura during the left internal thoracic artery (LITA) harvest allows the drainage of postoperative effusion to the thorax. However, if PE recurs, it generally accumulates laterally and posteriorly to the apex of the heart. We cautiously evaluated the CT images to determine an inferior connection to the right side in these cases. If we saw a link, we performed TPD from the right side. Otherwise, localized effusions around the apex without connections were drained from the left side of the thorax, avoiding adhesions near the LITA graft.

CONCLUSION

Subxiphoid tube insertion is the standard procedure for the treatment of PE that could not be treated percutaneously. Notably, TPD has the potential to provide similar favorable results for the treatment of post-surgical PEs with difficult locations. TPD is a safe and valuable alternative and is a justified procedure in case of failed subxiphoid surgery. Therefore, it should be considered more proactively as an alternative to thoracotomy in patients with prior cardiac surgery.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Kavaklıdere Umut Hospital.

Informed Consent: Informed consent was obtained from each patient.

Peer-review: Externally peer-reviewed.

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

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

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Original Research

Malignant Transformation of Mature Cystic Teratomas: A Retrospective Analysis of 181 Cases

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ABSTRACT

Objective: Mature cystic teratoma (MCT) constitutes 20% of all ovarian tumors and is the most prevalent ovarian germ cell tumor. Notably, any of its component tissues may undergo a malignant transformation (MT). This study aimed to retrospectively analyze the malignancies that arise from MCT of the ovary.

Methods: Data of histopathological analysis of ovarian masses resected from adult patients and diagnosed at our laboratory between January 2012 and December 2018 were reviewed.

Results: Of our 181 cases, 7 (3.86%) were detected to have MT. Among the MT cases, five had papillary thyroid carcinoma, one had squamous cell carcinoma, and one had a strumal carcinoid.

Conclusion: The diagnosis of malignancies arising from MCT is crucial to decide on the follow-up and treatment options for patients. Reporting data obtained from cases that demonstrate MT will aid in the pre- and postoperative management of patients.

Keywords: Malignancy, mature cystic teratoma, ovarian tumor

INTRODUCTION

Mature cystic teratoma (MCT) of the ovary is the most prevalent ovarian germ cell tumor and originates from two or three germ layers (ectoderm, mesoderm, and endoderm). It constitutes 20% of all ovarian tumors (1).

The prevalence rates of these tumors, encountered in patients of ages ranging from childhood to postmenopause, typically peak around ages 20–40 years (2). MCTs are generally clinically asymptomatic and are detected either incidentally or because of the pressure from the mass during gynecological examination. Transvaginal ultrasonography is the primary diagnostic tool used in the diagnosis of MCT (3). Notably, tumor markers have a limited role in diagnosis (4).

Mature cystic teratomas have a typical macroscopic appearance; most are cystic and contain sebaceous material. Tumor components include various tissues, including hair, fat, bone, cartilage, glial tissue, gastrointestinal epithelium, respiratory epithelium, and thyroid tissue (3).

Mature cystic teratoma is a benign tumor; however, it may rarely undergo a malignant transformation (MT) (at a rate of 1%–2%), typically encountered in more advanced ages (5). MT may arise from any of the MCT tissue components. The most common MT reported in a case series is squamous cell carcinoma (SCC), and the literature contains reports of various types of sarcoma and adenocarcinoma, melanoma, and basal cell carcinoma arising from MCT (6-13).

This study aimed to retrospectively evaluate 181 adult patients with MCT who were analyzed and diagnosed by our laboratory between 2012 and 2018 concerning their histopathological findings and MT.

METHODS

Results of histopathological diagnoses of ovarian masses resected from adult patients between January 2012 and December 2018 were reviewed. Data of 181 patients who were diagnosed with MCT or dermoid cysts were evaluated in terms of patient age, tumor location, tumor diameter, macroscopic properties of the tumor, and MT observed after histopathological diagnosis. The evaluation was based on hospital file records, as well as pathology reports. Besides, archived pathology slides were re-evaluated where necessary.

RESULTS

The ages of our patients ranged from 17 to 72 years (mean: 30.47 years), and 62.43% of MCT patients were aged between

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Figure 1.a-c. Follicular architecture of thyrocytes with nuclear overlapping, crowding, and nuclear enlargement. Hematoxylin and Eosin (H&E) ×200, ×400 (a, b). Immunohistochemical membranous positivity with HBME-1, HBME-1 ×400 (c)

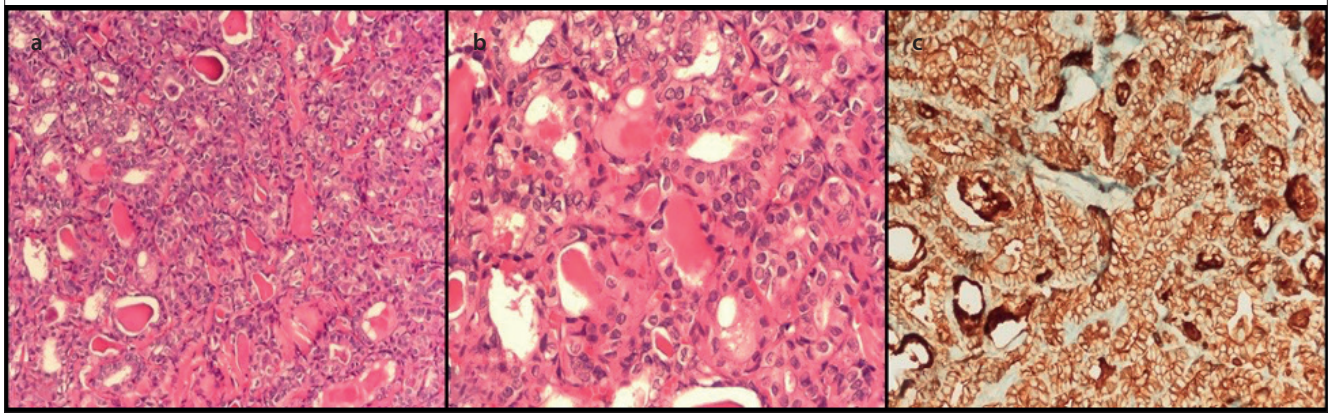
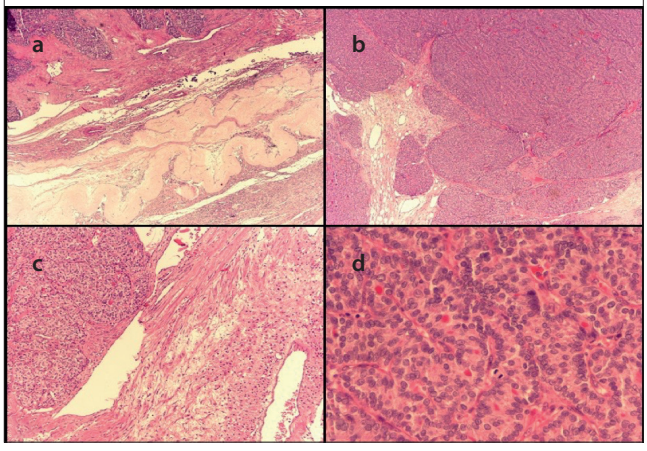


Figure 2. a-d. Solid nests composed of small uniform cells with round hyperchromatic nuclei, H&E ×40 (a, b). Tumorous area (left side) adjacent to the ovarian stroma (right side), H&E ×200 (c). Increased mitotic figures, H&E ×400 (d)

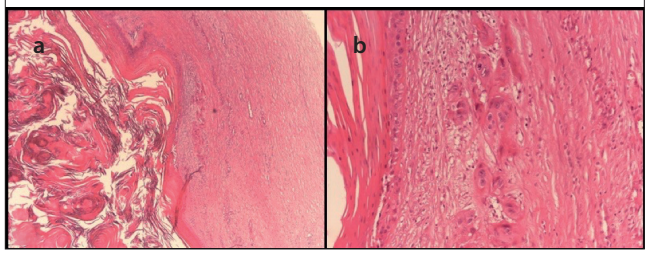


20–40 years. The tumor was localized in the right ovary in 85 cases (46.96%), left ovary in 77 cases (42.54%), and bilateral in 19 cases (10.49%). Evaluation of macroscopic tumor characteristics revealed that the diameter of the tumor was smaller than 10 cm in 124 cases (68.5%), between 10 and 20 cm in 53 cases (29.28%), and greater than 20 cm in 4 cases (2.2%). Macroscopically, the surface of the section was cystic in 68 cases, solid in 41, and solid-cystic in 72. Macroscopically, the solid component was observed to increase at greater tumor diameters.

Main Points:

- Although MCT is a benign tumor, it may rarely undergo a malignant transformation from any of the tissue components. Macroscopically, the ovarian mass with increased solid component and tumor diameter must be examined carefully for MT.
- The diagnosis of malignancies that arise from mature cystic teratomas is important for the follow-up and treatment options of the patients.
- Reporting the data obtained from cases of MT will be informative for the pre/postoperative management of the patients.

Figure 3. a, b. Squamous lining epithelium with prominent keratin, H&E ×40 (a). Irregular and atypical squamous islands in the stroma, H&E×400 (b)



In our series, 7 (3.86%) of the 181 patients manifested MT. Of these, five had papillary thyroid carcinoma (PTC), one had SCC, and one had a strumal carcinoid (Figures 1-4). Three of the PTC cases had microcarcinomas (<1 cm). Clinical complaints included pelvic pain in five cases, pelvic tenderness in one case, and a clinically asymptomatic mass that was incidentally detected during a routine check-up. We could access the tumor marker levels of only two cases, and both these cases were determined to have high CA 125 levels. Three cases were operated for a malignant preliminary diagnosis, whereas the rest were operated for a preliminary diagnosis of a dermoid cyst. The majority of cases demonstrated unilateral localizations and solid cut surfaces. The clinicopathological findings of our MT cases are presented in Table 1.

DISCUSSION

Mature cystic teratoma is the most prevalent ovarian germ cell tumor and constitutes 20% of all ovarian tumors (1). The most common complications associated with it include torsion, infection, and rupture. MT of the MCT is a rare (1%–2%) but serious complication (5, 14). Per the literature, a 35-year study by Ayhan et al. (14) determined an MT rate of 1.4% among their 501 study patients. Rathore et al. (15) determined this rate to be 3.5% in

their 230-patient series, whereas Bal et al. (16) identified an MT rate of 6.6% in their 75-patient series. In our series, 7 of the 181 cases were observed to have MT, and the MT rate was 3.8%.

Nevertheless, the clinical prediction of MT is difficult. Studies have not observed a correlation between MT and serum tumor markers; however, more than 70% of cases were reported to have high CA 125 or CA 19-9 levels (4). In our case series, of the three cases suspected of having malignancies, tumor marker levels of only two could be accessed, and both these cases were determined to have high CA 125 levels.

Most often MTs of the MCT are unilateral (16). In our series, three cases underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy, whereas others underwent unilateral excision for the unilateral masses. Notably, patients who underwent bilateral salpingo-oophorectomy also had unilateral tumors.

Notably, SCC is the most common MT of MCT. However, among the seven MT cases in our series, only one had SCC (0.55%). Nonetheless, the most common MT was PTC arising from struma ovarii, as observed in five cases (2.76%). One of our cases (0.55%) had a strumal carcinoid.

Struma ovarii is a monodermal teratoma of the ovary and constitutes 2% of all germ cell tumors. Notably, 5%–10% of struma ovarii cases are malignant, and typically manifest as unilateral masses during the reproductive period. However, their incidence increases with age. The most prevalent MT in struma ovarii is PTC, followed by follicular carcinoma and follicular variant of papillary carcinoma (17). Five of our cases were noted to have a follicular

Figure 4. a-f. Monomorphic cells growing in solid nests and trabeculae admixed with thyroid microfollicles containing colloid, H&E×40, ×100 (a, b). Solid islands of cells forming rosettes, H&E×40 (c). Follicular nuclear TTF-1 positivity in the thyroid component, TTF-1×100 (d). Low proliferation index with Ki 67, Ki67×100 (e). Inhibin negativity of the tumor, Inhibin×100 (f)

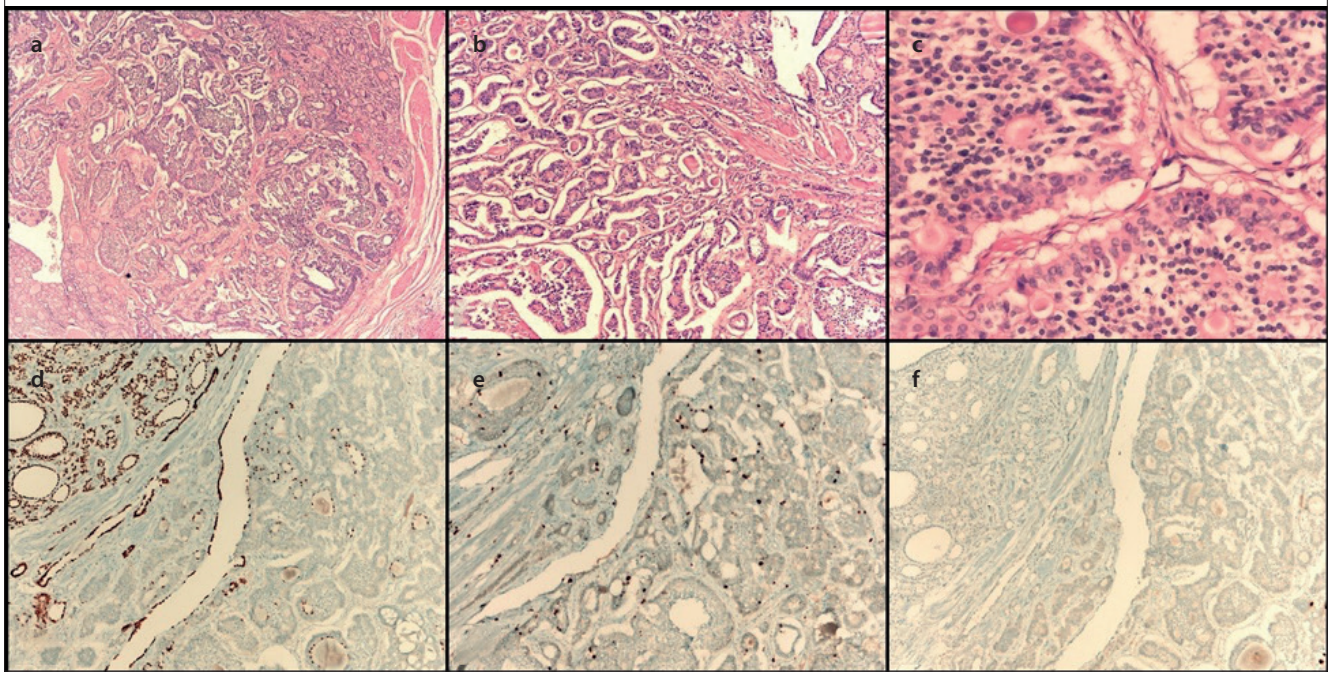


Table 1. Clinicopathologic parameters of 7 cases with malignant transformation

Case	Age	Localization	Symptom	Tm marker	Clinical diagnosis	Macroscopy	Histopathologic Diagnosis
1	47	R	Pelvic pain	None	Malignant	11 cm	SCC
2	27	R	Control	None	DC	3 cm	PMC
3	20	R	Pelvic pain	None	DC	4.5 cm	PMC
4	47	L	Pelvic pain	None	DC	7.2 cm	PMC
5	62	R	Pelvic tenderness	CA 125:80.6 U/ml	Malignant	7.5 cm	PDTC
6	72	L	Pelvic pain	CA 125:3648 U/ml	Malignant	10 cm	PTC
7	24	R	Pelvic pain	None	DC	9 cm	Strumal Carcinoid

R: right; L: left; DC: dermoid cyst; SCC: squamous cell carcinoma; PMC: papillary microcarcinoma; PDTC: poorly differentiated thyroid carcinoma; PTC: papillary thyroid carcinoma

variant of papillary carcinoma, whereas one case had a poorly differentiated thyroid carcinoma arising from PTC. All these cases were unilateral, with two in the second decade, one in the fourth decade, and the other two in the sixth and seventh decades. Two cases of advanced age were detected to have PTC and poorly differentiated thyroid carcinomas arising from PTC, whereas three cases had tumors in the form of a microcarcinoma. The presence of microcarcinoma led to PTC being the most prevalent MT in our series, contradicting the literature and our expectations. We reasoned that the detection of microcarcinoma cases was probably linked to the high number of macroscopic samples.

Malignant struma ovarii cases may clinically manifest thyrotoxicosis, and studies have reported cases of hypothyroidism after resection (17). The clinical data of our cases were inspected concerning their thyroid states, and it was observed that none had a history of any thyroid-related clinical complaints.

Nevertheless, there is no consensus regarding the clinical approach for malignant struma ovarii cases. According to various approaches in the literature that have been described for cases and case series, patients must be screened for metastasis after diagnosis, and their serum thyroglobulin and iodine 131 levels must be monitored. Notably, thyroidectomy has been reported in some cases (17, 18). We could access the postoperative follow-up data of three cases (cases 5 and 6), and thyroid examinations of these cases did not indicate any pathologies.

The second most prevalent MT in our series was SCC, which is the most common MT of MCT (19). In a study by Kikkawa et al. (20) that investigated a series of 37 patients with SCC arising from MCT over 17 years, the mean age was determined as 55.2 years. Notably, our case was 47 years old. Regarding the epidermal component, MT may arise from squamous, ciliated, and non-ciliated columnar epithelia. When dealing with ovarian SCC, metastatic carcinomas must certainly be eliminated. Notably, the most common primary cancers are cervical or vaginal SCC in ovarian metastases. Immunohistochemically, SCC arising from MCTs were reported to show HPV and strong P16 positivity. Based on these findings, HPV was thought to be a risk factor for this malignancy (19). Upon gynecological examination, our case did not have any cervical or vaginal pathologies. Our patient was not immunohistochemically evaluated for HPV and P16 expression. The conventional treatment for SCC arising from MCTs is total hysterectomy, bilateral salpingo-oophorectomy, surgical staging (omentectomy, appendectomy, peritoneal biopsies, pelvic and paraaortic lymphadenectomy) in early-stage cases, and optimal cytoreductive surgery in advanced stage cases. Chemotherapy is recommended for those with more advanced disease, but the efficacy of radiotherapy is uncorroborated. Notably, the prognosis is poor in cases with extra-ovarian spread (19).

Strumal carcinoid tumor of the ovary is a rare form of ovarian teratoma composed of carcinoid and thyroid tissue (21). Strumal carcinoid was first described in 1970 by Robboy et al. (22). Clinically, the symptoms associated with the mass may be accompanied by symptoms of carcinoid syndrome. Our case did not clinically manifest symptoms of carcinoid syndrome. Strumal carcinoids can present an admixture of thyroid tissue and the carcinoid tumor component. The thyroid component is composed of micro and macrofollicles containing colloid. The carcinoid component demonstrates a pure trabecular or a mixed trabecular-insular pattern in most cases. Notably, the thyroid and carcinoid components can be differentiated based on immunohistochemical markers, as well as cellular properties (21). Our case showed an admixture of thyroid tissue and the carcinoid component presenting trabecular and solid patterns. Furthermore, in our case, the carcinoid component exhibited synaptophysin and chromogranin positivity, and the thyroid component exhibited follicular nuclear TTF-1, cytoplasmic and colloidal thyroglobulin positivity. When diagnosing ovarian stromal carcinoid tumors, the following differential diagnoses must be excluded: thyroid carcinomas, ovarian granulosa, and Sertoli Leydig cell tumors arising from stromal carcinoids. Strumal carcinoids almost always have a benign manifestation (23). Kurabayashi et al. (24) reported detecting bone and breast metastases in cases who had shown marked cellular atypia, high mitotic activity, and focal necrosis 3.5 years earlier. Nevertheless, our case did not have cellular atypia, high mitotic activity, or necrosis, and is currently undergoing the first year of postoperative follow-up.

CONCLUSION

The diagnosis of malignancies that arise from MCT is crucial in deciding the follow-up and treatment options of patients. MCTs are encountered quite frequently in the pathological routine and must be inspected thoroughly for MT. Reporting data obtained from cases with MT can aid in the pre- and postoperative management of patients.

Ethics Committee Approval: Ethical committee approval was obtained from Gaziantep University School of Medicine ethical board (Approval No: 2019/323).

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

Peer-review: Externally peer-reviewed.

Author Contribution: Concept- Z.B., N.B.T.; Design- Z.B; Supervision- N.B.T.; Resources-Z.B., N.B.T; Materials-Z.B; Data Collection and/or Processing- Z.B., N.B.T.; Analysis and/or Interpretation- Z.B.; Literature Search- N.B.T.; Writing Manuscript- Z.B., N.B.T.; Critical Review-Z.B., N.B.T.

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

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Original Research

Clinical Significance of CBCT Findings in the Treatment of Maxillary Cysts Expanded Into the Nasal and Sinus Cavities

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ABSTRACT

Objective: We aimed to retrospectively assess the importance of the radiological findings from the cone-beam computed tomography (CBCT) data on the treatment of maxillary cysts extending into the nasal and maxillary sinus cavities.

Methods: Thirty-three consecutive patients with maxillary intraosseous odontogenic cysts that extended into the nasal and maxillary cavities were included in the present study. The CBCT signs of the lesions were classified into three subgroups for lesions extending into the maxillary sinus and divided into four subgroups for lesions extending into the nasal cavities. Age, gender, cyst type, location, presence of cortical bone expansion/resorption, root displacement, lacunarity (unilacunar and multilacunar), and lesion dimensions were also evaluated. All the patients were treated with only enucleation, only decompression, or decompression after enucleation. Here, $p<0.05$ was considered to be statistically significant.

Results: Patients (13/39.4% females and 20/60.6% males) were in the age range from 8 to 65 years (mean age: 30.42±12.74 years). Here, 23 cases, (69.7%) exhibited both buccal and palatine bone resorption as compared to only cortical resorption in the coronal CBCT slices. The cysts' dimensions were calculated from the axial, coronal, and sagittal slices as 24.58±8.56, 24.94±9.74, and 26.45±7.88 mm, respectively. There were no statistically significant differences between both the subgroups of the CBCT findings of lesions extending in the nasal area or maxillary sinus as well as the three treatment modalities ($p>0.05$).

Conclusion: The resorption of the lateral nasal wall and cortical floor, particularly in the nasal region, and the findings of narrowing of the airway may affect treatment planning, even if the obtained results were not statistically significant.

Keywords: Cone-beam computed tomography, decompression, enucleation, odontogenic cyst

INTRODUCTION

Odontogenic cysts are frequently encountered lesions in the maxillofacial region. Such entities that appear in the maxillary region may lead to the formation of granuloma-like lesions when restricted within the alveolar bone; sometimes, they can result in the resorption and/or expansion of the buccal and palatine cortical bones, as well as cause root resorption or displacement when related to the tooth-bearing areas (1, 2). For cases in which the lesions extend into the maxillary sinus or nasal cavity or both, more complicated findings may arise even without any symptoms. In particular, the potential cavity of the maxillary sinus results in lesion enlargement. It has been reported that large-volume lesions, such as radicular cysts (3), dentigerous cysts (4), and odontogenic keratocysts (5, 6), which are not confined within the maxillary alveolar bone, can extend toward the sinus and nasal areas.

It has also been reported that cases involving the maxillary sinus are related to symptoms such as facial swelling, epiphora related to nasolacrimal duct obstruction, orbital proptosis, and tooth displacement to the orbital floor caused by dentigerous cysts (3, 6, 7). Symptoms in the nasal region are generally limited to nasal floor resorption and/or expansion, leading to nasal airway narrowing and septal deviation (7).

Conventional radiographic techniques are inadequate to diagnose cystic lesions seen in the middle face region because of the region's complex three-dimensional structure. Therefore, computed tomography (CT) is the most popular technique that is preferred for observing details of the bone structures (1). However, in the recent years, cone-beam computed tomography (CBCT) has become increasingly popular than conventional CT in the dental field. In particular, it provides better image resolution

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with less radiation and cost as compared to those resulting from conventional CT (8).

The present study aims to determine the different radiological signs of maxillary odontogenic cysts that extend into the maxillary sinus and nasal cavities observed on the CBCT scans to help clinicians in appropriate treatment planning in favor of the patient.

