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Gülhane Tıp Dergisi

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Gülhane Tıp Dergisi

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Gülhane Tıp Dergisi

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Gülhane Tıp Dergisi

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Medical Journal
Gülhane Tıp Dergisi

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Gülhane Tıp Dergisi

GÜLHANE MEDICAL JOURNAL
Gülhane Tıp Dergisi

Gülhane Medical Journal

Gülhane Tıp Dergisi

Contents

Gülhane
Medical Journal
Gülhane Tıp Dergisi

Message from the Editor-in-Chief

Message from the Editor-in-Chief,

It is my pleasure to announce the successful closing of 2022 at GMJ. In this year, as every year, GMJ has published four issues, as scheduled, with highly quality articles.

In the fourth issue of GMJ in 2022, we have selected interesting four original articles, two reviews articles, and one case report for our readers. As the journal's publishing team, we try to keep covering a wide range of articles from different scientific disciplines.

I would like to express my gratitude to all submitting authors, reviewers, and editors for their contributions.

Also with this opportunity, I wish our community good health, happiness and success in the new year.

M. Ali Gülçelik, M.D., Prof.
Editor-in-Chief

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Effect of probiotics on oral *Candida*-a review

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ABSTRACT

Oral candidiasis is a frequently encountered fungal infection of the oral cavity. Its etiology is multifactorial, attributable to the disparity in the microorganisms inhabiting the oral cavity. Probiotics are live microorganisms that can have antagonistic effects on other pathogens. The benefits of probiotics in the management of gastrointestinal disorders are well known. Probiotics have been tried as a newer treatment option for managing oral candidal infections. Therefore, the present review attempts to summarize the significant data available from clinical trials about the efficacy of probiotics on oral candidal infections. A computerized search in PubMed and the Cochrane Library was conducted using the terms Candidosis (OR) Candidiasis (OR) oral *Candida* (AND) probiotics, to identify randomized controlled trials published from 2010 to April 2020. We identified forty-six articles and six of them fulfilled the inclusion criteria for this review. The studies have assessed the role of probiotics in oral *Candida* by measuring the candidal counts in samples from various intraoral sites, the clinical cure rate, or both. The probiotics used in most studies were of *Lactobacillus* species and *Streptococcus salivarius*. This review concludes that probiotics have preventive and curative effects on oral candidal infections, but the efficacy depends on the dosage, duration, and type of the probiotic strains.

Introduction

Oral candidiasis is an opportunistic fungal infection caused by *Candida* species. Under normal circumstances, *Candida* exists in the oral mucous membrane as a beneficial oral flora. When there is an imbalance in the microbial flora of the oral cavity, *Candida* species, most predominantly *Candida albicans* cause infections. Old age, an immunocompromised state, and prolonged consumption of broad-spectrum antibiotics are the predisposing factors. The pseudomembranous form of oral candidiasis is the most common, which presents as a scrapable curdy white patch in the oral mucosa and tongue (1). Live microorganisms that can provide health benefits to the host are called probiotics (2). Several probiotic microorganisms, such as lactic acid bacteria and yeasts, are well known for their therapeutic benefits. Probiotic therapy is the administration of a single or a combination of beneficial strains of microorganisms for managing various diseases such as gastrointestinal disorders and candidal vaginitis (2,3). In the management of oral candidiasis, antifungal drugs are the most commonly preferred line of therapy. However, the administration of antifungal drugs has various limitations because of the emergence of resistant strains to conventional antifungals and the recurrent nature of the disease in affected individuals (4). Probiotics are one of the newer therapeutic options tried in the management of various candidal infections. This article aims to review the available literature to determine the efficacy of probiotics in oral candidal infections.