METHODS

Patients

We designed a retrospective study comprising preoperative CBCT images of 33 patients. The patients were diagnosed and surgically, conservatively, or conservative+surgically treated with maxillary intraosseous odontogenic cysts at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Gaziantep University, between December 2012 and November 2018. Informed consent forms were not required. since it was a retrospective study and archive data was used. The inclusion criteria were as follows: 1) maxillary cyst that resorbed or expanded at least one cortical region (buccal, palatal, or nasal/sinus floor cortices or anterior/lateral nasal wall), 2) the cyst extending into the maxillary sinus that changed the sinus membrane thickness, and 3) the cyst extending into the nasal cavity that resorbed the lateral nasal wall or nasal floor or restricted the nasal airway.

Cone-beam computed tomography images with inadequate data (e.g., the field of CBCT images did not extend into the entire cyst cavity) or that showed signs of previous operations, non-osseous jaw cysts, and presence of technical artifacts that could complicate the evaluations of the maxillary sinus and nasal cavities were excluded. Case records were retrieved from the archives, and each patient's age, gender, cyst type, location, presence of cortical bone expansion/resorption, root displacement, lacunarity, dimensions of lesions, and treatment modality were also evaluated.

A total of 33 cases with CBCT records (24 radicular cysts, 5 dentigerous cysts, 2 odontogenic keratocysts, 1 nasopalatine duct cyst, and 1 glandular odontogenic cyst) obtained from 20 males and 13 females (age range: 8–65 years; mean age: 30.42±12.74 years) were ultimately included in the present study. The ethical approval for this retrospective study was obtained from the Ethics Committee of the Gaziantep University (2018/271, November 21, 2018).

CBCT Acquisition

All the CBCT images were obtained using the same scanner (Planmeca, ProMax, Helsinki, Finland) using a voxel size of 200 µm, field of view of 200 × 170 mm, and high resolution. The axial, sagittal, and coronal sections were imaged, and the images were analyzed using special CBCT software (Romexis, Planmeca, Helsinki, Finland). The exposure settings were 5.0–7.0 mA and 80 kV for an exposure time of 17.5 s. The data were reconstructed with slices at an interval of 1.2 mm.

Main Points:

- CBCT provides detailed radiographic data before surgical intervention.
- Huge cysts in maxilla may cause narrowing of the nasal airway.
- CBCT examination prevents permanent complications.

Assessment of CBCT Images

The observations were carried out under dimmed lighting and a black background. Images were viewed with a 24 inch UltraSharp LED TFT Monitor (Dell, USA) that displayed 2 megapixels at a pitch of 0.27 pixels. All the images were evaluated by the first author.

Determination of Cyst Dimensions and Localization

Intraosseous cysts that were located in the anterior/posterior maxillary regions expanding to the nasal cavity or maxillary sinus were radiographically identified as having a well-demarcated radiolucent unilocular or multilocular appearance, and their dimensions were measured on CBCT slices across all the three planes (coronal, axial, and sagittal) in millimeters. The longest diameter in the vertical direction was measured in the coronal section. The longest buccopalatal direction in the axial section and the most anterior–posterior dimension in the sagittal view were calculated.

The localization of cysts was mainly divided into three groups: anterior (canine to canine), posterior (canine to posterior), and anterior–posterior (lesion located on both the anterior and posterior regions).

Examination of Maxillary Sinus Affected by Cystic Lesions

On the axial CBCT slices, pathological findings obtained from the cystic lesions that expanded into the maxillary sinus were categorized as follows: 1) No pathological changes (MS_1); 2) resorption on the sinus wall and expansion into the sinus cavity with chronic sinusitis (MS_2); and 3) resorption on the sinus wall and expansion into the sinus cavity with total opacification (MS_3) (Figure 1a-c), respectively.

Examination of Nasal Cavity Affected by Cystic Lesions

On the coronal CBCT slices, the pathological findings obtained from the cystic lesions that expanded into the nasal cavity were categorized as follows: 1) No pathological changes (NC_1); 2) only resorption into the nasal floor cortical or lateral nasal wall (NC_2); 3) nasal floor expansion into the cavity with narrowing of the nasal airway (NC_3); and 4) resorption in the nasal floor cortical with expansion into the cavity, leading to narrowing of the nasal airway with septum deviation or thickness in the nasal mucosa (NC_4) (Figure 2a-d), respectively.

Surgical Procedures

All the patients included in this study underwent three different treatment modalities: surgical; conservative enucleation (removal of the cyst epithelial lining) and decompression (decreasing the intracystic pressure by stimulating new bone formation); conservative+surgically (decreasing the cyst volume by decompression and subsequent enucleation). All the treatments were performed under local anesthesia.

Figure 1. a-d. Pathological findings of cysts invading into the maxillary sinus. (a) no pathological changes (MS_1). (b) resorption on the sinus wall and expansion into the sinus cavity with chronic sinusitis (MS_2). (c) resorption on the sinus wall and expansion into the sinus cavity with total opacification (MS_3)

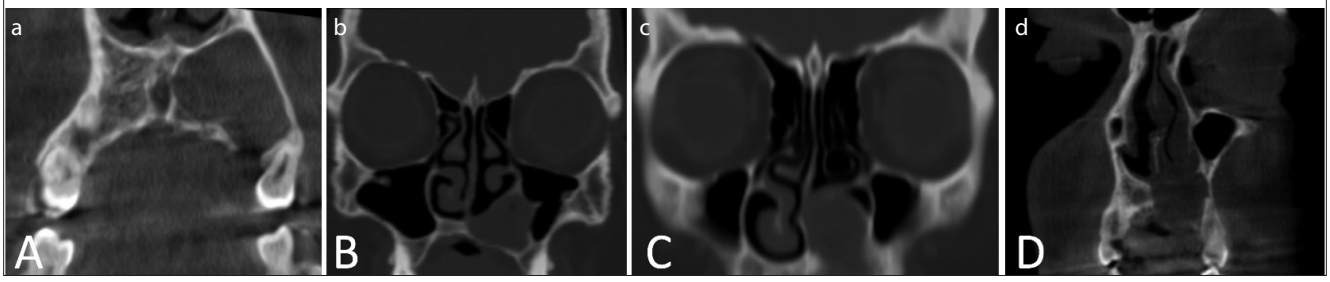
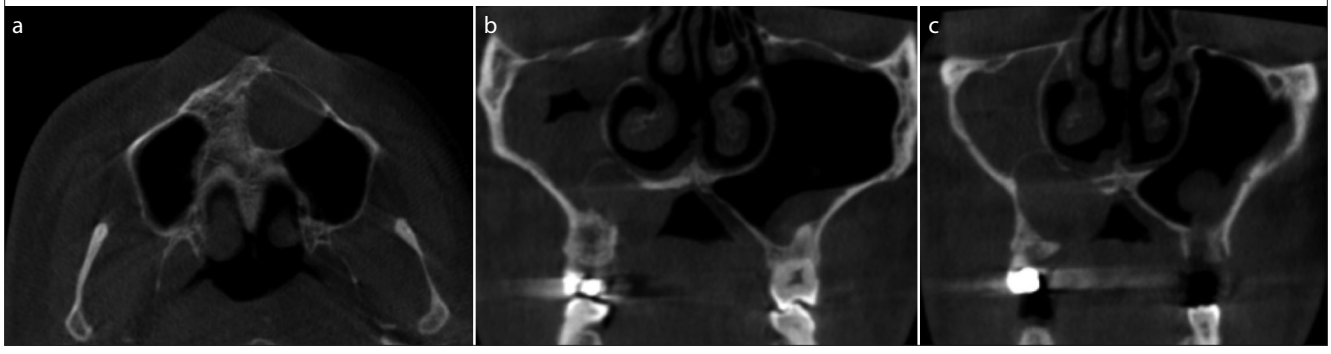


Figure 2. a-c. Pathological findings of the cysts invading into the nasal cavity. (a) no pathological changes (NC_1). (b) only resorption in the nasal floor or lateral nasal wall cortices (NC_2). (c) nasal floor expansion into the cavity with narrowing of the nasal airway (NC_3). (d) resorption in the nasal floor cortical region with expansion into the cavity, leading to narrowing of the nasal airway with septum deviation or thickness in the nasal mucosa (NC_4)



Statistical Analysis

All the values were shown as mean±deviation and all the analyses were performed using the IBM Statistical Package for the Social Sciences (IBM SPSS Corp.; Armonk, NY, USA) version 25.0 software. One-way ANOVA test was used for comparing the independent groups and post-hoc Tukey's test was performed for subgroup comparisons. The intraoperator reliability of the scores recorded by the same observer was examined by the intraclass correlation coefficient for an interval of at least 2 months, which yielded a 95% agreement rate. Here, $p<0.05$ was considered to be statistically significant.

RESULTS

The CBCT images of 33 patients were evaluated, which included 13 females (39.4%) and 20 males (60.6%). The age ranged from 8 to 65 years (mean age: 30.42±12.74 years). Here, 24 cases were radicular cysts (72.7%), 5 were dentigerous cysts (15.2%), 2 were odontogenic keratocysts (6.1%), 1 was nasopalatine duct cyst (3%), and 1 was glandular odontogenic cyst (3%). Further, 9 cases (27.2%) were located in the anterior region, 12 (36.4%) were in the posterior region, and 12 (36.4%) cases were extended in both the regions. More cases (23 cases, 69.7%) were present with both buccal and palatine bone resorption as compared to only cortical resorption in the coronal CBCT slices. Similarly, expansions in both the cortical regions were seen in more cases (19 cases, 57.6%) as compared to only cortical expansion. Such cases show only one finding in the buccal or palatine region (expansion or resorption), namely, these lesions tended to expand to the buccal side or undergo resorption on the same side (buccal expansion: 7/21.2% cases; buccal resorption: 5/15.1%). Only 9 cases (27.3%) exhibited root displacement. All the cases exhibited the unilocular radiographic appearance. With regard to the cyst dimensions, the mean axial, coronal, and sagittal measurements were calculated to be 24.58±8.56, 24.94±9.74, and 26.45±7.88 mm, respectively. Table 1 lists the clinical and radiological findings of 33 maxillary cysts with axial, coronal, and sagittal dimensions.

When evaluating the CBCT findings of the cysts that extend into the nasal cavity and maxillary sinus, 9 cases (27.3%) exhibited the MS_1 finding; 17 (51.6%) cases, the MS_2 finding; and 7 (21.1%) cases, the MS_3 finding. No pathological finding in the nasal cavity (NC_1) was found in 8 (24.3%) cases. The CBCT findings of NC_2, NC_3, and NC_4 were seen in 14 (42.5%), 5 (15.1%), and 6 (18.1%) cases, respectively. There were no statistically significant differences between both the subgroups of the CBCT findings of lesions extending into the nasal area or maxillary sinus as well as the three treatment modalities.

Out of the 33 treated cases, decompression was performed in 3 cases, enucleation in 15 cases, and decompression after enucleation in 15 cases. Follow-up was performed for at least one year; no recurrence was observed in all the lesions.

Data regarding the distribution of the different treatment methods applied to the lesions according to the maxillary sinus or nasal cavity findings are listed in Tables 2 and 3, respectively.

Table 1. Clinical and radiological findings of 33 maxillary cysts with axial, coronal, and sagittal dimensions (mm)

Characteristics	
Age range, years (mean±SD)	8–65, 30.42±12.74
	(n/%)
Gender	
Male	20/60.6
Female	13/39.4
Diagnosis of cyst	
Radicular cyst	24/72.7
Dentigerous cyst	5/15.2
Odontogenic keratocyst	2/6.1
Nasopalatine duct cyst	1/3
Glandular odontogenic cyst	1/3
Location	
Anterior	9/27.2
Posterior	12/36.4
Both regions	12/36.4
Cortical bone expansion	
No resorption	3/ 9.1
Buccal only	7/21.2
Palatine only	0/0
Buccal and palatine	23/69.7
Cortical bone resorption	
No resorption	8/24.3
Buccal only	5/15.1
Palatine only	1/3
Buccal and palatine	19/57.6
Root displacement	
Displacement	9/27.3
No displacement	24/72.7
Radiographic appearance	
Unilocular	33/100
Multilocular	0/0
Cyst dimensions (mean±SD, mm)	
Axial	24.58±8.56
Coronal	24.94±9.74
Sagittal	26.45±7.88

DISCUSSION

The maxillary region, which is in the midface area, makes it difficult for surgeons to treat cystic lesions due to the complicated anatomical bone structure in this region. Although the radiographical features of the pathological findings caused by maxillary cysts are reported in detail in other case reports (3, 5-7, 9), case series are limited (10, 11).

It is well known that clinical and radiographical findings affect the treatment planning of odontogenic cysts. For cysts in which the lesions expand into larger volumes with weak cortical bones or when surgical intervention causes inferior alveolar nerve damage and tooth buds or inadvertent injury to the adjacent structures, decompression or marsupialization is highly recommended in many studies employing CBCT or CT techniques (12, 13). These radiographical techniques yield more detailed bone structures, particularly in the midface region, as compared to those obtained from conventional 2D graphs. CBCT is superior to CT because of its advantages such as low radiation dose, high resolution, easy handling, and low cost in the evaluation of maxillofacial pathologies.

In this study, we retrospectively aimed to evaluate if the radiographical findings obtained from CBCT slices influence the treatment planning of maxillary cysts extending into the maxillary sinus and nasal region. It was found that the maxillary sinus and nasal region findings divided into different subgroups did not yield a statistical difference between the enucleation, decompression, and enucleation following decompression treatments applied to such cysts. The low number of cases in these subgroups may lead to this outcome; therefore, this can be considered as a demerit of the present study.

As mentioned earlier, the detailed radiographic data on large-volume maxillary cysts are usually limited by the individual case reports. The clinical experience of the surgeon performing the treatment with different clinical and radiographical findings of the lesion can result in a lack of standardization in the treatment planning of such cases. In similar cases, one surgeon may perform decompression (14), while another surgeon may perform enucleation (9) or assist with endoscopic enucleation (5). On the other hand, the different histopathological features of the cysts may affect their clinical behavior, and the results of similar treatments may be different. Gao et al. (15) reported that an increase in the bone density was more significant in radicular cysts than that in keratocystic odontogenic tumors (KCOTs). We believe that the lack of standardization in the cyst type is another limitation of the present study. This is due to the retrospective nature of this study.

Anteriorly located maxillary lesions expanding into the nasal cavity and posterior lesions mostly expand into the sinus. Lesions extending into the sinus may occasionally fill the sinus, but ophthalmologic complications may rarely occur, such as proptosis, exophthalmos, diplopia, ptosis, or decreased visual acuity by resorbing the orbital floor (16). Since no ophthalmologic symptoms or radiographical findings were detected in any of these cases, the classification of the findings in this study was limited to the maxillary sinus and nasal region.

Table 2. Distribution of different treatment methods for cysts according to maxillary sinus findings

		Decompression	Enucleation	Decomp+Enuc	p
Maxillary sinus findings (n/%)	MS_1 (9/27.5)	2/6.2	4/12.2	3/9.1	>0.05
	MS_2 (17/51.3)	1/3	9/27.1	7/21.2	
	MS_3 (7/21.2)	0/0	2/ 6.1	5/15.1	
	Total	3/9.2	15/45.4	15/45.4	33/100

Table 3. Distribution of the different treatment methods according to the findings of lesions extending into the nasal cavity

		Decompression	Enucleation	Decomp+Enuc	p
Nasal cavity findings (n/%)	NC_1 (8/24.2)	0/0	6/18.1	2/6.1	>0.05
	NC_2 (14/42.4)	2/6.2	6/18.1	6/18.1	
	NC_3 (5/15.1)	1/3	1/ 3	3/9.1	
	NC_4 (6/18.3)	0/0	2/6.2	4/12.1	
	Total	3/9.2	15/45.4	15/45.4	33/100

Inflammatory cysts may affect the sinus mucosa, with or without perforating the cortical bone of the maxillary sinus from areas where the bone is weak (17). In this study, chronic sinusitis or total opacification with maxillary sinus invasion was detected in a majority of the cases (n=24). Further, as indicated in the literature, a thin cortical radiolucent boundary may be recognizable on the CBCT or CT slices between the sinus cavity and the cyst in the lesions extending into the sinus, unless the cyst becomes infected. This thin cortical line is important in the diagnosis of cysts (18). This also explains why 72.5% of such lesions, which extend into the maxillary sinus, are radicular cysts originating from the nonvital tooth. These cysts may cause chronic infection in the sinus. While decompression was applied to only 1 of these cysts, the 12 remaining lesions (MS_2 findings: 7 cases; MS_3 findings: 5 cases) were treated with enucleation following decompression.

The lateral nasal wall or nasal cortical floor resorption and narrowing of the airway findings caused by resorption were effective in our treatment planning. In large-volume cases treated without decompression, the cyst epithelial remnants may remain as it is difficult to completely remove the cyst epithelium from the mucosa of the nasal base, resulting in recurrence in the postoperative period. Due to the rich vascular supply of the nasal mucosa, bothersome bleeding may occur during surgery. In the treatment of such lesions, it was primarily aimed to reduce the lesion by decompression (NC_2: 6 cases; NC_3: 3 cases; NC_4: 4 cases) and then the cyst epithelium was removed by enucleation.

CONCLUSION

Although the results obtained are not statistically significant, it can be stated that the resorption of the lateral nasal wall and cortical floor, particularly in the nasal region, and findings of narrowing in the airway affect treatment planning. The large volume of the cyst can be reduced by decompression once a sufficient amount of bone is regained in order to prevent damage to the adjacent vital structures in the midface and therefore the subsequent surgery becomes safer.

Ethics Committee Approval: The ethical approval for this retrospective study was obtained from the Ethics Committee of the Gaziantep University (2018/271, 21.11.2018).

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept – M.Y., M.D.; Design – M.Y., M.D.; Supervision – M.D.; Data Collection and/or Processing – M.Y.; Analysis and/or Interpretation – M.Y.; Literature Search – M.Y.; Writing – M.D.; Critical Reviews – M.D.

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Original Research

Turkish Translation, Accreditation, and Validation of the Otitis Media–6 Questionnaire

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ABSTRACT

Objective: Otitis media is one of the most common ear disorders occurring in childhood, which causes alterations in communication between parents and children, thereby having a significant effect on the health-related quality of life. Otitis Media-6 questionnaire (OM-6) is a Likert-type scale used to assess the health-related quality of life in children with otitis media. In this study, our aim was to create a consistent, reliable, and valid Turkish version of the OM-6.

Methods: Children with otitis media presenting with effusion and healthy children were enrolled in this study between February 2019 and September 2019. The OM-6 test was administered to three different groups, including preoperative, postoperative, and control, among the children who had undergone ventilation tube insertion operation and among the healthy children.

Results: The overall scores of children with otitis media presenting with effusion exhibited a changing pattern, indicating a statistical significance in the evaluation of the health-related quality of life. The Turkish version of OM-6 includes the scores of construct validity (Spearman values between 0, 58-0, 89), internal consistency (Cronbach's alpha values between 0, 88-0, 96), and reliability (Pearson correlation value between 0, 67-0, 91). These scores exhibited a statistical significance.

Conclusion: The Turkish version of OM-6 is a stable, valid questionnaire with internal consistency.

Keywords: Otitis media, quality of life, surveys and questionnaires

INTRODUCTION

Otitis media is one of the most commonly diagnosed and antibiotic-prescribed diseases in the pediatric population worldwide (1). Long-term complications of otitis media include hearing loss and language development disorders (2). To define in a more specific manner, children with otitis media display lower levels of phonological awareness, semantic knowledge, narration, and reading ability (3). Thus, the presence of hearing loss and language development disorders in children with otitis media has a negative impact on their health-related quality of life (4).

Constructing a validated questionnaire is a typical method for assessing the health-related quality of life (5, 6). For this purpose, a six-item questionnaire termed the Otitis Media-6 questionnaire (OM-6) was developed by Rosenfeld. This questionnaire has been translated and validated in several different countries and languages (7-9). The primary aim of this study was to create a consistent, reliable, and validated Turkish version of the OM-6.

METHODS

This prospective study was conducted at the Otorhinolaryngology Department of Gaziantep University between February 2019 and September 2019. After the Turkish cultural adaptation and translation of OM-6, the test was applied to three groups of children. The preoperative group consisted of children with otitis media presenting with effusion (OME). In a 3-month follow-up period, the diagnosis of OME was made by physical examination and tympanometry tests. After obtaining informed consent for the surgical approach, a ventilation tube was inserted into children in the preoperative group. Three months after the ventilation tube insertion, the OM-6 test was reapplied to the OME group to evaluate the test–retest reliability. This retested group formed the postoperative group. The hospital staff's children aged between 4 and 12 years constituted the control group.

A consent response e-mail was received from the first author of the original version of OM-6 for the translation and application (Table 1: OM-6) (10). According to the Declaration of Helsinki, the Gaziantep University ethical committee approved this study.

Otitis Media-6 was applied to the caregivers or parents of children aged 4-12 years. OM-6 is a six-item Likert-type scale addressing the quality of life concerning hearing. These items are listed as follows: physical suffering, hearing loss, speech

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Table 1. Sample of Otitis Media Quality of Life Survey

	None (1)	Quite (2)	Somewhat (3)	Moderate (4)	Hardly (5)	Very Much (6)
Physical Suffering (Ear pain, ear discomfort, ear discharge, ruptured ear drum, high fever, or poor balance)						
HEARING LOSS Difficulty hearing, questions must be repeated, frequently says "what," or television is excessively loud						
SPEECH IMPAIRMENT: Delayed speech, poor pronunciation, difficult to understand, or unable to repeat words clearly						
EMOTIONAL DISTRESS: Irritable, frustrated, sad, restless, or poor appetite						
ACTIVITY LIMITATIONS: Playing, sleeping, doing things with friends/family, attending school or day care.						
CAREGIVER CONCERNS: How often have you, as a caregiver, been worried, concerned, or inconvenienced because of your child's ear infections or fluid over the past 4 weeks?						

impairment, emotional distress, activity limitation, and caregiver concerns. Verbal informed consent was obtained from all participants before applying OM-6. Each participant was kindly asked to fill the test by choosing points from 0 to 7 for each criterion separately. The mean point of these six criteria is the final score. A lower score correlates with a high quality of life.

Validation of the Turkish questionnaire was achieved in three steps. The first step included the translation of the original OM-6 from English to Turkish by two native Turkish translators. Then, OM-6 was retranslated from Turkish to English by two native English translators. The translators in both stages were unaware of the other independent translators. In the final step, the translation and the adaptation of the final OM-6 form were reviewed. This final form was applied to the Turkish parents and caregivers.

Main Points:

- Hearing loss and language impairment in children with otitis media lower the health-related quality of life.
- The otitis media -6 is a constructed and validated questionnaire for the assessment of the health-related quality of life at the children with otitis media.
- Turkish version of OM-6 is a validated and the accredited form of the original OM-6 that could enable to evaluate the health-related quality of life in Turkish children with otitis media.

The inclusion criteria were as follows: children aged between 4 and 12 years, children diagnosed with chronic OME, and parents or caregivers who can speak native Turkish speakers. The exclusion criteria were the presence of conductive, sensorineural, or mixed-type hearing loss that was not caused due to the OME. The other exclusion criterion was children with parents or caregivers who cannot read and/or understand the Turkish language. In addition, patients noticed with a ventilation tube on the physical examination were excluded. We assumed that these patients could not be available for assessing the test–retest reliability.

Otitis media presenting with effusion diagnosis was established by two independent specialists who performed monthly physical examinations for 3 months. In addition to physical examinations, monthly tympanometric tests were conducted for 3 months. After obtained the informed consent, patients diagnosed with OME underwent ventilation tube insertion under general anesthesia. Three months after the operation, OM-6 was reapplied for assessing the test–retest reliability.

Table 2. Overall scores of the Turkish version of OM–6 and demographic variables

Group	Preoperative OME	Postoperative OME	Control	p
Age		6.29±2.14	6.11±1.72	0.821
Male		28	28	0.484
Female		40	27	
Q1*	4.26±1	1.91±0.93	1.62±0.68	<0.05
Q2	4.63±1.06	2.21±0.89	1.8±0.65	<0.05
Q3	4.5±1.14	2.18±0.93	1.71±0.68	<0.05
Q4	4.44±1.16	2.24±0.92	1.8±0.7	<0.05
Q5	4.46±1.11	2.25±0.76	1.67±0.66	<0.05
Q6	4.13±1.11	2.6±0.8	1.65±0.67	<0.05

* Question 1 of the OM–6 Turkish version

Table 3. The construct validity evaluation of the Turkish version of OM–6

Item description	Spearman correlation preoperative	Spearman correlation postoperative	Spearman correlation control	p
Q1*: Physical Suffering	0.643	0.691	0.789	<0.001
Q2: Hearing Loss	0.679	0.872	0.829	<0.001
Q3: Speech Impairment	0.679	0.898	0.817	<0.001
Q4: Emotional Distress	0.638	0.898	0.829	<0.001
Q5: Activity Limitations	0.597	0.872	0.852	<0.001
Q6: Caregiver Concerns	0.58	0.811	0.809	<0.001

Statistical Analysis

After the application of the Turkish version of OM-6 according to the abovementioned criteria, the consistency and the reliability were analyzed. The mean scores of the six items were compared between patients with OME and the control group to assess the construct validity of the test. The internal consistency of any association between the items was evaluated using the Cronbach's alpha method. Pearson correlation test was applied to assess the test–retest reliability.

The mean OM-6 scores of the control group and the preoperative OME group were compared using an independent samples Student's t-test. The comparison of OM-6 values between preoperative and postoperative time periods was accomplished using a paired sample Student's t-test. All statistical analyses were performed using IBM Statistical Package for the Social Sciences (IBM SPSS Corp.; Armonk, NY, USA) version 18.0 software.

RESULTS

Over a period of 6 months, a total of three groups (two paired and one independent) were tested for the validation of the Turkish version of OM-6. The preoperative group consisted of 68 children diagnosed with OME who formed the initial group assessed using OM-6. After 3 months, this OME group, renamed as the postoperative group, was reevaluated using the same OM-6 form. The third group consisted of 55 healthy children. The overall scores and the demographic variables of all groups are shown in Table 2. The Spearman correlation coefficient values to assess the construct validity identification of the OM-6 Turkish version are presented in Table 3. The Spearman correlation values were identified separately for each item of OM-6 among the three groups. The Spearman correlation values showed statistical significance among these groups.

The internal consistency of the Turkish version of OM-6 was evaluated by calculating Cronbach's alpha values, which are shown in Table 4. After assessing the normality of the OM-6 scores of the preoperative and postoperative groups using the Kolmogorov–Smirnov test (p=0.283 for the preoperative group, p=0.426 for the postoperative group). Pearson correlation coefficient values were estimated to evaluate the test–retest reliability. These results are presented in Table 5.

DISCUSSION

An appropriate analysis of the effect of otitis media on the quality of life of children can achieve effective communication between patients and clinicians. For this purpose, numerous valuable health-related quality of life questionnaires have been developed (10, 11).

In the present study, a general method (translation, back-translation, and synthesis) was implemented for the cross-cultural adaptation of the Turkish OM-6 version (12). According to Spearman validity scores of 0, 89 points, the Turkish version of OM-6

Table 4. The internal consistency values of the Turkish version of OM–6

	Cronbach's alpha*			
	preoperative	postoperative	control	p
Q1*: Physical Suffering	0.901	0.962	0.953	<0.001
Q2: Hearing Loss	0.889	0.948	0.942	<0.001
Q3: Speech Impairment	0.889	0.940	0.945	<0.001
Q4: Emotional Distress	0.891	0.947	0.941	<0.001
Q5: Activity Limitations	0.900	0.942	0.944	<0.001
Q6: Caregiver Concerns	0.903	0.948	0.945	<0.001

*These Cronbach's alpha values represent the value when the item is removed

Table 5. Test–retest reliability of the Turkish version of OM–6

	Pearson correlation values		
	preoperative	postoperative	p
Q1: Physical Suffering	0.67	0.747	<0.001
Q2: Hearing Loss	0.757	0.893	<0.001
Q3: Speech Impairment	0.757	0.914	<0.001
Q4: Emotional Distress	0.704	0.914	<0.001
Q5: Activity Limitations	0.699	0.893	<0.001
Q6: Caregiver Concerns	0.683	0.851	<0.001

has been shown to be a valid tool for evaluating children with OME. Previous studies using different languages have reported similar construct validity scores (7-9).

Postoperative scores of each OM-6 items exhibited a decreasing pattern compared with preoperative scores. To determine whether there was a single item that has a major impact on the overall score, the internal consistency was assessed using Cronbach's alpha scores. When one item was removed respectively in OM-6, the scores exhibited good internal consistency. This finding was also consistent with previous reports (13).

In this study, the stability of the Turkish version of OM-6 was evaluated on children diagnosed with OME. Several reports have mentioned that ventilation tube insertion improves the health-related quality of life in children (14, 15). To analyze the reason, we preferred to evaluate the stabilization of the Turkish OM-6 version on children diagnosed with OME. The Pearson correlation values also confirmed that the Turkish version of OM-6 is a stable test that can be applied in different time periods.

The application of the Turkish version of OM-6 in only one center could be assumed as a limitation, and similar questionnaires for assessing the health-related quality of life in further multicentric studies could be considered. One of the limitations was that we did not use the overall analog scale of OM-6 in addition to the six-item. This analog scale is a clinician perspective regarding the extent of children's suffering. In our study, our aim was to reveal the patients' perspective. Another limitation could be that only the reliability of OM-6 was assessed in children with a single subtype of otitis media. However, the literature has several reports indicating that OM-6 is a useful clinical tool for assessing other forms of otitis media in addition to OME (4, 16). We agree that after the validation of OM-6, it could be useful for evaluating other types of otitis media.

CONCLUSION

The Turkish translation of OM-6 is a stable, valid questionnaire that demonstrated an internal consistency. In addition, the clinical usage of this questionnaire may enable evaluating the clinical outcome for the treatment of otitis media in Turkish children. Finally, according to our opinion, the outcomes of the health-related quality of life could bring out a new path for evaluating the communication between the clinician and the patient.

Ethics Committee Approval: Ethics Committee approval was received for this study from the ethic committee of Gaziantep University of School of Medicine (Decision Date: 02.10.2019 Decision No: 2019/329).

Informed Consent: Verbal informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The author has no conflict of interest to declare.

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Original Research

Validity and Reliability of the Turkish Version of the 8-Item Morisky Medication Adherence Scale in Patients With Type 2 Diabetes

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ABSTRACT

Objective: Diabetes mellitus (DM) is a common disorder that causes undesirable medical and economic consequences. A simple, reliable, and validated self-report instrument could provide a better understanding of non-adherence to treatment and may help identify new treatment modalities. Thus, the eight-item Morisky Medication Adherence Scale (MMAS-8) was developed. The aim of this study was to evaluate the validity and reliability of the MMAS-8 among Turkish diabetes mellitus patients.

Methods: This cross-sectional descriptive study enrolled 199 patients. The Turkish translation of the Morisky-8 item scale consisted of forward translation, reconciliation, back translation, back translation review, developer review, pilot testing, and final translation.

Results: Cronbach's alpha test of internal consistency was calculated at alpha=0.890 for the eight items of the MMAS-8 scale. CFA demonstrated the scale fitted CMIN/DF=1.194, GFI=0.970, CFI=0.995, RMSEA=0.031. Poor glycemic control (HbA1c<7) was significantly higher in the low-adherence group than in the high- and medium-adherence groups (p=0.001, LR=21.79). Approximately 94% of the low-adherence group patients were in the poor glycemic control group.

Conclusion: The MMAS-8 Turkish version was found to be a valid and reliable scale in diabetic patients. This self-reported scale could function as a screening tool in busy clinics to identify patients with low adherence to medication treatment. Moreover, the MMAS-8 Turkish version could help improve adherence and develop new treatment strategies.

Keywords: Diabetes mellitus, reliability, treatment adherence, validity

INTRODUCTION

Diabetes mellitus (DM) is a common disorder that causes undesirable medical and economic consequences. The International Diabetes Federation has predicted that there will be 380 million people with diabetes in 2025 (1). Poor glycemic control leads to increased mortality and morbidity with significant direct and indirect costs to the healthcare system. Therefore, effective DM treatment is essential (2-4). Various factors affect the glycemic control of diabetic patients. Several studies have demonstrated that therapy with multiple drugs, poor patient physician communication, low patient education, local culture, religious affiliation, and medication adherence status are factors that affect the treatment outcome (5, 6). Non-adherence to treatment is a major problem faced by physicians today. Several studies have reported unsatisfactory medication adherence among type 2 DM patients (7-9). Many studies have tried to improve methods for assessing adherence to therapy (10, 11). One of the methods to evaluate adherence is to measure the patient's plasma drug level. However, it is difficult to access the drug levels; further, drug levels are not measured at every center; thus, this method appears impractical (12-14).

Prescription and pill-count follow up are other methods; however, their methodology and practicability require teamwork, and so they are rarely used (15). A simple, reliable and validated self-report instrument could provide a better understanding of non-adherence and may identify new treatment modalities (16). Therefore, the 8-item Morisky Medication Adherence Scale (MMAS-8) was developed (17). The MMAS-8 has been validated in some studies with patients diagnosed with type 2 DM (18,

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19). However, few studies have been conducted among Turkish diabetic population, and the scale has not been validated with diabetic patients in Turkey. A cross-sectional population based survey showed that the prevalence of the type 2 DM was 13.7% in the Turkish population in 2010 (20). Consequently, it is essential to improve the treatment outcome and facilitate the evaluation of medication adherence status for Turkish patients. To our knowledge, this is the first study of MMAS-8 validation and reliability survey among diabetic patients in Turkey. The aim of this study was to evaluate the validity and reliability of the MMAS-8 in Turkish diabetes mellitus patients.

METHODS

Study Design

This cross-sectional descriptive study aimed to evaluate the validity and reliability of the MMAS-8 in Turkish diabetes mellitus patients. The study was performed at the Gaziantep University Department of Endocrinology and metabolism. The study was performed from November 2013 to March 2014. The study was approved by Gaziantep University Council's Ethic Committee (NO: 408). All patients provided informed consent for study participation. The study design included patient selection, screening, interview, self-reported questionnaire survey, and data collection. The data were collected primarily using self-administered questionnaires.

Participants

Patients were selected from the Gaziantep University Internal Medicine outpatient clinic. The inclusion criteria for this study were as follows: 1. age ≥ 18 years 2. diagnosis of diabetes (type 1 or 2) established at least 1 year previously 3. literate status 4. consumption of at least one anti-diabetes drug 5. willingness to participate in the study and provision of written informed consent 6. willingness to schedule blood test at the laboratory at the time of the visit 7. and ability to understand the questions and instruction.

A target sample size of 160 patients was estimated by a ratio of 20:1 for each item but a larger sample size of 199 patients was enrolled to increase the reliability of the conclusion (21).

Instrument: The eight-item Morisky Medication Adherence Scale (MMAS-8)

The MMAS-8 is a diagnostic adherence assessment instrument, consisting of 8 items. The range of the scale is from 0-8 with 0 indicating low adherence and 8 showing high adherence. The first 7 questions require a dichotomous response, and the last item has a Likert scale. A categorical frequency distributes the scale into the following three parts: 0 to <6 is low adherence, 6 to <8 is moderate adherence, and a score of 8 indicates high adherence. The Turkish version was obtained with the permission of the scale copyright owner (Appendix 1).

Main Points:

- Diabetes mellitus (DM) is a common disorder that causes undesirable medical and economic consequences.
- A simple, reliable and validated self-report instrument could provide a better understanding of non-adherence.
- The MMAS-8 Turkish version was found to be a valid and reliable scale in diabetic patients.
- This self-reported scale could function as a screening tool in busy clinics to identify patients with low adherence to medication treatment.

Instrument translation

Step 1 concept elaboration

The agency project manager develops a concept elaboration document that describes the intentions of each question in the scale and offers definitions of key words and terms. These aided the translators in choosing the appropriate wording in the target language. This report is typically reviewed by the instrument developer before being sent to the translators.

Step 2 forward translations

The source scale is translated by two translators (T1, T2). The translators are both native speakers of the target language or are qualified to translate into that language by a creditable institution. The translators work independently of each other.

Step 3 reconciliation

The first translator (T1) combines the two forward translations into a third translation (T3) to maximize harmonization with the source document.

Step 4 back translation

The reconciled translation (T3) is translated back into English by two translators (T4, T5). The translators are both native speakers of English or qualified to translate into English by a creditable institution. The translators work independently of each other and work with no prior knowledge of the source version.

Step 5 back translation review

The Oxford outcomes project manager reviews the back translations (T4, T5) against the source documents and works with the first translator (T1) in order to a) refine the translation (T6) where necessary and b) clarify any ambiguities that have resulted from the back translations.

Step 6 developer review

The instrument developer reviews the back translation review. Any questions or comments are reviewed by the first translator (T1) and the project manager, and discussions continue until the time all the involved experts are satisfied with the outcome (T7).

Step 7 cognitive debriefing (pilot testing)

The first translator (T1) recruits 5 patients in the target population and asks them to complete a copy of the translated scale (T7). After they have completed the scale, the subjects are asked a series of questions to gauge their understanding of the translation. Any issues are discussed among the translator (T1) and the project manager until resolved (T8).

Step 8 the final translation

T8 is formatted in the preferred format of the client/developer and sent to two proofreaders. The proofreaders work sequential-

ly and independently. Both the proofreaders are native speakers of the target language and are briefed to avoid making suggestions that would invalidate the previous work. Thus, they are only required to point out spelling mistakes, and refrain from making stylistic or preferential changes.

Step 9 step 8 results in the final translation
This version is sent to the client and the instrument developer who reviews each item in the scale for its face and constructs validity.

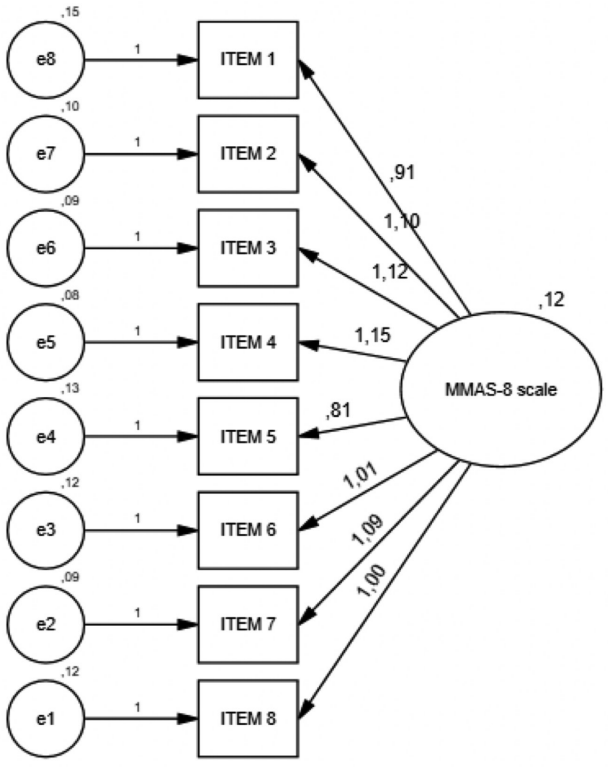
This translation process was performed by Dr. Morisky, the developer of the MMAS-8.

Patient Recruitment Procedure
We enrolled 200 diabetes patients, 199 of whom completed the full questionnaire. Patients were chosen from the internal medicine outpatient clinic. While obtaining written consent, we assessed the participants' literacy level by asking them whether they were able to complete the questionnaires independently or had used assistance. The patients who needed assistance mostly had a literacy issue; therefore, we excluded them from the study. Each survey required 15-20 min to complete.

Statistical Methods
The psychometric properties of the MMAS-8 were evaluated by using confirmatory and explanatory factor analysis (Figure 1).

Figure 1. Path diagram for the MMAS-8

[a]The MMAS (8-item) content, name, and trademarks are protected by US copyright and trademark laws. Permission for use of the scale and its coding is required. A license agreement is available from Donald E. Morisky, ScD, ScM, MSPH, 14725 NE 20th St Bellevue, WA 98007, USA; dmorisky@gmail.com.



Principle component analysis was used as the extraction method in the explanatory factor analysis. Cronbach's alpha was used to assess the reliability of the scale. In order to evaluate the correspondence between the MMAS-8 adherence groups and other clinical parameters, chi-square tests were used. All the univariate analyses and explanatory factor analysis were performed in IBM Statistical Package for the Social Sciences (IBM SPSS Corp.; Armonk, NY, USA) version 22.0 software. and confirmatory factor analysis was performed by using IBM AMOS version 22.0 package. A two-sided p-value <0.05 was considered to indicate statistical significance.

RESULTS

Clinical and Demographic Data
Recruitment was performed between September 2013 and March 2014, and 199 of the 200 participants completed the questionnaire. For the study group, 60.3% of the patients were women, within the age range of 18-87 years. The mean age of the study population was 55.02 years (SD 13.05). The socio-demographic and clinical characteristics of the participants are shown in Table 1.

Exploratory Factor Analysis and Internal Consistency
The Kaiser-Meyer-Olkin measures of sampling adequacy was 0.912, demonstrating marvelous inter-correlation among items for factor analysis. Bartlett's test of sphericity showed statistical significance (p=0.001), indicating that the inter-correlation matrix comes from a population wherein the variables are collinear. Table 2 presents factor loadings, which are the correlation between a variable and a factor that has been extracted from the data, for clinical samples for Morisky scale. According to the result of the explanatory factor analysis, only one component was extracted, and the solution cannot be rotated. Total variance explained by single factor solution was 58.60%. Internal consistency was assessed by using Cronbach's alpha, and values >0.8 indicate satisfactory internal consistency. The Cronbach's alpha value of our sample was 0.89, indicating high internal consistency (22). The Cronbach's alpha values decreased for each deleted item (Table 2). In addition, that item total correlation coefficients were high for each item, ranged from 0.599 to 0.758 (Table 2).

Confirmatory Factor Analyses
In the confirmatory factor analysis, user model versus baseline model p-value must be <0.05 for an acceptable model. Our model was statistically significant (p=0.001) according to the result of the confirmatory factor analysis. Many different criteria were considered to evaluate the result of the confirmatory factor analysis. The thresholds were determined from Hu and Bentler. CMIN/DF was 1.194 smaller than 4, the comparative fit index was 0.995 higher than the desired level of 0.90, the Tucker-Lewis Index was 0.993, and the GFI was 0.970 also higher than the desired level of 0.95. The root mean square error of approximation was 0.031 (90% CI=0.000-0.072); the desired level is <0.05. Furthermore, standardized root mean square residual was 0.028, quite smaller than 0.08. According to all the evaluated criteria, the reliability and validity of the scale were very high.

Table 1. Socio–demographic and clinical characteristic of the participants

Characteristics (n=199)		n	%
Sex	Female	120	60.3
	Male	79	39.7
Marital status	Married	160	80.4
	Single	39	19.6
Education level	Primary school	132	66.3
	High school or higher	67	33.7
Employment status	Employed	64	32.2
	Non–employed	135	68.8
Monthly income ($)	<500	123	61.8
	>500	76	39.2
Disease duration (years)	1–5	60	30.2
	5–10	57	28.6
	>10	82	41.2
Treatment modalities	Oral antidiabetics	74	37.2
	Oral antidiabetics+insulin	83	41.7
	Insulin	42	21.1
Number of anti–diabetic drugs	1	49	24.6
	2	95	47.7
	3	42	21.1
	≥ 4	13	6.5
Insulin administration Frequency (during the day)	0	74	37.2
	1	30	15.1
	2	49	24.6
	3	14	7.0
	4	32	16.1
Regular control visit	Yes	149	74.9
	No	50	25.1
Co–morbidity	Yes	89	44.7
	No	110	55.3
End organ damage	Yes	77	38.7
	No	122	61.3

External (Known Groups) Validity

This study assessed the known group validity through an association of glycemic control state. Poor glycemic control was defined as fasting plasma glucose (FPG) <130 and HbA1c <7 (20). The total score of MMAS-8 ranged from 0-8 for adherence. The MMAS-8 scores were categorized into three groups as follows: high adherence (score=8), medium adherence (score, 6 to <8), and low adherence (score, <6) (23).

Table 2. Factor loadings, item total correlations, and Cronbach's alpha values if item was deleted

Variables	Factor loadings	Item total correlation	Cronbach's alpha if the item was deleted
Question 1	0.698	0.599	0.893
Question 2	0.791	0.722	0.881
Question 3	0.816	0.739	0.880
Question 4	0.813	0.758	0.878
Question 5	0.689	0.580	0.894
Question 6	0.749	0.661	0.887
Question 7	0.811	0.736	0.880
Question 8	0.747	0.660	0.887

Table 3. Relationship between the level of adherence and glycemic control

Patient characteristics	Low adherence (<6)	Medium adherence (6 to <8)	High adherence (=8)
Good glycemic control	6 (6%)	26 (30%)	1 (34%)
Poor glycemic control	102 (94%)	62 (70%)	2 (66%)
Total	108 (100%)	88 (100%)	3 (100%)

In our sample, 18.2% of the participants had low adherence, 78.8% had medium adherence, and 3% had high adherence. The mean score for medication adherence was 4.3.

MMAS-8 categories using Chi square and likelihood ratio, assuming that patients with poor adherence level also report poor glycemic control. As shown in Table 3, Chi square test showed a significant relationship between the adherence levels as determined by the MMAS-8 and glycemic control (p=0.001). Poor glycemic control (HbA1c <7) was significantly more common in the low adherence group than in the high- and medium-adherence groups (p=0.001, LR=21.79). Around 94% of the low-adherence group patients were in the poor glycemic control group. Using a cutoff point of <8, the sensitivity of the MMAS-8 for identifying patients with poor glycemic control was estimated to be 61% and specificity was estimated at 81%.

DISCUSSION

To our knowledge, this is the first report on the translation and validation of the MMAS-8 into the Turkish language for use in diabetic patients. The Turkish version of the MMAS-8 has provided satisfactory evidence of the reliability and validity features in diabetic patients. Studies have reported the following Cronbach's alpha values for the translated versions of MMAS-8: 0.61, 0.73, and 0.68 (18, 24-26). In addition, only three studies that were performed on patients with diabetes mellitus have used the MMAS-8 (27, 28). The overall Cronbach's alpha for the Turkish MMAS-8 was 0.89, higher than that reported previously. The original MMAS-8 was tested by Morisky et al, in 1367 hypertension patients; the mean value was 6.6 (SD=1.6), and Cronbach's alpha was 0.61 (28).

In this study, the internal consistency of the MMAS-8 was higher than that in previous studies and the original MMAS-8 reported by Dr. Morisky. One reason for this might be the homogenous distribution of our participants' features. This study was performed at the University hospital, potentially resulting in a homogenous distribution of subject characteristics. Socio-cultural and health system differences may be other reasons for the scores. In contrast, 7 out of 8 items on the scale used binary responses (yes/no), and this tends to lower the Cronbach's alpha score (28); the score may be improved by increasing the number of response choices. In another study conducted on hypertension patients in Uganda, the Cronbach's alpha score was 0.65, lower than our value (29). These differences may also be attributable to differences in the physicians' education level as well as the cultural and educational level of the study participants. Moreover, in Uganda, there is limited supply of medication, making it less easily accessible to the patients. This situation does not exist in Turkey where patients have easy access to their drugs. Another study from sub-Saharan Africa reported a Cronbach's alpha score of 0.47, much lower than our Cronbach's alpha score (30). A Persian study on hypertensive patients reported a Cronbach's alpha value of 0.69 (31). However, most of the subjects in this study were illiterate, while our study excluded illiterate patients.

A Portuguese study on 937 subjects (more than that in our study) reported a Cronbach's alpha value of 0.68 (32). Moreover, the Portuguese study enrolled patients from 6 different centers across the country. The diversity of the patients may have caused the difference in the Cronbach's alpha values.

Because the factor loadings of the survey questions are very close to each other, we consider that there will not be significant difference in Cronbach's alpha score even if this question is removed. This situation can be assessed as positive evidence related to the reliability of the survey. The specificity and sensitivity of the MMAS-8 was 81% and 61%, respectively (likelihood ratio=21.79, p=0.001). These results showed that the MMAS-8 was strongly reliable for patients with high medication adherence and moderately reliable for those with low medication adherence. In many studies by Dr. Morisky, the sensitivity of MMAS-8 was higher than its specificity (28); this result may be attributable to the sample distribution and disease characteristics.

For known groups validity, three studies with patients with diabetes mellitus showed a significant association between adherence levels and glycemic control (27, 29, 33). In our study, the Turkish version of the MMAS-8 was able to differentiate strongly between patients with controlled and uncontrolled blood glucose levels based on their HbA1c levels. In our study, the number of subjects with high adherence was significantly lower than that of those with moderate and low adherence. One explanation for this could be the fact that Gaziantep (the city where this study was conducted) has one of the highest diabetic populations in Turkey (30, 34).

Study Limitations

Cronbach's alpha score is affected by sample characteristics; therefore, it is important to test the internal reliability for each different sample group.