Methods

A computerized search in PubMed and the Cochrane Library was conducted using the following search terms: (candidosis OR candidiasis OR oral *Candida*) AND (probiotics). Filters that were used in the PubMed database included "Randomized Controlled trails" and "Humans," and in the Cochrane Library, the filter was "Trials". Only the articles published in the English language between January 2010 to April 2020 were included. The inclusion criteria were as follows: 1. Randomized controlled trials to treat or prevent oral candidal infections using probiotics, with or without a placebo. 2. Studies have reported on candidal assessment after the administration of probiotics. 3. Studies on oral candidal infections with no other coexisting oral lesions. The review was performed following the PRISMA guidelines. The database search yielded 46 articles, out of which 22 were selected and screened after the removal of duplicates (Figure 1). Following the screening, 7 articles were assessed for eligibility and after excluding those that did not fulfill the exact aim of our present review, 6 articles were selected.

Characteristics of the included trials

The studies included in our review assessed the *Candida* counts in samples from various intraoral sites, the clinical cure rate, or both. The included studies differed widely concerning the age group, background, nutritional status, ethnicity, systemic factors of the study population, and the type, form, and duration of probiotic administration (Table 1).

Candida colonization in the oral cavity

The human oral cavity serves as a habitat for a wide variety of microorganisms, and interkingdom interactions among those microorganisms play a direct role in oral health. *Candida albicans* and a few other fungi of *Candida* species frequently colonize the oral cavity and participate in various complex microbial reactions like biofilm formation (5). Candidal colonization occurs sequentially by acquisition, adhesion, replication, and establishment of a stable population of yeast forms. The adhesion sites include epithelial surfaces, extracellular matrix proteins, bacterial cell-surface molecules, and dental acrylic. Basic proline-rich proteins and other salivary molecules promote the adherence of *Candida albicans*. Lectin, protein-protein, and hydrophobic interactions aid in the active adhesion of the fungal colonies. They also express alternate modes of adhesion in the oral cavity. They can escape host defense mechanisms and they can adhere to and colonize new environments by penetrating tissues, thereby establishing successful colonization (6).

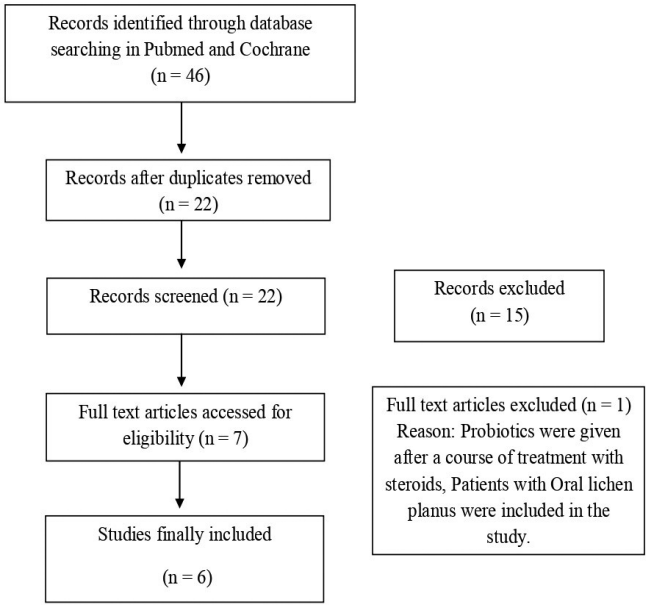

Figure 1. PRISMA flow diagram

Mechanism of action of probiotics on oral *Candida*

Probiotic bacteria adhere to the oral mucosa and compete with *Candida* species for nutrition and adhesion sites. They inhibit *Candida* by the production of various metabolites, stimulate the innate immune response of the host and decrease the production of inflammatory factors (7).

Lactobacillus species, which is the main constituent of most probiotic supplements, is known for its cell surface hydrophobicity and can directly form a mechanical barrier, and prevents candidal adhesion to the epithelium (8). Probiotics increase the levels of salivary immunoglobulin A and promote immunomodulation (9). Reuterin, a hydrogen peroxide-like toxin produced by live strains of *Lactobacillus reuteri*, is known to inhibit oral *Candida* by selective inhibition (10). Probiotic bacteria also produce various organic acids, hydrogen peroxide, bacteriocins, and antifungal peptides, thereby causing inhibition of candidal adherence and colonization in the host tissue (11,12).