CONCLUSION

The MMAS-8 Turkish version was determined to be a valid and reliable scale in diabetic patients. This self-reported scale could function as a screening tool in busy clinics to identify patients with low adherence to medication treatment. Moreover, the MMAS-8 Turkish version could help improve adherence and develop a new treatment strategy.

You can reach the questionnaire of this article at https://doi.org/10.5152/eurjther.2020.19132.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (number 408 year 2013).

Informed Consent: All patients provided informed consent for study participation.

Peer-review: Externally peer-reviewed.

Acknowledgment: Authors agreed to adhere all copyright requirements. The MMAS (8-item) content, name, and trademarks are protected by US copyright and trademark laws. Permission for use of the scale and its coding is required. A license agreement is available from Donald E. Morisky, ScD, ScM, MSPH, 14725 NE 20th St Bellevue, WA 98007, USA; dmorisky@gmail.com.

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Conflict of Interest: Donald E. Morisky receives honorarium for use of the copyrighted MMAS-8 diagnostic assessment instrument.

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APPENDIX 1

The required citations and copyright acknowledgement for the Morisky scale are available on the final license contract and copyright agreement. Required citation and acknowledgement for the 8-item MMAS are as follows:

Morisky DE, Ang A, Krousel-Wood M, Ward H. Predictive validity of a medication adherence measure for hypertension control. J Clin Hypertens (Greenwich) 2008; 10: 348-54

Krousel-Wood MA, Islam T, Webber LS, Re RS, Morisky DE, Muntner P. New medication adherence scale versus pharmacy fill rates in seniors with hypertension. Am J Manag Care 2009; 15: 59-66.

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Original Research

Immunosuppressants and Ischemic Postconditioning in the Management of Brain Ischemia in Rats: The Role of Pharmacologic and Nonpharmacologic Treatments

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ABSTRACT

Objective: Ischemic postconditioning in limb skeletal muscle may decrease the size of the cerebral hypoperfused area after stroke. We compared the effects of ischemic postconditioning with the effects of the pharmacologic agents infliximab and leflunomide in the management of stroke.

Methods: Thirty-two rats were divided into four groups: postconditioning, infliximab, leflunomide, and saline (control). Global cerebral ischemia was induced by clamping the bilateral common carotid arteries for 20 min, and subsequently reperfusion was allowed for 2 h. Rats in the infliximab group received 7 mg/kg of infliximab immediately after and 6 h after the induction of stroke. Rats in the leflunomide group received 10 mg/kg of leflunomide immediately after and 6 h after the induction of stroke. In the postconditioning group, the unilateral limb muscle was clamped for 180 min immediately after the induction of stroke, and subsequently reperfusion was allowed for 120 min. Rats in the control group received saline immediately after and 6 h after the induction of stroke. Glutathione peroxidase, malondialdehyde, and ischemia-modified albumin were measured, and histopathologic evaluation of cerebral tissue was performed.

Results: The area of hemorrhage was significantly decreased in the infliximab group. Loss of the gray matter–white matter boundary was significantly decreased in the infliximab and leflunomide groups. Brain glutathione peroxidase was significantly increased in the infliximab group. There were no significant differences between the infliximab, leflunomide, and postconditioning groups and the control group in serum malondialdehyde and ischemia-modified albumin

Conclusion: Immunosuppression by infliximab and leflunomide, but not ischemic postconditioning, may attenuate brain ischemia–reperfusion injury. Although the curative effects of postconditioning treatment on brain injury were documented, the lengths of the postconditioning cycles are important for its efficacy.

Keywords: Brain ischemia, infliximab, ischemic postconditioning, leflunomide

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INTRODUCTION

Ischemic stroke is a major health problem due to its high mortality and morbidity (1). Cerebral ischemia initiates numerous molecular events triggered by the energy deficit along with decreased cerebral perfusion. In cerebral ischemia, energy metabolites related to glucose metabolism, such as *adenosine* 5›-*triphosphate* (ATP) and phosphocreatine, are decreased and lactate levels are increased. This results in a metabolic imbalance. The reduction in ATP increases membrane depolarization and permeability, leading to increased levels of sodium, calcium, and chloride ions inside the cell and of potassium ions outside the cell. Glutamate activates *N-methyl-D-aspartate* (NMDA) channels, resulting in increased levels of intracellular calcium. Overactivation of NMDA receptors also contributes to the initiation of apoptosis. Previous studies have investigated the reduction or slowing down of this apoptotic process by medications and its recovery by reperfusion (2).

One of the most important results of the increase in intracellular calcium in cerebral ischemia is the release of free radicals by the formation of oxidants and subsequent activation of the *nitric oxide* synthase (NOS) pathway. Several methods have been used to determine the transcriptional factors implicated in the hypoxic or ischemic brain (3).

Previous studies reported that experimental animals exposed to short-term hypoxia were more resistant to cerebral ischemia and that ischemic preconditioning might be protective in ischemic brain injury models. According to these studies, short-term hypoperfusion had a neuroprotective effect against long-term ischemic injury (4). However, the risks associated with ischemic preconditioning of brain tissue and its very narrow therapeutic range limit the use of this method in humans (5). In contrast to ischemic preconditioning, cerebral protection by ischemic postconditioning is a relatively recent model. However, data regarding the protective mechanism of postconditioning against cerebral ischemia are very limited.

We hypothesized that ischemic postconditioning of skeletal muscle might ameliorate the hypoperfused penumbra in existing brain ischemia. We conducted a study to compare the effects of ischemic postconditioning on hypoperfused cerebral tissue with the effects of two immunosuppressive agents: infliximab and leflunomide. Infliximab is an inhibitor of *tumor necrosis factor*-α (TNF-α), which is one of the most important factors in initiating cell death in cerebral ischemia. Leflunomide, an inhibitor of pyrimidine synthesis and a derivative of isoxazole, has immunosuppressive and anti-inflammatory properties.

Main Points:

- Short-term hypoxia and ischemic preconditioning were reported be protective in ischemic brain injury models previously.
- Risks associated with ischemic preconditioning of brain tissue and its very narrow therapeutic range limit the use of this method in humans.
- Immunosuppression by infliximab and leflunomide resulted in significant reduction in brain ischemia after vascular occlusion.
- The model of Ischemic postconditioning may be also effective and reliable. However, the lengths of the postconditioning cycles are important for its efficacy.

Thus, the aim of this study was to evaluate the effects of infliximab, leflunomide, and ischemic postconditioning on the amelioration of ischemic regions in the cerebrum.

METHODS

The study was carried out in the Kahramanmaraş Sütçü İmam University Medical Faculty Pharmacology Department. All experiments were conducted in strict accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All protocols in this study were approved by the local Animal Experimentation Ethics Committee of Kahramanmaraş Sütçü İmam University (file no. 2018/07/02, approval date 10.04.2018). The rats were housed and maintained at a temperature of 22°C, a relative humidity of 60±5%, and a 12-h/12-h light/dark cycle with access to food and water *ad libitum*.

Drugs and Chemicals

Leflunomide (Abdi Ibrahim, Turkey) was prepared at 10 mg/kg, mixed with drinking water, and given twice in a volume of 1 mL/kg by oral gavage. Infliximab (Merck Sharp Dohme, Singapore) was prepared at 7 mg/kg, dissolved in sterile 0.9% saline, and administered intraperitoneally twice in a volume of 1 mL/kg. At the end of the experiment, the rats were anesthetized with ketamine HCl (80 mg/kg) and xylasine (10 mg/kg).

Induction of Global Cerebral Ischemia and Postconditioning in Skeletal Muscle

Thirty-two Wistar albino rats were equally and randomly divided into four groups (n=8 per group): group 1, postconditioning (PC); group 2, infliximab (Infx); group 3, leflunomide (Lef); and group 4, saline (control). All rats underwent induced global cerebral ischemia by occlusion of the bilateral common carotid arteries, as described by Zhou et al. (6). Briefly, the rat was fixed on an operating table in a supine position after being anesthetized with ketamine HCl (80 mg/kg) and xylasine (10 mg/kg). After superficial microdissection with a midline incision, a deep microdissection proceeded toward the common carotid arteries. Both common carotid arteries were exposed through the midline incision in the neck and temporarily clipped for 20 min with cross-clamps. After 20 min, the clips were removed to restore the blood flow for recirculation, and reperfusion was allowed for 2 h. Control rats underwent the same surgical procedure and occlusions, but the drugs were not administered.

In the PC group, ischemic postconditioning was induced by applying a tourniquet to the upper third of the right leg, as described by Ergün et al. (7), for 180 min immediately after reperfusion by the carotids. The ischemic period of the limb muscle was selected to be 180 min, so that the distal pulse of the compressed limb could not be taken. After 180 min, limb muscle reperfusion was allowed for 120 min. Rats in the Infx group received 7 mg/kg of infliximab intraperitoneally twice, immediately after and 6

h after reperfusion by the carotids. Rats in the Lef group received 10 mg/kg of leflunomide orally twice, immediately after and 6 h after reperfusion by the carotids. Rats in the saline (control) group received saline intraperitoneally twice, immediately after and 6 h after reperfusion by carotids.

Verification of Skeletal Muscle Ischemia

Skeletal muscle ischemia in the compressed limb was verified by determining elevated levels of serum creatine kinase (CK) and lactate dehydrogenase (LDH) due to muscle destruction. Reperfusion was verified by regaining the distal pulse and normalization of skin color. Serum CK and LDH activities were evaluated spectrophotometrically by a Siemens ADVİA 1800 Chemistry Autoanalyser (Siemens Healthcare GmbH, Germany).

Collection of Cerebral Tissue Samples

At the end of the experiments, the rats were sacrificed under general anesthesia, and the brains were removed, immersed in fixative (10% formalin solution) for 24 h, and embedded in paraffin, monitored the tissue processing by autotechnicon device (Leica ASP 300, Germany), and microsectioned at a thickness of 4 µm (Leica Microtome RM 2145, Germany). The brain samples, stained with hematoxylin and eosin, were examined for infarct-associated morphological changes (Olympus BX53 polarizing microscope, Germany).

Evaluation of Histopathological Ischemic Changes in Brain Tissue

An overall score of the severity of cerebral tissue damage was semiquantitatively assessed taking account of vascularization (proliferation of vascular structures secondary to healing), macrophages (inflammatory component increased in wound healing), necrosis (infarct-induced coagulation necrosis), edema (in the interstitial area during the stages of inflammation), hemorrhage (red blood cells in the extravascular area), loss of the gray matter–white matter boundary (loss of the distinction between gray and white matter in the brain), neutrophilic infiltration (increased numbers of neutrophils in the area surrounding necrosis), and small red neurons (changes in glial cell morphology affected by ischemia). The severity of cerebral tissue damage was semiquantitatively scored as 0 (normal), 1.0 (mild), 2.0 (moderate), or 3.0 (severe).

Assessment of Hypoxia-Induced Oxidant Markers in Brain Tissue and Serum

Glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and ischemia-modified albumin (IMA) levels were investigated in serum and tissue homogenates. Blood was collected from the rats by cardiac puncture. Serum was obtained by centrifugation at 4000 rpm for 10 min. Brain tissue samples were taken quickly and washed in cold saline. Brain tissues were homogenized with cold 0.15 M KCl (10%, w/v). Tissue homogenates were centrifuged at 600×*g* for 10 min at 4°C to remove crude fractions. The supernatants were then centrifuged at 10,000×*g* for 20 min to obtain the postmitochondrial fraction. GSH-Px activities were determined in the postmitochondrial fraction. MDA levels in homogenates and serum were determined using thiobarbituric acid. IMA levels in serum were colorometrically measured. GSH-Px activity was measured with cumene hydroperoxide as substrate. In this method, GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase, and the oxidation of NADPH was followed spectrophotometrically at 340 nm at 37°C. The results were calculated using extinction coefficient (6.22×10^3/M cm).

Statistical Analysis

The data were presented as arithmetic means and standard deviations. In order to apply parametric tests, the Kolmogorov–Smirnov test was used to determine whether the results were normally distributed and whether the variances were homogeneous. For multiple groups, analysis of variance with a post hoc Tukey's test for the significance of differences was used for normally distributed data. The Kruskal–Wallis test with the Mann–

Figure 1. Serum creatine kinase (CK) levels after compression of limb skeletal muscle

*Significant increase in the postconditioning (PC) group compared with the control group (p=0.014)

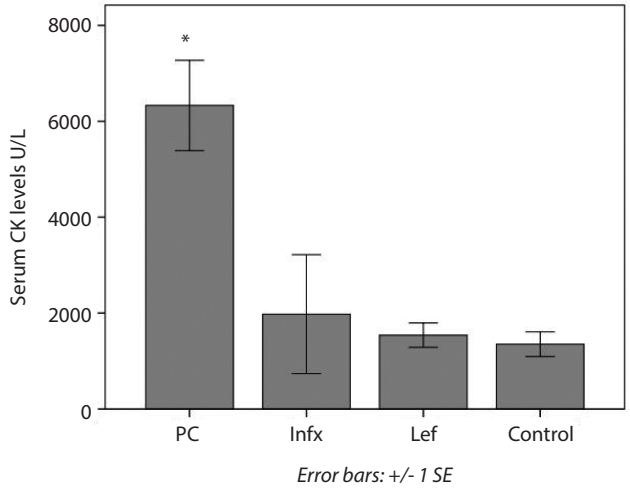
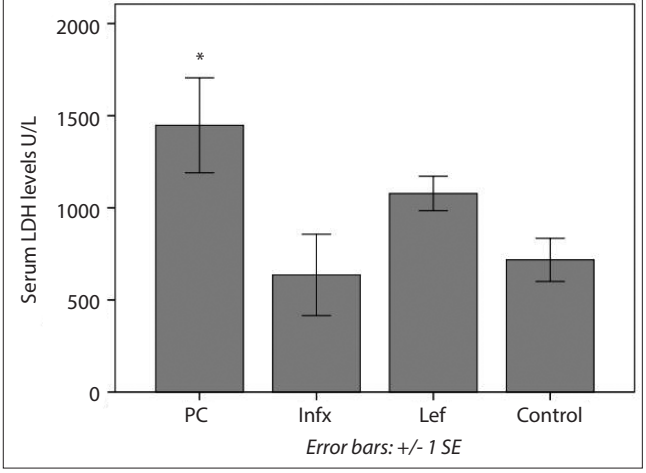


Figure 2. Serum lactate dehydrogenase (LDH) levels after limb skeletal muscle compression

*Significant increase in the postconditioning (PC) group compared with the control group (p=0.013)



Whitney U test under Bonferroni correction was used for analysis of non-normally distributed data. A $p<0.05$ was considered to indicate a significant difference. The data were evaluated at a 95% confidence interval. The Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) 17.0 program was used for statistical analysis.

RESULTS

Effects of Postconditioning on Skeletal Muscle Enzymes

Skeletal muscle ischemia in the compressed limb was verified by significant increases of serum CK and LDH levels in the PC group compared with the control group ($p=0.014$ and 0.013, respectively). The mean serum levels of CK and LDH were 6329.00±1884.65 and 1447.60±576.06 U/L, respectively, in the PC group and 1352.20±575.91 and 714.40±261.55 U/L in the control group (Figures 1 and 2). These findings confirm that ischemic postconditioning was induced in lower limb skeletal muscle, as described.

Effects of Treatments on Histopathological Changes in Brain Tissue

The hemorrhagic area was significantly less in the Infx group than in the control group ($p=0.002$) (Figure 3). In addition, loss of the gray matter–white matter boundary was significantly less in the Infx and Lef groups than in the control group ($p=0.002$ for both comparisons) (Figure 4). There were nonsignificant decreases in macrophages, coagulation necrosis, interstitial edema, neutrophilic infiltration in the area surrounding necrosis, and small red neurons affected by ischemia in the Infx and Lef groups compared with the control group.

Figure 3. a, b. Appearance of hemorrhagic areas in cerebral tissue after global cerebral ischemia reperfusion

*Significant decrease in the infliximab (Infx) group (a) compared with the control group (b) ($p=0.002$)

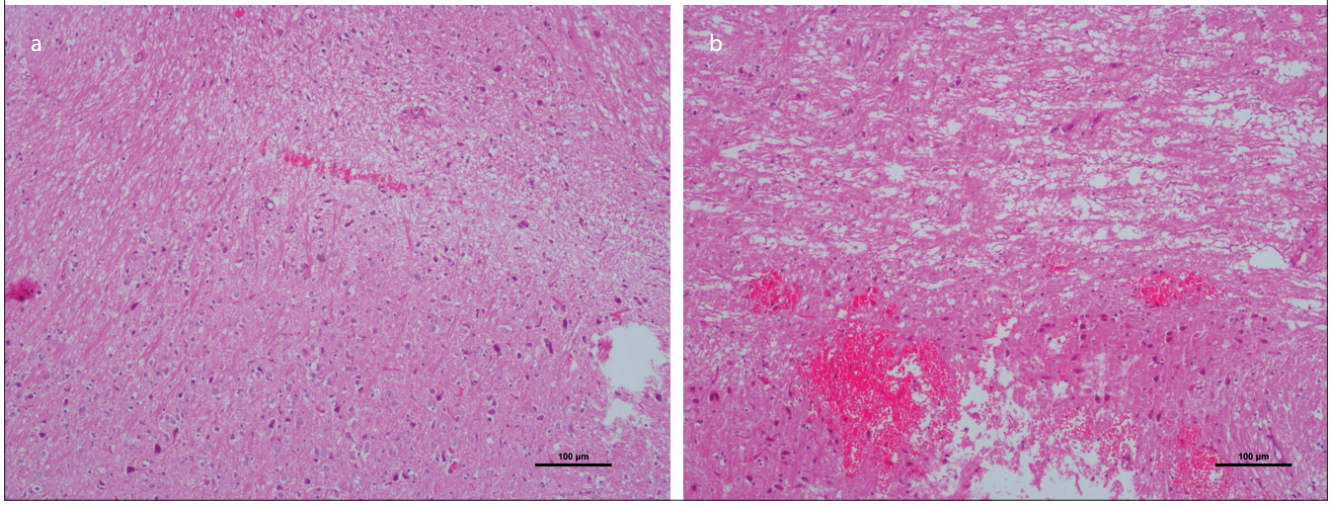


Figure 4. a, b. Loss of the gray matter–white matter boundary after global cerebral ischemia reperfusion

*Significant decrease in the infliximab (Infx) group (a) compared with the control group (b) ($p=0.002$)

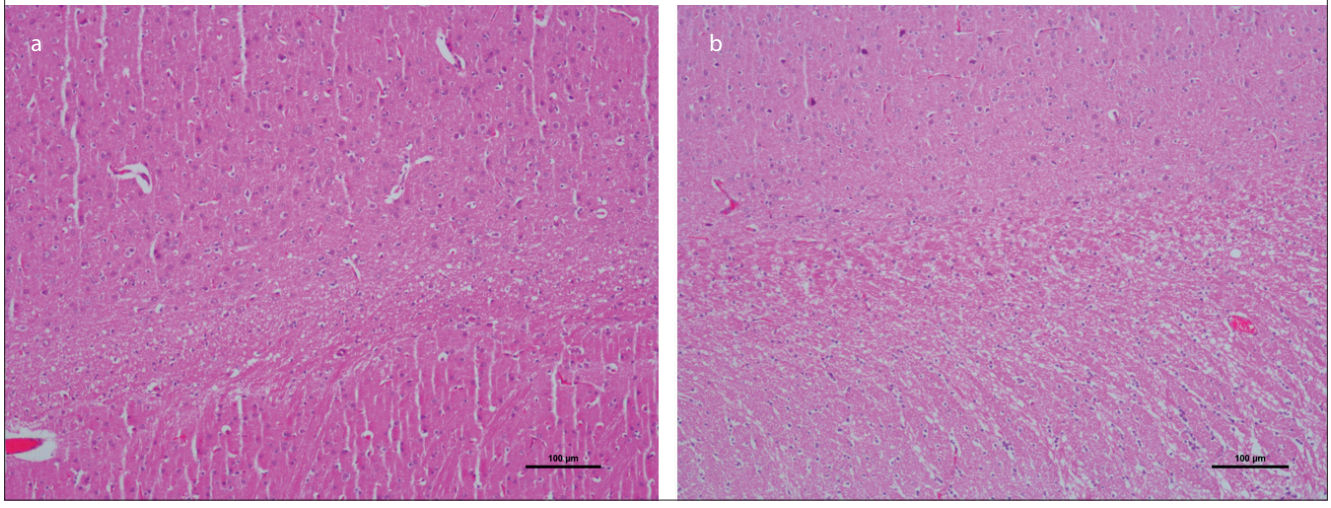


Table 1. Hypoxia–induced oxidant markers in the brain and serum of rats with pharmacologic (infliximab or leflunomide) and nonpharmacologic (ischemic postconditioning) treatments after global cerebral ischemia

Variable	PC	Infx	Lef	Control
Brain GSH–Px (nmol/g)	288.17±30.51	293.10±30.29*	282.93±58.37	244.92±29.12
Serum GSH–Px (nmol/mL)	56.27±9.10**	72.54±5.33	66.45±10.67**	75.12±3.13
Brain MDA (nmol/g)	14.64±2.78***	13.07±2.50***	6.49±1.24	6.85±0.94
Serum MDA (nmol/mL)	6.73±1.07	6.78±1.06	6.39±2.17	6.69±5.36
Serum IMA (absU)	0.34±0.05	0.31±0.08	0.33±0.07	0.35±0.02
Serum CK (U/L)	6329.00±1884.65[a]	1978.00±2479.08	1540.25±509.14	1352.20±575.91
Serum LDH (U/L)	1447.60±576.06[b]	635.75±442.01	1077.75±186.72	714.40±261.55

PC: postconditioning group; Infx, infliximab group; Lef: leflunomide group; GSH–Px: glutathione peroxidase; MDA: malondialdehyde; IMA: ischemia–modified albumin; CK: creatine kinase; LDH: lactate dehydrogenase

*Significant increase in the Infx group compared with the control group (p=0.035). **Significant decrease in the PC and Lef groups compared with the control group (p=0.023). ***Significant increase in the PC and Infx groups compared with the control group (p=0.003 and 0.002). [a,b] Significant increase in the PC group compared with the control group (p=0.014 and 0.013).

Figure 5. Brain glutathione peroxidase (GSH-Px) levels after global cerebral ischemia reperfusion

*Significant increase in the infliximab (Infx) group compared with the control group (p=0.035)

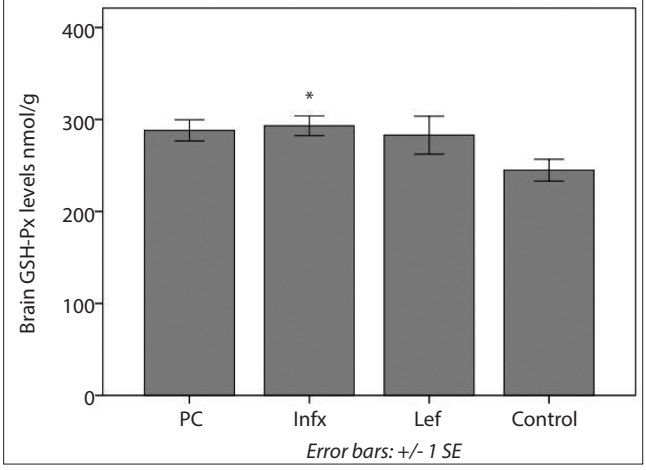


Figure 6. Serum malondialdehyde (MDA) levels after global cerebral ischemia reperfusion

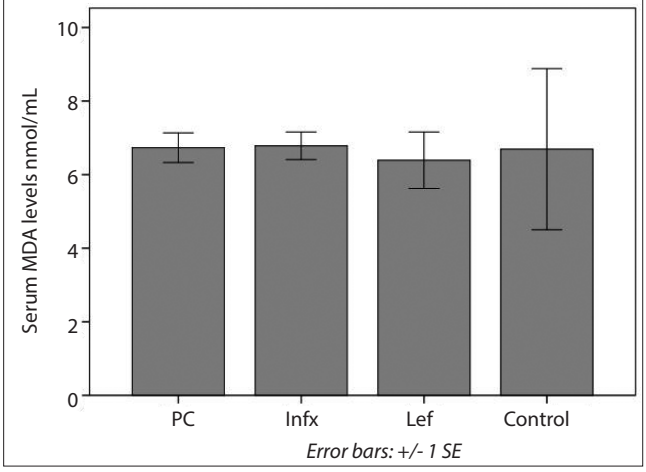
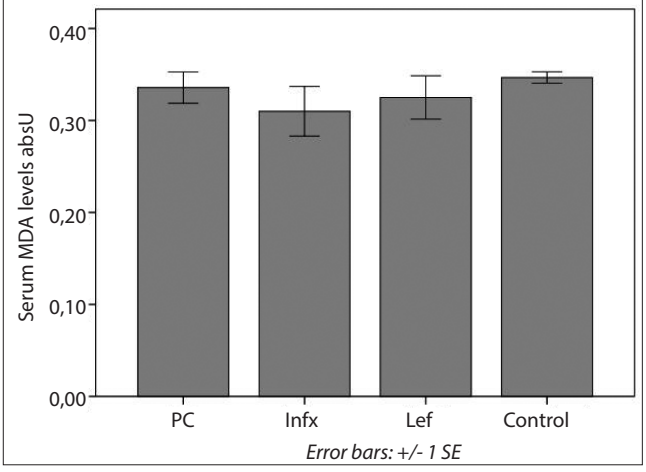


Figure 7. Serum ischemia-modified albumin (IMA) levels after global cerebral ischemia reperfusion



Effects of Treatments on Hypoxia-Induced Oxidant Markers in Brain Tissue

We evaluated the brain tissue levels of GSH-Px and MDA in the PC, Infx, and Lef groups compared with the control group. The mean level of brain GSH-Px was significantly greater in the Infx group (293.10±30.29 nmol/g) than in the control group (244.92±29.12 nmol/g) (p=0.035) (Table 1 and Figure 5). However, the mean levels of brain MDA in the PC group (14.64±2.78 nmol/g) and the Infx group (13.07±2.50 nmol/g) were significantly greater than that in the control group (6.85±0.94 nmol/g) (p=0.003 and 0.002, respectively).

Effects of Treatments on Hypoxia-Induced Oxidant Markers in the Serum

We evaluated the serum levels of GSH-Px, MDA, and IMA in the PC, Infx, and Lef groups compared with the control group. The mean serum levels of GSH-Px were 56.27±9.10 nmol/mL in the PC group, 56.73±24.49 nmol/mL in the Lef group, and 75.12±3.13 nmol/mL in the control group. These findings indicate that serum GSH-Px was significantly reduced in the PC and Lef groups

(p=0.023 for both comparisons). There were no significant differences in the serum levels of MDA or IMA between the treatment groups and the control group (Figures 6, 7).

DISCUSSION

This study compared the effects of ischemic postconditioning on hypoperfused cerebral tissue with the effects of infliximab and leflunomide. First, we demonstrated that postconditioning resulted in significant increases in the serum levels of CK and LDH, verifying the destruction of skeletal muscle in the compressed limb in the ischemic postconditioning model. Second, we found that immunosuppression by infliximab and leflunomide, but not ischemic postconditioning, may lead to significant induction of antioxidant activity in serum and ischemic brain tissue after vascular occlusion.

There are many factors in the pathogenesis of stroke caused by cerebral ischemia. Studies have shown that cytokines may have a role in this process. In cerebral ischemia, the release of various local and systemic cytokines, in particular TNF-α, is induced. TNF-α may cause either the protection or damage of nerve cells following ischemia. In the inflammation process, TNF-α is involved in the release of chemoattractant cytokines, up-regulation of endothelial adhesion molecules, migration of leukocytes, and induction of the endothelium to the prothrombotic stage (8). TNF-α is involved in ischemic injury, and blockade of endogenous TNF-α is claimed to be protective against neuronal injury. Studies showed that high serum levels of TNF-α persisted for 7 days after stroke and were correlated with the severity of the cerebral infarction area (9, 10). We found that the use of the TNF-*α* inhibitor infliximab decreased the area of the hemorrhagic region in the rat brain after global cerebral ischemia. The number of red blood cells in the extravascular area was less in rats treated with infliximab when the agent was administered immediately after and 6 h after the induction of ischemia, compared with untreated rats. Infliximab was administered at two different times because of changes in the dynamics of TNF-*α* and its receptors, depending on the time of its administration (8). Similarly, we showed that loss of the distinction between gray and white matter of the brain after stroke was significantly decreased in the Infx group.

TNF-α also acts as a potent inducer of reactive oxygen species through impairment of mitochondrial biogenesis and activation of NADPH oxidase (11). In previous studies, treatment of ischemia with infliximab increased *superoxide dismutase (*SOD) activity and decreased GSH levels in spinal cord tissue (12). In a rat model of the ischemic kidney, SOD and GSH levels were significantly higher in the infliximab groups than in the ischemia group (13). In our study, the TNF-α inhibitor infliximab increased GSH-Px levels in brain tissue during stroke.

Leflunomide, an isoxazole derivative and pyrimidine analog, suppresses proinflammatory cytokines as a main target in anti-inflammation and immune regulation. Our findings indicated that loss of the distinction between the gray and white matter of the brain was significantly decreased in the Lef group, in association with and likely mediated by suppression of proinflammatory cytokines.

Several studies indicated that leflunomide increased the expression of antioxidant enzymes such as NAD(P)H quinone dehydrogenase 1 (NQO1), catalase, and SOD. These results supported the hypothesis that leflunomide decreases oxidative stress in human pulmonary arterial endothelial cells via SOD2- and catalase-dependent, but aryl hydrocarbon receptor- and NQO1-independent, mechanisms (14, 15). However, we found that leflunomide treatment did not improve the serum or brain antioxidant levels of rats that received 10 mg/kg of leflunomide immediately after and 6 h after stroke. This short period may not be sufficient for improvement of the redox state. The mechanisms by which this drug induces NQO1 in vivo are unknown. Leflunomide was reported to cause significant induction of the expression of pulmonary CYP1A1 and NQO1 in neonatal mice. Interestingly, the dose at which leflunomide increased NQO1 was significantly higher than that required to induce CYP1A1 enzyme (15). We may find improvement in the antioxidant state if we explore the levels of leflunomide in the later period, since leflunomide has a long half-life.

Preconditioning is a protective condition in which subinjury stress can lead to protection against the effects of stroke. Ischemic preconditioning acts in a variety of tissues, including the brain. Hypoxic preconditioning increases disruption of the blood–brain barrier through a vascular endothelial growth factor (VEGF)-related pathway and suggests the possibility of aggravation of brain edema by hypoxic preconditioning in the early stages of cerebral ischemia (16). Preconditioning is a form of defense that stimulates endogenous protective mechanisms. Many pharmacologic agents, such as metabolic inhibitors, volatile anesthetics, K^+ATP channel activators, inflammatory mediators, and some natural dietary compounds, are defined as preconditioning stimuli. Some nonpharmacologic conditions, such as repetitive hyperbaric oxygen, normobaric hyperoxia, hypo- and hyperthermia, depression, acupuncture, and exercise, may also trigger cerebral preconditioning that reduces ischemic brain damage (5). In animals, preconditioning has been well documented as an effective mechanism for protecting the brain from injury. However, there are concerns over how these results will translate to humans with chronic diseases.

Ischemic postconditioning consists of a series of rapid, intermittent interruptions of blood flow during reperfusion, and it stimulates the same protective mechanisms as preconditioning. Since most cerebral ischemic events occur unpredictably, postconditioning could provide more therapeutic benefits than preconditioning. Up-regulation of the expression of antiapoptotic factors and neurotropins and modulation of the activities of several protein kinases and transcription factors, such as hypoxia-inducible factor-1 (HIF-1), are considered the most important aspects of the neuroprotective potential of postconditioning (17). Multiple mechanisms have been suggested to contribute to the neuroprotective mechanisms of ischemic postconditioning, such as regulation of synaptic signaling, reduction in oxidative stress and inflammation, maintenance of mitochondrial integrity, decrease in endoplasmic reticulum stress, activation of the phosphoinositide 3-kinase/Akt pathway, inhibition of apoptosis, and protection of the neurovascular unit (18). In the study of Chu et al. (19),

ischemic postconditioning, similar to preconditioning, improved the functions and transmembrane potential of mitochondria in rats after ischemia/reperfusion. They found that ischemic postconditioning reduced the release of cytochrome C by inhibiting the decrease in the transmembrane potential of mitochondria, thus reducing the occurrence of apoptosis. Schewe et al. (20) reported that reduced efflux of thromboxane B2 was a possible mechanism for the effect of ischemic postconditioning. Sun et al. (18) claimed that activation of autophagy was involved in the beneficial effects of ischemic postconditioning by reducing infarct volume.

In our study, however, ischemic postconditioning had no significant positive effects on the recovery of neurons in the penumbra region in the early period in rats with ischemic stroke. Our findings may be consistent with the presence of dysfunctional autophagic flux during reperfusion, possibly due to impaired autosomal degradation. Gao et al. (21) found that rapamycine, an inducer of autophagy, attenuated the effects of ischemic postconditioning. In support of this finding, in biochemical analysis we found that MDA levels in brain tissue were increased and serum GSH-Px was reduced in the PC group compared with the control group. Similar to our findings, in a study of an acute ischemia–reperfusion kidney injury, ischemic preconditioning and ischemic postconditioning, together or separately, were unable to preserve kidney function and did not exert a protective effect against tubular cell injury (22). These findings suggest that despite being essential for the survival of ischemic tissue, postconditioning may lead to additional cellular injuries. This dual effect of post-conditioning may depend on the lengths of the postconditioning cycles in the experimental protocols, as we induced ischemic postconditioning in skeletal muscle for 180 min, and subsequently reperfusion was allowed for 120 min. Shorter- and intermediate-length cycles of postconditioning were reported to enhance the mucosal microcirculation and redox state. Furthermore, milder histopathologic lesions and lower concentrations of serum proinflammatory cytokines were observed in previous studies with shorter- and intermediate-length postconditioning cycles (23). Li et al. (24) studied brief ischemic postconditioning and found that a brief episode of global brain ischemia (3 min) conducted at 1, 3, or 7 days provided neuroprotection against amyloid-β peptide neurotoxicity. The underlying mechanism was reported to be up-regulation of NMDA receptor signaling and down-regulation of mixed lineage kinase-3 (MLK3)–mitogen-activated protein kinase signal events. Most studies so far have been related to models of so-called rapid ischemic postconditioning, when reperfusion interruption is conducted in early stages (seconds and minutes) after ischemia; however, in addition to early ischemic postconditioning, "delayed" and distant ischemic postconditioning of the brain are recognized (17). Hence, the efficacy of ischemic postconditioning may be due to the lengths of the postconditioning cycles.

CONCLUSION

Although ischemic postconditioning is a technique that was found to be effective in previous experimental studies, the lengths of the postconditioning cycles are important for its efficacy. Postconditioning is not a part of current treatment protocols, as there is not enough evidence to suggest its routine use to treat ischemic injury. Furthermore, immunosuppression by infliximab and leflunomide results in significant reduction in brain ischemia after vascular occlusion. Larger studies are required to demonstrate the positive effects of postconditioning on clinical outcomes.

Ethics Committee Approval: Ethics committee approval was received for this study from the local Animal Experimentation Ethics Committee of Kahramanmaraş Sütçü İmam University (file no. 2018/07/02, approval date 10.04.2018).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – D.A.A.; Design – D.A.A., B.T.Y. ; Supervision – D.A.A.; Resources – D.A.A., B.T.Y., T.K., A.U.; Materials – D.A.A., Ü.K., M.S.; Data Collection and/or Processing – D.A.A., Ü.K., M.S.; Analysis and/or Interpretation – Ü.K., M.S., A.U.; Review – D.A.A., T.K., A.U.; Other – B.T.Y., T.K.

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

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Original Research

Effect of Peri-Implant Disease on Adropin Levels: A Cross-Sectional Pilot Study

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ABSTRACT

Objective: Adropin is a peptide hormone related to the inflammatory process of several systemic diseases. The inflammatory characteristics of peri-implant diseases are known. This study aimed to measure and compare the level of adropin in peri-implant sulcus fluid (PISF) in patients with peri-implant disease.

Methods: Overall, 75 individuals were included in this cross-sectional study. Each of the three study groups [healthy (H), peri-implant mucositis (PM), and peri-implantitis (PI)] consisted of 25 patients. During the periodontal examination, periodontal pocket depth (PD), plaque index (PI), and gingival index (GI) scores were recorded, and marginal bone loss (MBL) was measured using ImageJ. PISF samples were collected from dental implants. Adropin level in PISF was analyzed using the enzyme-linked immunosorbent assay (ELISA) technique. All clinical and biochemical parameters were statistically analyzed using SPSS v22.

Results: PD, PI, and GI parameters were noted to be significantly higher in the PM and PI groups compared with the H group. PI group showed statistically significant differences related to MBL compared with the other groups ($p<0.05$).

Conclusion: Therefore, adropin may prove to be a useful diagnostic tool in the diagnosis of peri-implant disease. However, further studies are needed to explore the different biochemical parameters in PISF, thereby helping to understand the interaction of adropin with other mediators.

Keywords: Adropin, periodontal disease, peri-implant disease

INTRODUCTION

Dental implant treatment has become widespread and has garnered popularity because of various factors, such as prolonged life expectancy, aging, increased tooth loss, and discomfort from fixed dentures and removable prosthesis (1). Nevertheless, the widespread use of dental implants has led to an increase in biological complications. The most significant among these complications are peri-implant mucositis (PM) and peri-implantitis (PI) (2). PM is a reversible inflammation of the soft tissues around the implant without clinical and radiographical bone loss. On the other hand, PI is the decrease in alveolar bone associated with inflammation of the bone and soft supporting tissues of the dental implant. PM is considered an initial lesion and may return with patient self-care or periodontal therapy (3).

The development of infection and inflammation around the dental implants are closely related to the presence of microorganisms. The salivary glycoproteins may attach to the exposed inorganic surfaces shortly after implant placement. Subsequently, microorganisms colonize on this glycoprotein layer. Finally, in peri-implant diseases, a gram-negative anaerobic microflora, generally similar to periodontitis, becomes dominant (4). Although the primary etiological agent for the onset of periodontal and peri-implant diseases is dental plaque, the immune mechanisms triggered by host-bacterial interactions in the body also release a large number of inflammatory mediators, such as cytokines (by disrupting the balance between protective and destructive immune mechanisms), which are critical factors of destruction. Some biological mediators, such as cytokines, peptides, and hormones, were noted to be related to periodontal and peri-implant disease (5). Moreover, almost all mediators related to periodontal and peri-implant disease were noted to be present in the gingival crevicular fluid (GCF) and peri-implant sulcus fluid (PISF). Once the periodontal disease begins, the capillary vessels dilate, and serum proteins are released into the GCF and PISF, besides an increase in the fluid flow rate. Therefore, PISF has the potential to provide crucial information regarding the periodontal health and disease conditions owing to its unique structure (6).

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Adropin is a peptide hormone first discovered in 2008 by Kumar et al. (7) It has an approximate molecular weight of 7.927 kDa, consists of 76 amino acids, and is encoded through the energy-related gene code (*ENHO*-energy homeostasis associated). A study conducted by Kumar et al. (7) observed that blood serum adropin levels increased in high-fat nutritional conditions, thereby suggesting that adropin hormone may play a role in the metabolism of lipogenesis in the liver. In another study, the lack of adropin hormone was observed to be associated with increased adipose tissue and insulin resistance. Therefore, it was concluded that the adropin molecule might be related to glucose metabolism, insulin resistance, dyslipidemia, and metabolic syndrome. Proving the association of adropin with insulin resistance and obesity opened the avenues for several clinical studies on this subject (8). In such a study conducted by Celik et al. (9), the serum adropin levels of patients with gestational diabetes mellitus (GDM) and healthy controls were compared, and the blood serum adropin levels were determined to be significantly lower in patients with GDM compared with the control group. Moreover, low adropin levels may be involved in the pathogenesis of diabetes. In another study by Wu et al. (10), the relationship between coronary atherosclerosis and serum adropin levels was investigated in patients with and without diabetes and revealed that serum adropin levels decreased with an increase in the coronary atherosclerosis score in all patients. After these milestone studies, low levels of adropin were proven to be associated with the progression of coronary atherosclerosis and increased risk of cardiovascular disease. Besides, further studies that explored adropin have revealed its protective and regulatory role in endothelial function (9).

In recent years, adropin has been considered an essential mediator in the pathogenesis of some systemic diseases, such as obesity, diabetes, cardiovascular diseases, and atherosclerosis. Furthermore, these systemic diseases have been noted to be related to the severity of the periodontal disease. Nonetheless, upon a literature review, we found no studies that evaluated adropin levels in PISF in patients with peri-implant diseases.

Hence, considering all this information, this study aimed to evaluate the PISF adropin levels in healthy and diseased dental implants to obtain meaningful information regarding the role of adropin in the etiopathogenesis of peri-implant diseases.

METHODS

Study population

Overall, 75 non-smoker individuals (36 females, 39 males) who were admitted to the Gaziantep University Faculty of Dentistry Periodontology for periodontal treatment were included in this cross-sectional study. This study was conducted per the Helsinki Declaration, and approval was obtained from the Gaziantep University Clinical Studies Ethics Committee on March 13, 2017, (No.: 2017/101). All participants were provided verbal or written information regarding the study, and their consent was obtained.

Main Points:

- Peri-implantitis and peri-implant mucositis are inflammatory diseases, because of that; sign of inflammatory process could be observed in peri-implanter sulcus fluid.
- Adropin can be a new biomarker in the peri-implanter sulcus fluid to identify these inflammatory conditions.
- Peri-implanter sulcus fluid can serve as a reservoir for inflammatory biomarkers.

Inclusion and Exclusion Criteria

The inclusion criteria were age over 18 years, no systemic diseases (such as diabetes, cardiovascular disease, atherosclerosis, and obesity), no antibiotics and anti-inflammatory drug use in the preceding 6 months, and no dental or periodontal treatment in the preceding 6 months. Besides, it was ensured that the dental implants were functional for at least 12 months after loading and were not supported by overdentures and bridges, and only dental implants with a single crown were included in the study. The 2017 EFP and AAP workshop criteria were considered when diagnosing healthy implants (H), peri-implant mucositis (PM), and peri-implantitis (PI) (11, 12). For standardization, tissue-level implants were not included in the study, and all study groups had Straumann (Basel, Switzerland) and MIS (C1, Savion, Israel) implants with conical connections.

Clinical Examination

Pocket depth (PD), plaque index (PI), and gingival index (GI) scores were recorded. Clinical periodontal and peri-implant examinations were performed by an initially calibrated periodontist (HG). Intraexaminer k values were 0.95 (PD) and 0.82 (CAL). Bleeding on probing was considered positive if bleeding occurred 15 seconds after probing. The clinical parameters were measured using a plastic periodontal probe (Hu-Friedy, USA). Furthermore, to determine the presence of bone loss around the dental implants, all patients underwent periapical or panoramic radiographs, and radiological bone loss (MBL) was measured using the ImageJ program (National Institutes of Health, Bethesda, MD, USA).

PISF Sampling and Analysis

PISF samples were collected based on the radiographic data before performing the clinical measurements. Before sample collection, the implant was isolated with cotton pads, gently dried using air or water spray, and then the paper strips were placed into the pocket until slight pressure was felt. After waiting for 30 seconds, the paper strips (Periopaper®, OraFlow Inc., USA) were placed in a pre-calibrated Periotron 8000 instrument to measure PISF volume. The volume was then recorded after measurements. Samples were stored at –80°C until the analysis day. Adropin levels in PISF samples were measured per the manufacturer's instructions (Cloud-Clone Corp. Adropin kit) by using the enzyme-linked immunosorbent assay (ELISA) method (9).

Statistical Analysis

Statistical evaluation of the data was performed using IBM Statistical Package for the Social Sciences (IBM SPSS Corp.; Armonk, NY, USA) v22.0 for Mac. The Shapiro-Wilk test was used to assess whether the numerical data had a normal distribution. The Mann-Whitney U test was used to compare the non-normally distributed variables among groups. ANOVA and LSD multiple

Table 1. Differences, mean, and standard deviations of adropin levels in PISF

	PI (n=25) α	PM (n=25) β	H (n=25) γ	p
PI	2.42±0.55	2.39±0.38	0.23±0.29	*αγ, βγ
PD (mm)	6.56±2.25	5.45±2.31	1.32±1.63	*αγ, βγ
GI	2.73±0.06	2.36±0.35	0.16±0.21	*αγ, βγ
MBL (mm)	6.00±1.46	1.06±0.15	0.43±0.33	** αγ, αβ
PISF Volume (µL)	0.98±0.21	0.69±0.29	0.09±0.1	**αγ,*βγ
Adr (ng/30sn)	0.83±0.27	0.36±0.25	0.20±0.21	* αγ

PI(α): Peri–implantitis; PM(β): Peri–implant mucositis; H (γ): Peri–impalnter health; PI: Plaque index; PD: Probing depth; GI: Gingival index total; MBL: Marginal bone loss.
†mean±SD deviation.
*Statistically significant (p<0.05). **Statistically significant (p<0.001)

comparison tests were used for comparison of normally distributed numerical data, and Kruskal–Wallis and all pairwise tests were used for comparison of normally distributed data. Descriptive statistics were presented as mean±standard deviation. A value of p<0.05 was considered statistically significant.

RESULTS

The mean adropin levels in PISF and the standard deviations are listed in Table 1. Overall, 75 patients (H, n=25; PM, n=25; and PI, n=25) in the age range of 28–65 years completed the study. Although PD, PI, and GI parameters were observed to be significantly higher in the PM and PI groups compared with the H group, no statistically significant differences related to pocket depth (PD) and GI were noted between PM and PI groups. Regarding MBL, the PI group had significantly higher MBL than the other groups (p<0.05). A statistically significant difference was observed upon the comparison of H-PI and H-PM groups regarding the PISF volume parameter (p<0.05). Regarding the PISF adropin levels, a statistically significant difference was noted between PI and H groups (p>0.05), (p<0.05).

DISCUSSION

Currently, mediators like cytokines and hormones can be used to clinically diagnose periodontal diseases, as well as evaluate the treatment efficacy. Adropin is a peptide hormone involved in the endothelial function through activation of the vascular endothelial growth factor receptor-2 (VEGFR-2) and phosphatidyl inositol-3-phosphate kinase (PI3K) pathways in the vascular wall endothelium (13). Notably, endothelial wall dysfunction occurs in periodontal and peri-implant diseases (14). Therefore, we purposed to investigate the concentration of adropin in PISF samples in patients with the peri-implant disease, besides discerning differences, if any, between healthy and diseased implants. Furthermore, adropin levels in PISF has not been examined before, and this study is the first study on this subject. Based on this study findings, adropin levels in PISF were noted to be statistically significantly increased in patients of the PI group compared with the H group. Moreover, clinical data related to PI, GI, and PD values were concordant with other studies (15).

Leptin and adiponectin, which are peptide hormones like adropin, are involved in similar functions in the body. It has been recently discovered that leptin and adiponectin, known for their effects on body weight regulation, body metabolism, and proliferation, have a direct effect on the immune responses and may be part of some inflammatory diseases. Leptin is thought to be a pro-inflammatory cytokine in the inflammatory response, whereas adiponectin mediates anti-inflammatory effects and functions in cell proliferation, differentiation, and regeneration (16, 17). When the literature was examined, different results were obtained regarding the relationship between periodontitis and leptin and adiponectin (16, 18-20). The reason for this difference is that most studies on leptin and adiponectin were cross-sectional, and the number of comparative studies was limited. Therefore, the cause-effect relationship between periodontitis and leptin and adiponectin levels has rarely been explored. Hence, because of the variation in the sample sizes and the differences between the qualifications and methods of these studies, no definite result could be achieved. In addition, a study investigating the level of VEGF in patients with periodontitis determined that the total amount of VEGF in GCF collected from the diseased regions was higher than that collected from clinically healthy regions (21). Johnson et al. (22) investigated the VEGF and IL-6 levels in patients who were healthy or had gingivitis, 4–6 mm periodontal pockets, and periodontitis, and observed that VEGF and IL-6 levels were lower in healthy individuals compared with patients with periodontitis. Although IL-6 concentration increases with increasing PD, VEGF concentration in 4–6 mm periodontal pockets was greater than that in >6 mm pockets. This finding is probably because of the increased VEGF owing to the increased vascular network in the initial and progressive phases during the transition from gingivitis to periodontitis. Another study compared the VEGF levels in patients who were healthy or had gingivitis, chronic periodontitis, and periodontal treatment. In chronic periodontitis, the VEGF level was noted to be significantly higher than the other groups, and the VEGF level decreased after the periodontal treatment (21). Notably, there is a relationship between adropin and VEGF levels. Therefore, considering this fact, it is appropriate to compare the present study data with VEGF. According to all these studies, the increase in adropin levels in patients with PI is thought to be due to the increase in VEGF, which is a pro-inflammatory factor in inflammatory diseases. Because adropin is a new mediator, more comprehensive and multicenter studies are needed to

fully understand the relationship between adropin, a peptide hormone, and several systemic diseases, in relation to the periodontal tissues. Considering all the past studies that discussed the role of adropin levels in the field of medicine, adropin levels in PISF may be increased because of the inflammatory character of peri-implant diseases (especially, peri-implantitis). Consequently, it can be stated that these inflammatory processes and responses are not only destructive but also protective in peri-implant disease. In addition, in recent years, adropin has been considered a crucial mediator in the pathogenesis of some systemic diseases, such as obesity, diabetes, cardiovascular diseases, and atherosclerosis.

In the present study, PI (23), PD, GI (24), and MBL values were recorded for the assessment of oral care and periodontal status of patients. These indexes were preferred because they were widely used and could be compared with other studies (25). PISF is affected by several factors, such as tooth brushing, mechanical irritation, probing, circadian rhythm, saliva-blood contamination, ambient temperature, individual body temperature, medications, smoking, and diabetes. In our study, to minimize the change in the PISF flow because of mechanical irritation from clinical measurements, PISF was collected before clinical measurements, based on the radiographs. PISF measurements of all individuals within the scope of the study were performed in the morning (09.00 am to 12.00 pm) to prevent PISF amounts from being affected by the circadian rhythm.

CONCLUSION

Within the limitations of the present study, it can be concluded that PI is an inflammatory condition that can result in resorption of alveolar bone. Adropin may be a future diagnostic tool for the peri-implant disease, especially in PI; however, to prove this, multicenter studies are needed that can evaluate the various biochemical parameters of peri-implant diseases.

Ethics Committee Approval: Ethics committee approval was received for this study from the Gaziantep University Clinical Studies Ethics Committee on 13/03/2017 (no: 2017/101).

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

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Original Research

Comparison of Incisional Hernias with Other Type of Abdominal Hernias in Terms of Predisposant Factors

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ABSTRACT

Objective: Incisional hernia (IH) is one of the most common late complications of abdominal surgery. Factors such as wound infection, type of incision, wound closure technique, and suture material used as well as patient-related factors such as age, gender, body mass index (BMI), diabetes mellitus (DM), and smoking are also involved in the development of IH and other types of abdominal hernias (OTAH). In this article, we aimed to compare the predisposing factors for IH and OTAH.

Methods: We analyzed predisposing factors for IH and OTAH among 130 patients undergoing surgery for abdominal hernia between January 2015 and December 2018 at the Department of General Surgery of Gülhane Training and Research Hospital.

Results: The female/male ratio was 28/102, the mean age of the patients was 58.6 years, and the mean BMI was 29.3 kg. The prevalence of DM and smoking was also evaluated. The rate of drain application was 56.2% and 4.1%, and the duration of hospitalization was 8.6 and 5.3 days in the IH and OTAH groups, respectively.

Conclusion: We found male gender to be a dominant risk factor for OTAH and high BMI to be dominant for IH. Age, DM, and smoking were equivalent risk factors for both. Drain application for IH was statistically significant high and resulted in prolonged hospitalization. These results provide evidence for an important complication of DM and obesity and also conclude that obesity is a major risk factor for IH.

Keywords: Abdominal hernia, diabetes mellitus, herniorrhaphy, incisional hernia, obesity

INTRODUCTION

Incisional hernia (IH) is a type of abdominal hernia that occurs at a previous surgical incision site. The incidence of IH of midline incisions is higher than for incisions of other regions. Despite advances in abdominal wall closure techniques, the rate of development of IH following laparotomy ranges from 15% to 20% (1). It has been found that more than 50% of IHs that originate from abdominal incisions occurs within the first year after surgery and 80% within 3 years (2, 3). Other types of abdominal hernias (OTAH) are hernias that do not originate from previous abdominal incisions but rather from anatomical weak sites of the abdominal wall. Abdominal hernias that are not classified as IH or OTAH are associated with a risk of strangulation and may cause other life-threatening complications if they are very large in size, irreducible, or consist of abdominal luminal organs. In such cases, surgical treatment of the hernia is strongly recommended.

Factors such as wound infection, location/type of incision, wound closure technique, and suture materials as well as patient-related factors such as age, body mass index (BMI), presence of diabetes mellitus (DM), and smoking are considered as other important risk factors for the development of IH. In addition, poor nutritional status, chronic lung disease, renal failure, malignancies, and steroid therapies are also considered to be facilitating factors for the development of IH (1-4).

In this study, we examined data from 130 abdominal hernia patients operated between January 2015 and December 2018 at the Department of General Surgery of Gülhane Training and Research Hospital, University of Medical Sciences. Among 130 patients, 32 patients had IH and 98 patients had OTAH. The data from the two groups were compared statistically. The aim of the study was to determine the factors for the development of ab-

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Table 1. Sociodemographic and clinical features of patients with incisional hernia and other types of abdominal hernias

	IH (n=32)		OTAH (n=98)		Total (n=130)		
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**p**
Gender	19 (41%)	13 (59%)	83 (84%)	15 (16%)	102 (78%)	28 (22%)	0.02
Age distribution (mean)	24–75 (56.8±5.7) years		21–86 (59.2±8.1) years		21–86 (58.6±7.5) years		0.26
BMI distribution (mean)	18.8–39.8 (29.3±3.4)		19.5–40.1 (27.2±3.6)		18.8–40.1 (27.8±4.1)		0.03
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	
DM	6 (18.7%)	26 (83.3%)	14 (14.3%)	84 (85.7%)	20	110	0.57
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	
Smoking	14 (44.6%)	18 (55.4%)	47 (47.9%)	51 (52.1%)	61	69	0.67
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	
Drain application	18 (56.2%)	14 (43.8%)	4 (4.1%)	94 (95.9%)	22	108	<0.00
Duration of hospital stay (mean)	2–19 (8.6±3.2) days		1–18 (5.3±4.1) days		1–19 (7.2±4.0) days		<0.00

BMI: body mass index; DM: diabetes mellitus; IH: incisional hernia; OTAH: other types of abdominal hernias

dominal hernias and investigate whether the factors have the same impact on both IH and OTAH. In addition, we compared herniorrhaphy repair techniques, drain application, and patient hospitalization duration.

METHODS

Data of 130 abdominal hernia patients operated in the General Surgery Department of Gülhane Training and Research Hospital of the University of Medical Sciences between January 2015 and December 2018 were analyzed retrospectively. Analyzed patient data included age, gender, BMI, presence of DM, smoking, hernia repair technique, duration of hospitalization, and drain application. Thirty-two patients who underwent surgery had IH and 98 had OTAH. Because this study is a retrospective analysis of patient medical data that did not collect any personal data, no informed consent was obtained from the patients. All study procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments.

Statistical Analysis

Gender, age, BMI, presence of DM, smoking, postoperative drain application, and duration of hospitalization of the two groups were analyzed using Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA) for statistically significance. $p<\alpha=0.05$ was accepted as statistically significant. A nonparametric chi-square test was applied for gender, presence of DM, smoking, and postoperative drain application. Mann-Whitney U test was used to analyze age, BMI, and hospitalization duration, and these parameters were also evaluated for descriptive statistics. Gender, DM, smoking, and postoperative drain application parameters were evaluated for percentage distribution.

The statistical significance of the 3 different operation techniques used in the two groups was evaluated with SPSS version 16.0. The percentage distribution for the 3 different operation techniques was evaluated, and a nonparametric chi-square test was applied for statistical analysis. $p<\alpha=0.05$ was accepted as statistically significant.

Main Points:

- Age, DM, smoking are the risk factors for OTAH and IH.
- Male gender is the main risk factor for OTAH
- BMI is the dominant risk factor for IH
- Obesity increases the risk of postoperative late complication IH.

RESULTS

Most patients with abdominal hernia were male. The female/male ratio was 13/19 in the IH group and 15/83 in the OTAH group, with total of 102 male and 28 female patients (Table 1). There was a statistically significantly higher proportion of males in the OTAH group. The ages of the patients ranged from 24 to 86 years (mean±SD, 58.6±7.5 years); the age distributions of the groups are given in Table 1. There was no statistically significant difference in average age between the groups.

Patients were evaluated for BMI, DM, and smoking. The mean BMI was 29.3±3.4 in patients with IH and 27.2±3.6 in patients with OTAH. The BMI of the patients is given in Table 1. Statistically difference was found between two groups. DM presences for the patients in the two groups were compared, 6 (18.3%) of the patients in the IH group and 14 (14.3%) patients in the OTAH group had DM, which no statistically significant difference between the two groups were found (Table 1). One patient in each group was

Table 2. Distribution of hernia repair types

	IH (n=32)	OTAH (n=98)	Total (n=130)	p
Primary repair	4 (12.5%)	13 (13.2%)	17 (13.1%)	
Mesh herniorrhaphy	9 (28.1%)	60 (61.2%)	69 (53.1%)	<0.01
Fence darning	19 (59.4%)	25 (25.5%)	44 (33.8%)	

IH: incisional hernia; OTAH: other types of abdominal hernias

medicated for insulin and others were medicated for oral antidiabetics. The prevalence of smoking was found to be 44.8% in the IH group and 47% in the OTAH group (Table 1) with no statistical significance between the groups.

Regardless of the type of repair technique, the rate of drain application was 56.2% in the IH group and 4.1% in the OTAH group (Table 1). There was a statistically significant difference in the rate of drain use between the two groups.

The duration of hospital stay ranged from 1 to 19 days, with a mean duration of 8.6±3.2 days in the IH group and 5.3±4.1 days in the OTAH group. The duration of hospitalization was significantly longer in IH patients than in OTAH patients (Table 1).

Patients were categorized into three subgroups based on hernia repair technique: classical herniorrhaphy using primary suturing, herniorrhaphy using mesh repair, and fence darning technique (Table 2). Among the IH and OTAH groups, classical herniorrhaphy using primary suturing was applied in 12.5% and 28.1% of the patients, herniorrhaphy using mesh repair was used in 59.4% and 13.2%, and the fence darning technique was used in 61.2% and 25.5%, respectively. The statistically significant difference between the groups is shown in Table 2.

DISCUSSION

Both age and gender were found to be risk factors for abdominal hernias. Older age was associated with wound-healing impairment, as was the presence of DM, which will be discussed below. DM was also found to be a cause of IH. In addition, as patients get older, the strength of the connective tissue decreases, which causes a weakening in the abdominal anterior wall and OTAH. Thus, there is no doubt that age is a predisposing factor for abdominal hernia. However, in our study, we found no statistically significant difference in age between the two groups. We conclude that age is an equivalent risk factor for both IH and OTAH.

Gender is also known to be a risk factor for abdominal hernia; however, its effect on OTAH and IH is unknown. We compared the predisposing effect of gender in each group separately. We found that male gender is a stronger risk factor for OTAH than IH. This may be because males deal more with heavy works which cause abdominal muscles to contract and increased intra-abdominal pressure. When males undergo surgery, they tend to decrease the use of their muscles in line with the advice of the surgeon; therefore, male gender is ultimately not a dominant predisposing factor for IH.

Obesity is one of the most important risk factors for the development of IH after abdominal surgery, and it may cause problems with wound healing in a significant proportion of patients with a BMI greater than 30 kg/m^2 in the early or late postoperative period (2, 5, 6). Wound complications, including wound infections and wound separation, are frequently associated with obesity because of the poor vascularization of increased adipose tissue and the proliferation of proinflammatory tissue factors. Obesity increases the rate of IH by impairing wound healing or causing infections. In addition, the increase in the risk of IH development may be the result of increased intra-abdominal pressure in obesity. In many animal experiments, physical and pathological events that increase intra-abdominal pressure have been shown to cause herniation of the abdominal wall from weak areas or from sutured incision sites. The weak areas of the abdominal wall include not only the incisional scars but also physiological anatomical locations, such as the umbilicus and inguinal region. Thus, obesity is also considered to be a risk factor for OTAH (7). However, our study results showed that obesity is a more dominant risk factor for IH.

It is very difficult to conduct human experimental studies on intra-abdominal pressure and tissue resistance. The results of animal studies are not scientifically fully valid for humans because of their different anatomical structures. Therefore, Kroese et al. created a simulator called AbdoMAN and carried out different studies on this model, which has very similar features to the muscles and fascia of the human abdominal wall. They clearly demonstrated the importance of increased intra-abdominal pressure for the development of IH (8, 9). These studies have shown that factors that increase intra-abdominal pressure, such as coughing, straining, vomiting, obesity, and heavy physical exercise, may increase the risk of IH independent of other factors. Therefore, patients should be evaluated in the postoperative period for constipation, pulmonary infection, or urination difficulty, and if necessary, medical treatment should be initiated for the diseases causing these symptoms.

In our study, we evaluated obesity by measuring the BMI of patients in both the IH and OTAH groups. We found that the BMIs of the two groups were statistically different. This result also supports the data in the literature demonstrating that obesity can cause both IH and OTAH and is a more dominant risk factor for IH. From this result, it can also be stated that incisional scars are weaker than the anatomical weak areas of the abdominal wall. In conclusion, we believe that obesity is a risk factor for both IH and OTAH, and to reduce the risk of IH, patients with a high BMI should be advised to lose weight.

DM is one of the known risk factors for the development of IH and is responsible for many possible local and systemic complications (10, 11). It is known that the adverse effects of DM

occur through the disruption in vascular structures, resulting in ischemia in the tissues, or by further compromising the general condition of patients as a result of previously developed systemic complications related to the cardiac or nephrologic systems. DM, which causes a delay in wound healing and increases wound complications, also demonstrates these effects by impairing collagen synthesis. Similar to DM, smoking also has a negative impact on wound healing through the mechanism of collagen synthesis and is therefore considered to be a risk factor for the development of IH. Studies have shown that approximately 8% of patients with abdominal hernias have DM and 43% are smokers. Our results showed that the frequency of DM was slightly higher, but the remaining results were consistent with the data in the literature (9, 12-15). However, we did not observe a statistically significant difference in the presence of DM or smoking between the IH and OTAH groups. Because DM and smoking are associated with wound-healing impairment and weakening of normal tissue, we found that these factors have an equal effect on both IH and OTAH. This may be the result of both the effects on wound healing and also the weakening of the abdominal wall by the pathophysiology described above.

Studies on the role of obesity, DM, smoking, and related collagen synthesis disorders in the etiology of IH have yielded conflicting results. The connective tissue consists of three groups of extracellular proteins: proteoglycans, glycoproteins, and collagens. Proteoglycans regulate the structure and permeability of tissues, whereas glycoproteins are proteins that are effective in cell-to-cell interactions. Because collagen is responsible for matrix structure and connective tissue support, dysfunction of the connective tissue is associated with collagen synthesis disorders (16). In fact, some experimental studies have found that the most intense changes in collagen metabolism occur directly in the anterior sheath of the abdominal rectus muscle. However, despite these proven functional properties of collagen structures, many clinical observational studies showed no significant difference in age, presence of DM, or smoking between patients with different hernia types (9, 17). A published systematic analysis analyzed 55 original articles evaluating connective tissue changes in patients with abdominal hernias and showed no significant difference in collagen changes between IH and OTAHs (18). The findings of this previous research support the results of our study, namely, that there is no statistically significant difference in the presence of DM or smoking between patients with IH and OTAH.

Apart from DM and smoking, wound infection, location, type of incision, wound closure material, and wound closure technique are other important factors involved in the etiology of IH. The development of wound infection leads to the release of many mediators in the surgical area, disrupts the general resilience of the patient, delays the formation of granulation tissue in the wound area, and prevents wound healing by disrupting collagen synthesis. It has been shown that collagen synthesis is especially reduced after contaminated surgical procedures and in patients with infected wounds, and this result is defined as a risk factor for the development of IH. One study reported that IH developed in the first postoperative year in 21% of patients who underwent colorectal surgery (6). The published series reported that half of patients with IH had a history of wound infection in the postoperative period and that the risk of development of IH in the first postoperative year was fivefold higher in patients with wound infection than in those without wound infection (4, 8). In our study, none of the patients had postoperative wound infections, and thus, we cannot declare a result for the effect of would infection in the IH and OTAH groups. In addition, we did not evaluate the location of the incision, type of incision, or wound closure material in our patients. However, we found a statistically significant difference between the wound closure techniques applied for the repair of IH and OTAH.

The closure technique and materials used for abdominal incisions are thought to have an effect on the development of IH (19, 20). Although there are ongoing discussions about the proper wound closure technique and materials used in surgery, it is considered sufficient to use any nonallergic material that does not increase the risk of infection and can provide adequate tissue resistance. Abdominal closure is underestimated by most surgeons and is generally considered an educational activity for inexperienced residents. Closure of laparotomy should be taken as seriously as all prior operative procedures and handled with appropriate techniques and materials (9). One of the key techniques in preventing the development of IH is the use of fascia sutures, which can last for a long time and resist tissue resistance. In patients undergoing laparotomy, only 70% of the fascia's tensile strength can be recovered 1 year after fascia repair. Therefore, suture materials that are absorbed and lose their strength in a short time are not suitable for fascia repair. Although there are studies indicating that the wound-stretching force is higher with the use of the single-suture technique as compared with the continuous-suture technique for laparotomy closure, single or continuous monofilament/polypropylene sutures were not shown to have a significant effect on wound healing or the development of IH. Some authors have argued that the use of the continuous-suture technique increases the risk of IH based on studies indicating that this suture technique causes insufficient wound tensile strength (21, 22).

The data in the literature given above obviously state that the closure technique is a factor in abdominal hernias, and we compared the wound-closure technique used in both groups. Although we did not evaluate relapse rates after the individual wound closure techniques, we found that the primary saturation technique was applied in the same ratio for both IH and OTAH. However, the fence darning technique was applied more often in IH, and mesh herniorrhapy was applied more frequently in OTAH. This may be the result of surgeon habits for inguinal herniorrhapy, which is the Lichtenstein technique. Because the Lichtenstein technique is the gold standard for inguinal hernia repair, which is evaluated in the OTAH group, the rate of mesh herniorrhaphies was higher in the OTAH group than in the IH group. However,

fence darning is safer because there is no application of prosthetic material, resulting in a lower risk of wound infection. Thus, surgeons often choose the fence darning technique in reoperated wounds, such as IH.

In patients undergoing mesh repair, some precautions should be taken, such as the use of drains and antibiotics to avoid the risk of seroma, hematoma, and mesh reactions. For this reason, we compared the drain application in both groups. We found that the application of drains was statistically significantly higher in the IH group as compared with the OTAH group. However, the use of mesh herniorrhaphy was greater in the OTAH group, which is in contrast to the indication of drain application in the presence of mesh. This may be because the main indication for drain application is not the presence of mesh. Drains are also applied in cases with a risk of hemorrhage. Because reoperated IHs have a greater risk for bleeding, surgeons may choose to apply a drain to the incisional IH.-

The duration of hospitalization is related to the expected postoperative complications and also the patient's postoperative recovery. In our study, we found that the hospitalization of IH patients was longer that of OTAH patients. This may be because IH is more complicated than OTAH. We also found that the rate of drain application was higher in the IH group than in the OTAH group. This may also be the cause for the longer hospitalization of the patients, as the drain needs to be monitored. However, because no bleeding or wound infection was found in either group, it appears that the application of the drain was unnecessary and merely a result of the surgeons' habits (23).

CONCLUSION

Many of the factors described above are known to be predisposing for abdominal hernias, but the significance of these factors for IH and OTAH has not undergone much discussion. In this study, we aimed to specify the significance of the predisposing factors according to the type of abdominal hernia (i.e., IH or OTAH). We found that male gender is the dominant risk factor for OTAH and obesity is the dominant factor for IH, whereas age, presence of DM, and smoking are equivalent risk factors for both IH and OTAH. We also found a statistically significantly high frequency of drain application in IH patients, resulting in delayed hospitalization. All of these results indicate that age, DM, and smoking are not the only risk factors for IH but are also risk factors for OTAH. In addition, we found that abdominal hernias are a complication of both DM and obesity. Thus, physicians have an additional reason to treat obesity and DM aggressively. Finally, obesity was found to have a greater effect on the occurrence of IH, so we suggest that surgeons recommend that patients with a high BMI lose weight postoperatively in order to prevent IH.

Ethics Committee Approval: No Ethics committee approval was received because the study was done by collecting data retrospectively from the patient files and no personal data of the patients were used.

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

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Original Research

Knowledge of Dentistry Students about Local Anesthetic Systemic Toxicity and Intravenous Lipid Rescue Therapy: A Cross-Sectional Questionnaire-Based Study

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ABSTRACT

Objective: The aim of this study was to evaluate the level of consciousness of local anesthetic systemic toxicity (LAST) among dentistry students, which would provide helpful information for scheduling the educational content of future syllabus before graduation to prepare students for possible challenges in the future.

Methods: This study included 234 dentistry students, who were in the 3rd, 4th, and 5th degrees during the period 01 December 2018–01 April 2019, and was conducted using a cross-sectional, questionnaire-based design. The revised questionnaire form includes questions addressing the frequency of encountered LAST cases, signs of LAST they had seen, and treatments for LAST, particularly lipid treatment, they had used.

Results: The questionnaire was sent to 234 dentistry students in the 3rd, 4th, and 5th degrees at the Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey, of whom 215 (91.88%) responded. The majority of participants (93%, n=200) declared that they received training about local anesthetics (LAs). Only one LA agent was preferred among 38.60% (n=83) of participants, whereas other participants preferred multiple agents. A significant majority of the participants (79.5%; n=171) declared that they did not observe LAST before this study, whereas only 15 (7%) students mentioned that they had encountered LAST but used an alternative therapy rather than intravenous lipid rescue therapy. None of the students personally applied lipid rescue therapy.

Conclusion: The results of this study implicate the evident need for additional educational effort to create awareness about LA use and effective management of LAST among dentistry students.

Keywords: Dentistry, lipid emulsion, local anesthetic systemic toxicity, local anesthetics, toxicity

INTRODUCTION

Local anesthetics (LAs) are frequently used in routine clinical practice and sometimes may be associated with systemic toxicity. However, there is a lack of studies in the literature concerning the awareness of local anesthetic systemic toxicity (LAST) among different medical specialties due to misdiagnosis or underreporting of similar events (1-3).

Therefore, we conducted a cross-sectional questionnaire-based study to determine the level of knowledge about LA use and the effective management of LAST among dentistry students at the Faculty of Dentistry, Gaziantep University. Our aim was to evaluate the level of consciousness of LAST among dentistry students, which would provide helpful information for scheduling the educational content of future syllabus before graduation to prepare students for possible challenges in the future.

METHODS

After obtaining approval from Gaziantep University Clinical Researches Ethical Committee (2019/318), a total of 234 dentistry students, who were in the 3rd, 4th, and 5th degrees during the period 2018–2019, were included in this study. Verbal informed consent was obtained from these participants before they filled in their questionnaire form. This study was conducted in a cross-sectional, questionnaire-based manner, which was adapted from a previous study conducted by Oksuz et al. (4). Students are supposed to have one semester of a lesson entitled "Local anesthesia in dentistry" in the 3rd year and one semester in "General anesthesia in dentistry," including LA lessons in the 4th degree at the Faculty of Dentistry, Gaziantep University. All the 4th and 5th degree students use local anesthesia during their clinical practice on behalf of their preceptors in various divisions.

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The revised questionnaire form includes questions addressing the frequency of encountered LAST cases, signs of LAST they had seen, and treatments for LAST, particularly lipid treatment, they had used. The questionnaire contains multiple-choice questions that are shown at Appendix 1.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) for Windows version 11.5®, and the results are shown in tables presented as descriptive statistics.

RESULTS

The questionnaire was sent to 234 dentistry students, who were in the 3rd, 4th, and 5th degrees at the Faculty of Dentistry, Gaziantep University, of whom 215 (91.88%) responded. Mean age of the participants was 22.52±1.41 years (range 20–27 years). The majority of them (93%, n=200) declared that they received training about LAs. Most of them preferred LAs as shown in Table 1.

Only one LA agent was preferred among 38.60% (n=83) of the participants, whereas others preferred multiple agents. The degrees of knowledge of the participants about the LAs they used are presented in Table 2.

Table 1. Most commonly preferred LA agents among participants

Agent preferred	Number of participants	(%)
Lidocaine	160	74.41
Lidocaine+vasoconstrictor	4	1.86
Articaine+vasoconstrictor	2	0.93
Articaine	88	40.93
Bupivacaine	18	8.37
Prilocaine	9	4.18
Mepivacaine	7	3.25

LA: local anesthetic

A significant majority of the participants (79.5%; n=171) stated that they did not observe LAST before this study, whereas only 15 (7%) students mentioned that they had encountered LAST but used an alternative therapy rather than intravenous lipid rescue therapy. None of the students personally applied lipid rescue therapy.

We also observed that 42.8% (n=92) of the participants had heard about lipid rescue therapy for LAST, but they did not remember how to manage this clinical situation. Among the study participants, 12 (5.6%) mentioned that they knew how to use lipid rescue therapy with intravenous lipids. A total of 23 (10.7%) participants had read articles about the therapy, whereas 88 (40.9%) participants stated that they did not hear anything about this therapy. Table 3 shows the most common LA-related adverse effects observed in clinical practice.

DISCUSSION

The side effects frequently observed in the use of LAs are often minor and/or transient. The symptoms of side effects fall on a broad spectrum, ranging from mild to life-threatening severe ones, including cardiac arrest, to the involvement of the central nervous system.

Individual patient risk factors, concurrent medications, location and technique of block, specific LA compound, total LA dose, timing of detection, and adequacy of treatment are the risk factors that entail the severity of LAST. The history of articles on LAST published in the literature goes back to 1884, with the introduction of cocaine to clinical practice in 1884, bupivacaine in 1970s, and ropivacaine and levobupivacaine in late 1980s (5, 6). Research studies are aimed at lightening up the pathophysiology of LAST and on novel treatment modalities such as lipid emulsion. The first guideline regarding the role of lipid emulsion in the management of LAST was published by the Association of Anaesthetists of Great Britain and Ireland in 2007 (7). The American Society of Regional Anesthesia and Pain Medicine (ASRA) reported practice guidelines regarding the prevention and treatment of LAST in 2010 (8). These guidelines state that the treatment for refractory LAST can be performed using conventional therapies (airway management with 100% O_2, convulsion therapy, and cardiopulmonary resuscitation if cardiac arrest occurs) and lipid emulsions using 20% intravenous

Table 2. The degree of knowledge of the participants regarding LAs they use

	Know very well % (n)	Know well % (n)	Not sure % (n)	No idea % (n)
LA doses	10.7 (23)	33.5 (72)	46 (99)	9.8 (21)
LA contraindications	8.4 (18)	23.3 (50)	56.3 (121)	12.1 (26)
LA complications	8.4 (18)	25.1 (54)	52.6 (113)	14 (30)
LA maximum doses	4.2 (9)	34.9 (75)	45.6 (98)	15.3 (33)
Adverse effects of LA	8.4 (18)	21.4 (46)	56.7 (122)	13.5 (29)
Management of adverse events	11.2 (24)	40 (86)	37.7 (81)	11.2 (24)

LA: local anesthetic

Table 3. Most common LA–related adverse effects observed in clinical practice

Signs and symptoms	(%)	Number
Tachycardia–palpitation	52.09	112
Syncope	27.44	59
Irritability	24.18	52
Tinnitus	5.58	12
Metallic taste in the mouth	6.04	13
Allergic reactions	25.11	54
Hypotension	21.86	47
Hypertension	9.30	20
Stupor	4.18	9
Convulsion	0.93	2
None	0.93	2

LA: local anesthetic

lipid solutions with a dose of 1.5 mg/kg intravenously followed by 15 mL/kg/h infusion for maintenance. In case of persistent symptoms, a bolus dose can be applied twice more without exceeding a limit of 10 mL/kg.

The occurrence of LAST cases in dentistry is rare. However, they can become a serious issue if the clinical symptoms and signs are underestimated and appropriate steps are not taken. Unfortunately, to the best of our knowledge, there are no epidemiologic studies regarding the frequency of LAST in dentistry (9).

Inferior alveolar nerve blockade is relatively commonly performed in dentistry (15.3%); therefore, the expected risk of LAST may be higher while performing this nerve blockade procedure. The frequency of the use of ester-type LA agents is not high. Among amide-type LAs, lidocaine is the most commonly used LA agent, which has low potency (10).

According to our results, the most commonly used LA agent was lidocaine (74.41%). Bupivacaine is a long-acting LA agent with a severe cardiotoxic potential. Cardiac arrest cases caused due to bupivacaine-induced LAST are known as resuscitation-resistant cases (11). Among the amide-type LAs, the percentage of choice of bupivacaine was relatively low (8.37%). One has to consider that our study population consisted of dentistry students. The more they become experienced, the more they can treat complicated cases that may require long-acting nerve blockade with bupivacaine.

Even when the practitioner's choice is amide-type LAs, the risk of LAST is still present. Furthermore, if a patient is allergic to this drug, one has to choose the ester-type LAs that have high potency (9).

Unfortunately, most of the clinics that apply LAs do not readily have anesthesiologists in charge at their clinic. All nonanesthesiologist practitioners, including dentists, have to be alert of LAST symptoms and signs and hence the therapy modalities. In a study conducted by Oksuz et al. (4) among 600 dentists, 404 (67.3%) respondents mentioned that they had no idea about lipid treatment, 128 (21.3%) had heard about lipid treatment but said that they did not have sufficient knowledge about it, and 59 (9.8%) had read an article about lipid treatment, but only 9 (1.5%) knew how to use lipid treatment. Another study conducted among 124 dentists demonstrated that the subjects were aware of some side effects about LAs with vasoconstrictors; however, they had inadequate knowledge about the signs and symptoms of overdose of LAs (12).

Published case reports regarding LAST (13-15) in the literature most commonly depend on the experience of nonanesthesiologists. Interestingly, a Danish survey study conducted among anesthesiologists in 2011 concluded that the study subjects had limited knowledge about lipid rescue therapy for LAST (3). It can be speculated that the guidelines about lipid emulsion therapy were relatively new at the study time period. A study performed at a similar time period among dermatologists reported similar results, wherein the awareness of intravenous lipid rescue therapy was lower than expected (22%) (2).

Nurses who work in preoperative and postoperative care units, outpatient services, and labor and delivery units and even operation room circulating nurses generally do not receive formal education or training about the recognition and treatment of LAST events (16).

Ophthalmologists are another group of specialists who frequently use LAs. A questionnaire-based study performed among 104 ophthalmologists reported that 76% of the participants declared that they used LAs every day or more than twice a week, whereas 56.7% of them had no specific training about this clinical situation (17).

Dentistry practitioners who perform various nerve block procedures multiple times a day also have to be aware of LAST. A dentist who confronts a LAST case should accurately understand about rapid recognition and also consider about treatment with lipid emulsion therapy. Therefore, we have to incorporate education on LA safety as a treatment for LAST in mandatory training sessions. In addition, introduction of national guidelines on lipid rescue therapy would probably accelerate this process.

CONCLUSION

In this context, academic trainers have a very important mission to prepare their students to encounter possible challenges in the future. The content of local anesthesia lessons has to be reviewed and arranged in view of these concerns. The results of this study implicate the evident need for additional educational effort to create awareness about LA use and effective management of LAST among dentistry students.

You can reach the questionnaire of this article at https://doi.org/10.5152/EurJTher.2020.19094.

Ethics Committee Approval: Ethics committee approval was received for this study from Gaziantep University Clinical Researches Ethical Committee with approval number: (2019/318).

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Appendix 1. Study Questionnaire (revised from Oksuz et al.)

Thank you for participating in our questionnaire about local anesthetic systemic toxicity (LAST) and treatment.

1. **Age:**
2. **Degree of class:**
3. **Did you have training about local anesthesia (LA)?**
   Yes ( ) No ( ) Don't remember ( )
4. **Choose the local anesthetics that you most frequently use.**
   Articaine ( ) Bupivacaine ( ) Lidocaine ( ) Prilocaine ( ) Mepivacaine ( )
   Articaine with vasoconstrictor ( ) Lidocaine with vasoconstrictor ( ) Prilocaine with vasoconstrictor ( )
   Mepivacaine with vasoconstrictor ( )

Evaluation of degree of knowledge about local anesthetics.

5. **LA dose:** No idea ( ) Not sure ( ) Know well ( ) Know Very Well ( )
6. **LA contraindications:** No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
7. **LA complications:** No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
8. **LA maximum dose:** No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
9. **LA side effects:** No idea ( ) Not sure ( ) Know well ( ) Know Very Well ( )
10. **Treatment of LA side effects:** No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
11. **Recognize signs and symptoms:**

    Tachycardia ( ) Syncope ( ) Irritability ( ) Tinnitus ( ) Metallic taste in the mouth ( ) Allergic reactions ( ) Hypotension ( )
    Hypertension ( ) Stupor ( ) Convulsion ( )

12. **Have you ever seen LAST?**
    Yes ( ) No ( ) Unaware ( ) Don't remember ( )

13. **Do you know intravenous lipid treatment in LAST?**
    Had no idea about intravenous lipid rescue therapy ( )
    Had heard but did not have enough knowledge about it ( )
    Had read an article about lipid rescue therapy ( )
    Know how to use lipid rescue therapy ( )

14. **Have you ever used intravenous lipid treatment in LAST?**
    Had never seen local anesthetic toxicity ( )
    Had seen it but used treatments other than lipid rescue therapy ( )
    Had seen it and used intravenous lipid therapy ( )

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Original Research

Effects of Dapagliflozin on Serum Low-Density Lipoprotein Cholesterol and Triglyceride Levels

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ABSTRACT

Objective: The aim of this study is to assess the effects of dapagliflozin, a sodium/glucose cotransporter 2 (SGLT2) inhibitor, on serum triglyceride and low-density lipoprotein (LDL) cholesterol levels in patients with type 2 diabetes mellitus (DM).

Methods: A total of 40 patients with type 2 DM, who were followed up regularly in the Endocrinology and Metabolism Outpatient Clinic of State Hospital, were evaluated retrospectively. In these patients, dapagliflozin was added to their regular treatment for glycemic control. The patients' anthropometric measurements, glycemic regulation status, and serum LDL cholesterol and triglyceride levels were retrieved from the system records. A statistical analysis of drug effects was performed using the repeated measures analysis of covariance test, keeping the effects of HbA1c and body mass index (BMI) covariates constant.

Results: In addition to the improvement in fasting blood glucose levels, HbA1c, and body weight of the patients, a reduction by 10 mg/dL and 43.04 mg/dL was observed in serum LDL cholesterol and triglyceride levels, respectively. The evaluation of BMI and HbA1c covariates together revealed a statistically significant reduction in triglyceride levels (p=0.032 and p=0.008, respectively).

Conclusion: Besides glycemic control and weight loss, addition of dapagliflozin to the type 2 DM therapy is associated with an improvement in serum triglyceride levels, suggesting that together with other benefits, SGLT2 inhibitors appear to provide an additional benefit of reducing the risk of cardiovascular diseases.

Keywords: Cardiovascular diseases, dapagliflozin, hyperlipidemia, SGLT2 inhibitor

INTRODUCTION

Diabetes mellitus (DM) is associated with an increased risk of cardiovascular diseases (CVD), which is the major cause of morbidity and mortality in patients suffering from type 2 DM (1, 2). Other risk factors for CVD include hypertension, hyperlipidemia, obesity, and smoking. Hyperlipidemia is a common metabolic disorder among patients with type 2 DM (3). Particularly, an increase in triglyceride and low-density lipoprotein (LDL) cholesterol levels is pronounced (4).

Although the risk of CVD is reduced by decreasing LDL cholesterol to target levels, a substantial proportion of patients with type 2 DM fail to achieve target LDL cholesterol levels. Hence, a significant number of patients suffering from type 2 DM remain at risk of CVD (5).

With this in view, new therapeutic options for type 2 DM provide us with new opportunities. Sodium/glucose cotransporter 2 (SGLT2) inhibitors help to provide glycemic control by inhibiting glucose reabsorption through proximal renal tubules (6). In addition, SGLT-2 inhibitors show favorable effects on blood pressure, body weight, arterial stiffness, visceral adiposity, albuminuria, and plasma uric acid concentration (7). Considering the effects of SGLT-2 inhibitors on lipid parameters, in addition to studies showing an increase in both high-density lipoprotein (HDL) and LDL cholesterol levels, there are studies reporting an increase in HDL cholesterol but not in LDL cholesterol (7, 8).

In the present study, we evaluated the effects of dapagliflozin that was added for 24 weeks to current treatment plans of patients with type 2 DM, who were receiving oral antidiabetics (OAD) and/or insulin on lipid parameters.

METHODS

The present study included patients with type 2 DM aged 40–70 years, followed up in the endocrinology and metabolism outpatient clinic of Hatay State Hospital from August 2016 through March 2017. Dapagliflozin was added to the OAD and/or insulin therapy. We were able to reach a total of 58 patients. Among these patients,

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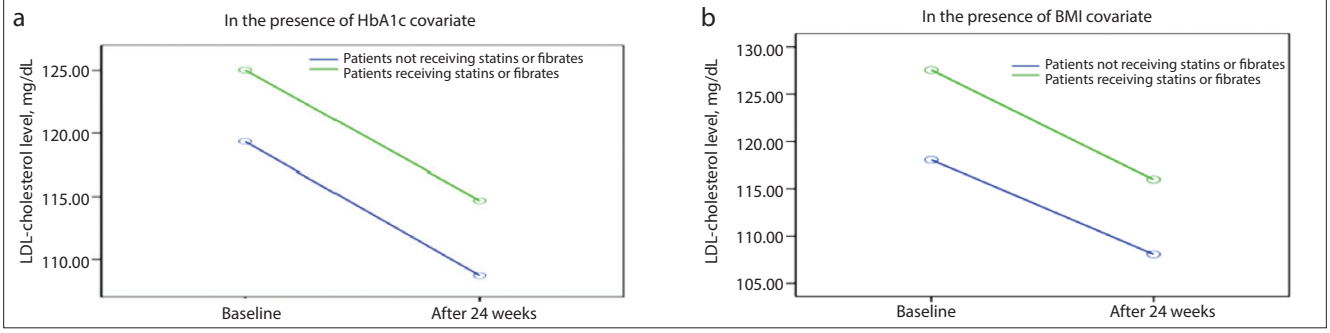
Figure 1. a, b. Change in low-density lipoprotein cholesterol levels with time and drug use in the presence of (a) HbA1c covariate and (b) body mass index covariate



those without the 3rd and 6th month follow-ups were excluded from the study. The data of the remaining 40 patients were retrospectively reviewed. Ethics committee approval was not obtained because of the retrospective study design. However, the present study was carried out in accordance with the World Health Organization Standards and Operational Guidance for Ethics Review of Health-Related Research with Human Participants (2011) and the principles of the Declaration of Helsinki of the World Medical Association (2013). All patients were followed up by the same specialist using the same follow-up and treatment protocol.

Weight measurements were performed in the morning on an empty stomach at baseline and follow-up visits. Body mass index (BMI) was computed as a ratio of weight to square of height (kg/m^2). Ambulatory blood pressure was recorded using automatic blood pressure monitors (Omron M2, HEM-7121-E) in sitting position after at least 5-minute rest.

For biochemical analyses, all blood samples were obtained from venous samples between 08:00 and 10:00 am after overnight fasting. Fasting blood glucose (FBG) and lipid profile were assessed using an automated enzymatic method, and HbA1c was assessed using the turbidimetric inhibition immunoassay (Roche Diagnostics, Mannheim, Germany). Estimated glomerular filtration rate was assessed using the Chronic Kidney Disease-Epidemiology (CKD-EPI) collaboration equation formula. Insulin administration and dosage regimens were unchanged at baseline and follow-up visits.

Main Points:

- SGLT-2 inhibitors show favorable effects on blood pressure, body weight, arterial stiffness, visceral adiposity, albuminuria, and plasma uric acid concentration.
- Substantial proportion of patients with type 2 DM fail to achieve target lipid levels.
- Results of studies on the effects of SGLT2 inhibitors on LDL-cholesterol and triglyceride have varied.
- Dapagliflozin, a SGLT-2 inhibitor, included in the treatment plan of patients with type 2 DM and high triglyceride levels not only provides glycemic regulation, but it also shows a beneficial effect on hypertriglyceridemia.
- The positive effect of Dapagliflozin on lipid parameters is an important result in reducing the risk of CVD in patients with type 2 diabetes.

Statistical Analysis

Continuous variables were expressed as mean±standard deviation, whereas categorical variables were expressed as frequency (%). The level of significance was predetermined to be 0.05 within the 95% confidence interval for all tests. The Shapiro–Wilk test was used for the Gaussian distribution. As for the univariate analysis, the chi-squared test and paired t-test were used, while the Wilcoxon signed-rank test was used when the condition for normality was not met. Keeping the effects of covariates HbA1c (difference as percentage) and BMI (numeric difference) constant, the change in LDL cholesterol and triglyceride levels between before and after treatment according to the medication was analyzed by repeated measures analysis of covariance (ANCOVA). After providing normality and homogeneity of variances for ANCOVA (using the Box-M test), the assumption of regression curves of the independent variable (drug) and covariates (HbA1c and BMI) being homogeneous (interactions >0.05) was provided. In addition, the linearity of LDL cholesterol and triglycerides with the covariates (HbA1c and BMI) was reviewed. All analyses were performed using the IBM Statistical Package for the Social Sciences Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The mean age of 40 patients, of whom 17 (42.5%) were female and 23 (57.5%) were male, was 52.85±9.08 years. The mean duration of DM was 8.07±4.12 years. Laboratory results and anthropometric measurements are summarized in Table 1. The mean weight loss was 1.63±0.32 kg. The pre- and posttreatment changes in FBG, HbA1c, LDL cholesterol, and triglyceride levels were significantly lower in our study group (Table 1).

Of the patients, 26 (65%) were not receiving statins or fibrates, and 14 (35%) were receiving either of the drugs (Table 2).

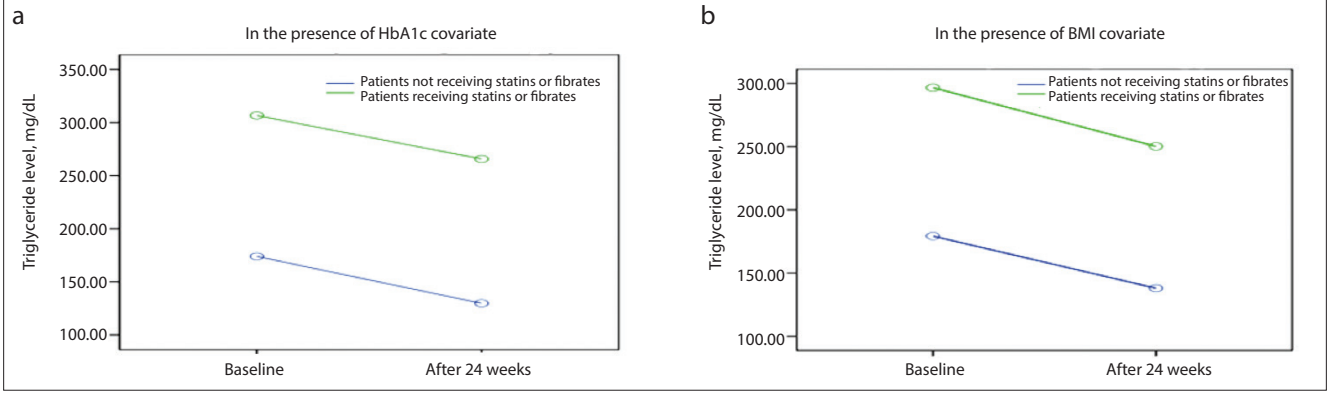
By keeping the HbA1c and BMI covariates constant over the 6-month treatment period from baseline, a decrease was observed in the LDL cholesterol levels during that time. However, this decrement was not caused by the drug (p=0.663 for the drug with HbA1c as covariate and p=0.525 for the drug with BMI as covariate) (Table 3, Figure 1).

Table 1. Univariate analyses and descriptive values for the parameters

Parameter	Baseline	After 24 weeks	p
Age (year), mean±SD	52.85±9.08		
Duration of DM (year), mean±SD	8.07±4.12		
Gender, n (%)			
Female	17 (42.5)		0.352*
Male	23 (57.5)		
Cigarette smoking, n (%)			
Nonsmoker	27 (67.5)		0.001*
Quitted	8 (20)		
Current smoker	5 (12.5)		
Body weight (kg), mean±SD	88.85±15.04	87.22±14.72	0.012**
BMI (kg/m^2), mean±SD	32.26±4.50	31.70±4.44	0.014**
SBP (mmHg), mean±SD	126.50±14.59	125.75±10.09	0.520**
DBP (mmHg), mean±SD	80.12±5.60	79.62±5.11	0.562**
FBG (mg/dL), mean±SD	219.62±70.38	172.48±53.08	0.001***
HbA1c (%), mean±SD	10.07±1.70	7.97±1.24	0.001**
LDL (mg/dL), mean±SD	121.23 ±35.81	110.70±35.95	0.041**
TG (mg/dL), mean±SD	219.72±150.63	176.68±125.84	0.002***
Creatinine (mg/dL), mean±SD	0.72±0.19	0.72±0.20	0.513**
eGFR (mL/min per 1.73 m^2), mean±SD	101.64±14.13	101.71±14.60	0.938**

*chi-squared test; **Student' s t-test; ***Wilcoxon signed-rank test. DM: diabetes mellitus; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; LDL: low-density lipoprotein; TG: triglyceride; eGFR: estimated glomerular filtration rate; SD: standard deviation

Figure 2. a, b. Change in triglyceride levels with time and drug use in the presence of (a) HbA1c covariate and (b) body mass index covariate



By keeping the HbA1c and BMI covariates constant over the 6-month treatment period from baseline, a decrease was also observed in triglyceride levels during that time. Drug use as well as time had an effect on this decrement (p=0.008 for the drug with HbA1c as covariate and p=0.032 for the drug with BMI as covariate) (Table 3, Figure 2).

DISCUSSION

SGLT-2 inhibitors cause a loss of approximately 240–320 calories/day by means of urinary glucose excretion at an average of 60–80 g/day (9). In a study conducted on the patients who were added SGLT-2 inhibitors to their treatment, a weight loss up to

Table 2. Drug groups, which might affect lipid levels, used by patients at the beginning of the study

Drug Groups	(%)
Metformin	87
DPP4 inhibitors	75
SU	40
Insulin	20
TZD	5
GLP–1 A	2.5
Statin	20
Fibrate	15
Thiazide	10
Beta blocker	10
LT4	7.5

SU: sulfonylurea; DPP4: dipeptidyl peptidase 4; TZD: thiazolidinedione; GLP–1A: glucagon–like peptit–1 analog; LT4: levothyroxine.

Table 3. Effects of dapagliflozin on LDL cholesterol and triglyceride levels according to statin and fibrate use in the presence of covariates (BMI and HbA1c)

		Mean±SD	p
Statin and fibrate nonusers	LDL baseline	118.30±37.22	0.145
	LDL final	107.40±36.53	
	TG baseline	180.63±75.00	0.006
	TG final	136.42±45.41	
Statin and fibrate users	LDL baseline	127.10±33.95	0.374
	LDL final	117.30±35.72	
	TG baseline	294.00±223.58	0.214
	TG final	253.20±187.66	

LDL: low–density lipoprotein; TG: triglyceride; SD: standard deviation

1.1–1.8 kg on average was observed in the 6-month follow-up period (10). In the present study, the mean weight loss was 1.63 kg, which is consistent with the literature.

Among the parameters of glycemic regulation, the expected reduction in FBG and HbA1c values with addition of SGLT-2 inhibitors is 20–30 mg/dL and 0.5%–1%, respectively (11). In the present study, the mean decrease in FBG and HbA1c was 47.14 ng/dL and 2.1%, respectively. In patients receiving DPP-4 inhibitors together with SGLT-2 inhibitors, the average reduction in HbA1c has been reported between 1.1% and 1.5% (12). In our study group, the substantial proportion of the patients was receiving DPP-4 inhibitors (75%). The response to antidiabetic medications is usually far above the expected levels in patients with high baseline HbA1c and FBG values.

Many studies have demonstrated that using statins for either primary or secondary prevention remarkably reduces cardiovascular events and related deaths (13, 14). SGLT-2 inhibitors, which are among the new generation OADs, are OADs with insulin-independent glucose-reducing effect. They lead to calorie loss while reducing glucose absorption through proximal tubules. In case of fasting, calorie deficit is compensated using lipids instead of glucose (15, 16). Various clinical trials performed with SGLT-2 inhibitors have reported increased LDL cholesterol (1.5%–6.3%) and HDL cholesterol (5.5%–9.2%) levels, but decreased triglyceride (1%–9.4%) levels (17). In the present study, we observed that the LDL cholesterol level decreased by 11.53 mg/dL (8.68%), and triglyceride levels decreased by 43.04 mg/dL (19.58%). The evaluation of LDL cholesterol alone revealed that the decrement reached the level of statistical significance; however, considering it together with the changes in BMI and HbA1c, the decrement was not statistically significant. In this sense, the results of the present study are consistent with the literature. The decrement in triglyceride levels was significant both alone and in the presence of other covariates (HbA1c and BMI). The improvement in triglyceride levels might be associated with weight loss and improved insulin sensitivity (18).

The present study has some limitations. First, it is a single-center small-scale study. Second, the study has a retrospective design. Moreover, HDL and total cholesterol measurements were not available as the patients were followed up according to their treatment protocol.

CONCLUSION

Addition of SGLT-2 inhibitors in the treatment of type 2 DM improves the lipid profile in addition to glycemic regulation. In this sense, it will be reasonable to mention an additional effect of SGLT-2 inhibitors in reducing the risk of CVD in patients with type 2 DM. Dapagliflozin, a SGLT-2 inhibitor, included in the treatment plan of patients with type 2 DM and high triglyceride levels not only provides glycemic regulation, but it also shows a beneficial effect on hypertriglyceridemia, which is one of the risk factors.

Ethics Committee Approval: Authors 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: Due to the retrospective design of the study, informed consent was not taken.

Peer-review: Externally peer-reviewed.

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

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Case Report

Coronary Embolism from Prosthetic Aortic Valve due to Incompliant Warfarin Use: A Rare Cause of Acute Coronary Syndrome

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ABSTRACT

Acute ST-segment elevation myocardial infarction (STEMI) is a life-threatening condition for which revascularization should be accessed emergently. Most STEMI cases result from atherosclerotic plaque rupture. However, rare causes such as coronary artery dissection, the vasculitic involvement of the coronary arteries, and coronary artery embolism may result in pathophysiological mechanism. This paper presents the case of a young male patient with subacute anterior STEMI secondary to thrombus embolism from prosthetic aortic valve due to incompliant warfarin use.

Keywords: Coronary artery embolism, incompliant warfarin use, prosthetic aortic valve

INTRODUCTION

Acute ST-segment elevation myocardial infarction (STEMI) develops after coronary artery occlusion due to ruptured coronary artery plaque and results in myocardial necrosis. Coronary artery embolism is a rare cause of STEMI without underlying atherosclerosis. Atrial fibrillation is the most common underlying disease related to coronary artery embolism (1).

This paper presents the case of a young male patient with a prosthetic aortic valve. The patient was admitted to our hospital with subacute anterior STEMI due to coronary artery embolism that potentially originated from prosthetic aortic valve owing to incompliant warfarin use.

CASE PRESENTATION

A 17-year-old male patient with compression type chest and back pain, which started 3 hours before admission, was admitted to our emergency department. His past medical history revealed Benthall procedure (prosthetic aortic valve and ascending aorta graft replacement) performed two years ago due to severe aortic regurgitation and ascending aorta dilatation related with bicuspid aortic valve. His only daily medication was warfarin, which was not consumed for the last four days. The patient reported that he had not used any other medications and substances during that period. His vital signs were in normal range; physical examination was unremarkable, except the metallic sound of S2. His electrocardiogram was consistent with subacute anterior STEMI (Figure 1). Hypokinesia at anterior wall mid-basal segments with estimated left ventricular ejection fraction of 50% was established by bedside echocardiographic evaluation. Prosthetic aortic valve functions were normal, and neither thrombus nor vegetation was detected on the valve surface. The international normalized ratio (INR) level was 1.23, which was below the therapeutic range. Cardiac biomarkers were mildly elevated (CK-MB:8.6 (0-6.3 ng/mL) and Tn-I: 0.113 (0-0.04 ng/mL), and coronary angiography was performed emergently. His right coronary artery, circumflex artery, and left main coronary arteries were normal. A huge thrombus obstructing the lumen was detected in the left anterior descending artery (LAD) (Figure 2). Bileaflet prosthetic valve motion was also normal at fluoroscopy. Intracoronary tirofiban was administered, and maintenance infusion of unfractionated heparin and tirofiban was continued for 24 hours. Therafter, subcutaneous enoxaparin 2×0.6 cc was initiated, in addition to the administration of 100 mg of aspirin and 75 mg of clopidogrel. Transeosophageal echocardiography revealed no thrombus at the heart valves, with prosthetic aortic valve and left atrial appendage and normal prosthetic valve functions. A control coronary angiography was performed 72 hours later, indicating that thrombus in the LAD had disappeared (Figure 3). Rheumatological markers and thrombophilia genetic panel were negative. Homocysteine level was also in the normal range. The patient did not describe dark urine in the morning, and hemo-

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Figure 1. Electrocardiography on admission indicating pathological Q waves and ST segment elevation in anterior leads consistent with subacute anterior myocardial infarction

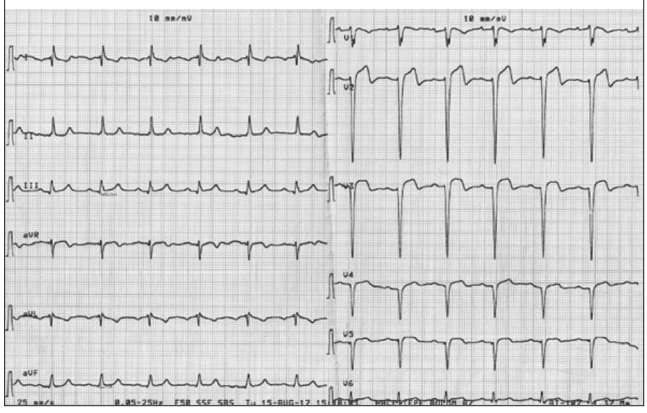
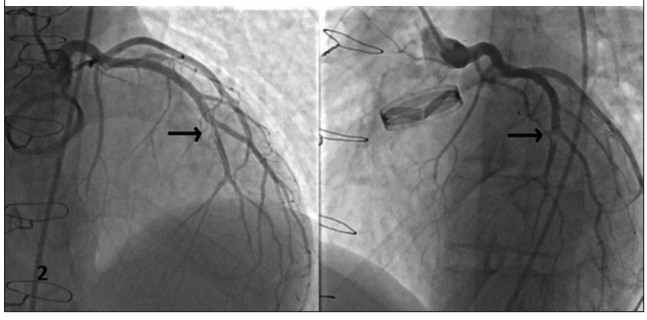


Figure 2. Emergent angiogram at right anterior oblique and left anterior oblique views indicating a huge thrombus at the mid portion of the left anterior descending artery

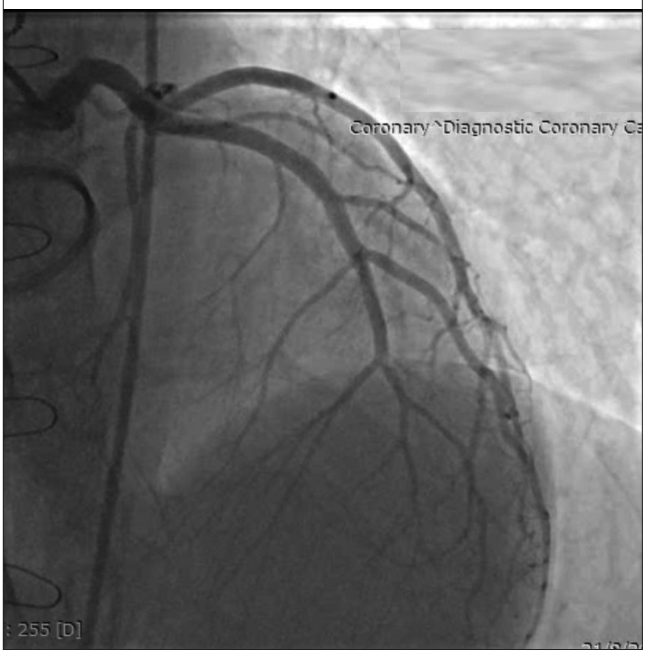


lytic parameters were normal. Other causes of arterial thrombus were excluded; coronary thromboembolism from the prosthetic aortic valve secondary to incompliant warfarin use and subtherapeutic INR level was proposed as the underlying mechanism. The patient was uneventfully discharged with a triple antithrombotic regimen (including warfarin, 100 mg of aspirin, and 75 mg of clopidogrel) for three months, and warfarin as well as aspirin was continued thereafter. As the past surgical reports were unavailable, the type of prosthetic valve could not be learnt from the patient. The target INR level during follow-up was determined as 3.0 owing to thromboembolic episode in conjunction with mechanical prosthetic aortic valve. Informed consent was obtained from the patient.

Main Points:

- An acute coronary syndrome due to coronary thromboembolism should be considered in differential diagnosis among patients with mechanical prosthetic heart valves.
- Effective therapeutic anticoagulation should be maintained in all patients with mechanical prosthetic heart valves.
- Monitoring of effective therapeutic INR levels is very important to prevent inadvertent embolic events in those patients.

Figure 3. Control angiogram at right anterior oblique view indicating the disappearance of thrombus at the left anterior descending artery after heparin and tirofiban infusion therapy



DISCUSSION

Plaque rupture is the most common cause of STEMI; of the other rare situations, coronary embolism results in STEMI. STEMI is a life-threatening condition that must be emergently treated with either mechanical or pharmacological revascularization. Primary percutaneous intervention is better than thrombolytic treatment to achieve thrombolysis in myocardial infarction grade 3 flow; accordingly, interventional treatment should always be preferred as the first option if available. Thrombus aspiration can be an option in selected patients with coronary artery embolism. There was a diversity between randomized controlled study results in regard to outcomes of thrombus apiration during primary PCI. Therefore, routine thrombus aspiration before PCI is not suggested by the American Heart Association (AHA) guidelines, and this option should be only considered in selected cases (1).

The prevalance of coronary artery embolism in patients with STEMI is estimated to be 13% by post-mortem series. Infective endocarditis, atrial thrombus, myxoma, prosthetic valves, calcific aortic stenosis, and biological glue used to repair aortic dissection are common causes of coronary artery embolism (2). Thrombus originating from aortic valve usually goes to left coronary artery presumably associated with aortic valve morphology (3). Anticoagulation with warfarin should be advised to the patients with mechanical prosthetic aortic valve, and the INR level should be maintained between 2.0–3.0 consistent with the recommendations of AHA/ACC guidelines to minimize the risk of thromboembolism. In patients with On-X aortic valve replacement, lower INR levels (1.5–2.0) are acceptable due to lower thromboembolic risk. Aspirin (75–100 mg/day) should be added in anticoagulant therapy (4). No consensus exists regarding the maintainence treatment of coronary embolism. Warfarin and as-

pirin were administered to the patient with STEMI considering mechanical prosthetic aortic valve, and clopidogrel was added for three months.

Similar cases with coronary artery embolism from the mechanical mitral valve, during and just after aortic valve replacement procedure and from blood cyst originated from mitral valve have been reported (5-7). To the best of our knowledge, no case report in the literature showing a coronary artery embolism from mechanical aortic valve as a result of incompliant use of warfarin and subtherapeutic INR level has been reported.

CONCLUSION

The most common cause of coronary artery occlusion is a ruptured unstable atherosclerotic plaque; however, coronary artery occlusion due to thromboembolic events should be included in the differential diagnosis of patients with acute coronary syndrome. Patients with prosthetic valves carry a high risk for thrombotic complications; accordingly, these patient groups should be effectively anticoagulated and monitored at regular intervals.

Informed Consent: Informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.Z.Ş., M.O., U.C.; Design – Y.Z.Ş., M.O., U.C.; Supervision – U.C., L.Ş., K.A.; Resource – Y.Z.Ş., M.O., U.C.; Materials – Y.Z.Ş., M.O., L.Ş.; Data Collection and/or Processing – Y.Z.Ş., M.O.; Analysis and/or Interpretation – Y.Z.Ş., M.O., U.C.; Literature Search – Y.Z.Ş., M.O., U.C.; Writing – Y.Z. Ş., M.O., U.C.; Critical Reviews – U.C., L.Ş., K.A.

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Case Report

A Rare Involvement of Left Main Coronary Artery Due to Woven Coronary Artery in a Patient with Behçet's Disease

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ABSTRACT

In general, woven coronary artery (WCA) is a benign congenital pathology; ocassionally, it may result in adverse cardiovascular events owing to myocardial ischemia. Though all coronary arteries may be affected, the right coronary artery is the most affected. This paper presents an extremely rare WCA effecting the left main coronary artery concurrent with Behçet's disease.

Keywords: Behçet's disease, left main coronary artery, woven coronary artery

INTRODUCTION

Woven coronary artery (WCA) is an extremely rare congenital anomaly with unexplained etiology (1). In this malformation, epicardial coronary arteries divide into long and thin channels. Thereafter, these channels merge to form an artery at the distal vascular bed (2). Normal blood flow after the abnormal coronary segment secures the relevant region, considered to be a good nature of coronary artery anomaly. Although this condition is considered to be benign, it occasionally causes angina pectoris, acute coronary syndromes, or possible sudden cardiac death owing to myocardial ischemia (3-5). Moreover, a few case reports have claimed no adverse cardiovascular events during long-term follow up (6, 7). This paper presents an extremely rare WCA effecting the left main coronary artery (LMCA) concurrent with Behçet's disease (BD).

CASE PRESENTATION

A 53-year-old male patient with a diagnosis of BD for 20 years was admitted to our department for unstable angina pectoris. The patient had a history of coronary artery bypass surgery 10 years ago. Resting electrocardiogram indicated q waves on D3 and aVF as well as nonspecific ST segment changes in precordial derivations with ventricular extrasystoles. Echocardiography indicated the systolic regional wall motion impairment. Coronary angiography was planned due to recurrent angina pectoris. Coronary angiography revealed a rudimentary right coronary artery (RCA) without a significant stenosis. Left coronary system angiography demonstrated a WCA of LMCA proceeding to the left anterior descending coronary artery (LAD) and circumflex artery (Cx). Furthermore, we observed a functional and well-developed left internal mammary artery (LIMA) to LAD anastomosis that associated dense collaterals with peripheral structures. Moreover, angiography revealed a collateral development between the left coronary system and RCA (Figure 1, 2). However, no information exists about the patient's coronary anatomy before his coronary artery bypass surgery. We believed that the patient's anginal complaints maybe associated with coronary steal owing to the dense collateral flow of LIMA. Furthermore, WCA of LMCA may have resulted from the decreased blood flow of Cx artery. So, we decided to optimal antianginal medical treatment to control of patient's symptoms. Written informed consent was obtained from the patient.

DISCUSSION

The etiology of WCA is unclear and is incidentally detected during coronary angiography. The literature indicates a male predominance (10:1) and reveals that RCA is the most affected (1, 4, 6). The recanalized thrombus, antegrade coronary collateral flow, or spontaneous coronary artery dissection should be considered for differential diagnosis (6). In particular, publications have indicated the effectiveness of optical coherence tomography (OCT) in differential diagnosis (8). We believed that the use of intravascular ultrasound or OCT for this patient is inappropriate owing to the diffuse involvement of LMCA. Depending on the affected segment of the coronary system, pharmacological treatment, percutaneous coronary intervention, or coronary artery bypass grafting may be the possible treatme/nt options.

The frequency of vascular involvement among BD patients ranges from 7.7% to 38% and is referred to as vascular BD (9). Males seem to be affected with arterial involvement than females. Vascular involvement more commonly affects the veins than the arteries, and coronary arterial involvement is extremely rare. Cardiovascular involvement in BD patients is estimated to range from 3% to 6%. This may result in pericarditis, myocarditis, coronary artery disease, valvular heart disease or intracardiac thrombus, endocarditis with valvular regurgitation, aneurysms of the coronary arteries or sinus of

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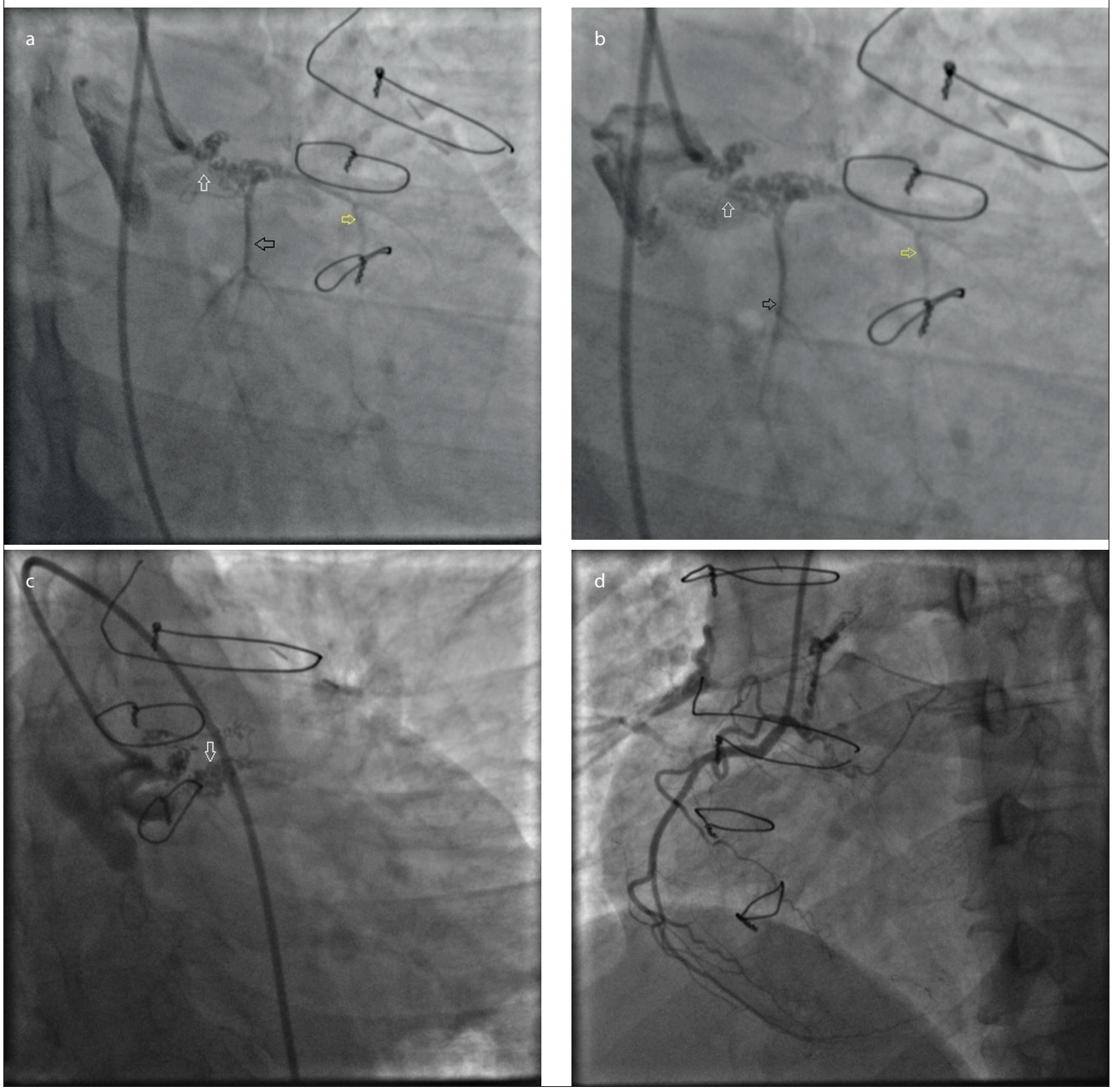
Figure 1. a-d. Coronary angiography depicts woven coronary artery of the left main coronary artery (white arrow), totally occluded left anterior descending coronary artery, dominant septal artery (yellow arrow), and circumflex coronary artery (black arrow) (a). Anteroposterior and caudal angiographic image of vessels [left main coronary artery (white arrow), septal artery (yellow arrow), and circumflex coronary artery (black arrow)] (b). Left anterior oblique caudal projection (spider view) demonstrating woven coronary artery of the left main coronary artery (white arrow) (c). Nondominant right coronary artery in a left anterior oblique view (d)

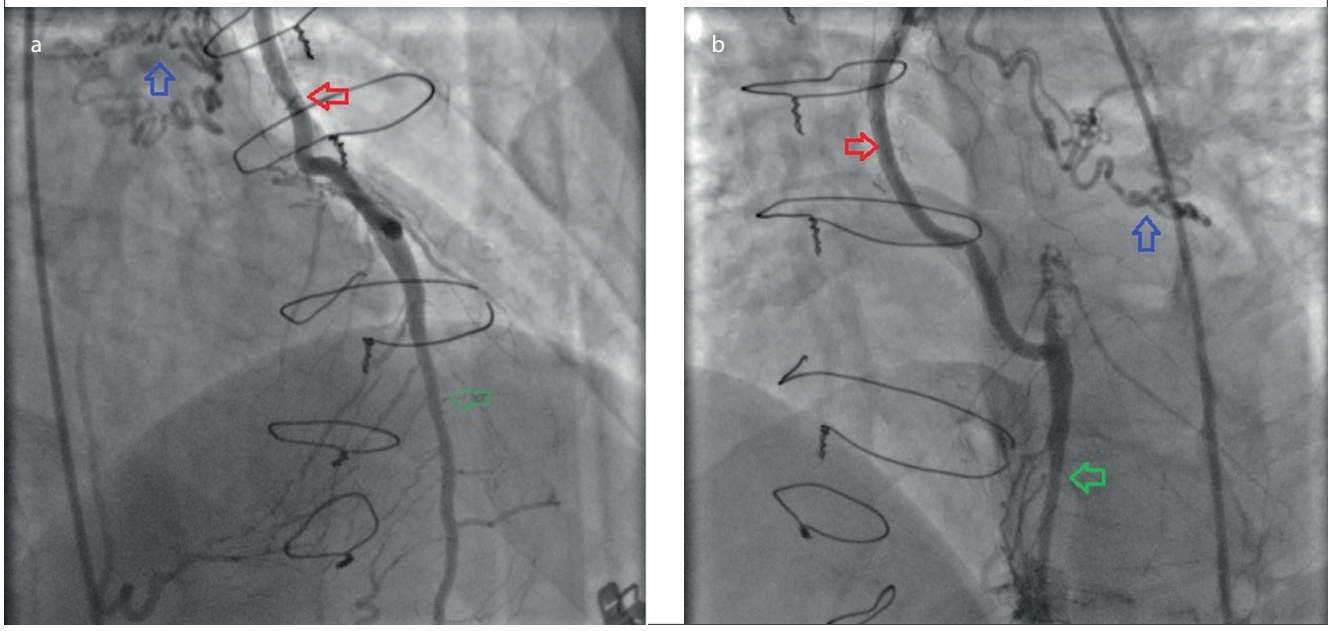


Main Points:

- The etiology of the woven coronary artery is still controversial.
- Involvement in the left main coronary artery is rarely reported.
- In this case, the presence of connective tissue disease (Behçet's Disease) with woven coronary artery may provide a different perspective for etiology.

valsalva, and advanced heart failure (10, 11). Lesions of coronary arteries include stenosis, occlusion, aneurysm, and pseudoaneurysm with or without myocardial infarction. A previously published study has demonstrated that silent myocardial ischemia rate was higher among BD patients compared with that of healthy controls at 19.5% and 2.9%, respectively (12). Peripheral arterial involvement in BD patients may range from 1.5% to 7% (9, 13). When a coronary artery pathology is detected in BD patients, the entire arterial or venous

Figure 2. a, b. Left internal mammary artery (red arrows) to left anterior descending coronary artery (green arrows) and anastomosis and well-developed collaterals (blue arrows) to the peripheral structures (a). Another image of left internal mammary artery (red arrows) to left anterior descending coronary artery (green arrows) and anastomosis and well-developed collaterals (blue arrows) (b)



vascular system must be evaluated for involvement. Doppler ultrasound scan of carotid arteries and iliofemoral arterial and venous system was observed to be in the normal range for this patient.

This case may be essential for two reasons. First, WCA can effect the isolated LMCA. To the best of our knowledge, this report is the first case to focus on the condition of WCA in BD patients in the literature. Since the patient's coronary anatomy before his coronary artery bypass surgery was unknown, commenting on congenital or the acquired appearance of LMCA seems impossible. As for the patient in this case, the LMCA lesion may have mortal consequences; thus, it is acceptable to explain this view through a chronic pathology. Furthermore, LMCA thrombosis may have resulted in coronary artery bypass grafting for this patient. Accordingly, this angiographical image may have resulted from chronic thrombosis recanalization. Second, the appearance of BD as a chronic vasculitics syndrome may result from chronic inflammation.

CONCLUSION

Finally, histopathological examination continues to be the gold standard technique for the diagnosis or differential diagnosis of WCA. This case indicates that the screening of connective tissue diseases may be beneficial in patients with WCA.

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

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