Strain specificity of probiotic bacteria

The most widely used probiotic has been the *Lactobacillus* species. According to the evidence from available literature, probiotics containing *Lactobacillus* colonize the oral cavity only at the time of consumption, hence are capable of exerting only a short-term effect. But probiotics must show a long-term effect to prevent candidal colonization for a longer period. *Lactobacillus rhamnosus GG* is known to exist in the oral cavity for an extended period (13). A study by Miyazima et al. (14) found *Lactobacillus acidophilus NCFM* to be superior to *Lactobacillus rhamnosus Lr-32* in reducing the number of candidal colonies in highly infected individuals. On the other hand, experimental studies in infected immune-suppressed mice models have shown *Lactobacillus rhamnosus Lr-32* to be more effective than *Lactobacillus acidophilus NCFM*. It is also more effective than the antifungal agent Nystatin, in reducing the oral colonization of *Candida Albicans* (15). *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 have been used

successfully in frail elderly patients to reduce oral candidal counts (16). *Lactobacillus reuteri* also inhibits various other pathogenic bacteria by affecting their tissue binding ability and inhibiting the release of proinflammatory cytokines (7).

Streptococcus salivarius K12 has a higher tendency to bind with the hyphae form compared to the yeast forms of *Candida* and suppresses the adhesion of *Candida*. They exert an indirect antifungal activity that cannot be related to the anti-microbial activity of the bacteriocin (17).

Li et al. (12) found a combination of probiotics including *Bifidobacterium longum*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* to be effective in patients who were already on topical antifungal therapy (2% Nystatin paste) in reducing *Candida* species. It was also found to decrease the number of Gram-negative bacilli and increase the detection rate for *Staphylococcus epidermidis* in the saliva samples (12). *Staphylococcus epidermidis* inhibits *Staphylococcus aureus, Streptococcus mitis*, and *Streptococcus sanguis*, which exert synergistic properties with *Candida albicans*. Thus, *Staphylococcus epidermidis* interferes indirectly with the candidal growth or their adhesion to the biofilms of the oral mucosa (18,19). Hence, strain specificity is one of the vital factors determining the efficacy of probiotics against oral *Candida*.

Mycological cure

The mycological cure can be assessed by the detection of *Candida* in the culture (Table 2). Hu et al. (20) demonstrated negative microscopy and no growth of *Candida* in culture by administering *Streptococcus salivarius K12* along with topical antifungal therapy with Nystatin (500,000 U) to patients with oral candidiasis, thereby achieving an enhanced mycological cure rate of about 90% compared to the use of antifungals alone. Thus, combined probiotics and conventional antifungals can shorten the course of treatment. Kraft-Bodi et al. (16) reported a major reduction of *Candida* in the saliva and plaque samples of frail elderly patients, up to 51% of the reduction was noted in the probiotic group at the end of treatment. All the studies included in this review have shown significantly reduced *Candida* after the intervention.

Clinical cure

Studies indicate that the severity of denture stomatitis (DS) is directly related to *Candida albicans* in 72% of the patients. The management of DS is complex as it requires identification, correction, and elimination of the local and systemic predisposing factors. Newton has classified DS into three types based on the clinical appearance of the lesion; type 1 comprises a simple, localized inflammatory lesion, type 2 comprises a diffuse, generalized inflammatory lesion on the mucosal contacting the denture, and type 3 is characterized by chronic inflammatory lesion with granulomatous papillary hyperplasia (21). Lee et al. (22) have successfully demonstrated clinical improvement in the severity of DS after intervention with probiotics. In their study group, 11 participants had type 1, 6 had type 2 and one participant had type 3 DS at the beginning of the trial. After a six-month probiotics intervention (T1), only six had type 1, and one had type 2 DS. On the other hand, one participant who had type 3 DS maintained the same severity. Finally, six months after the discontinuation of the intervention (T2), there was no change in the clinical type of DS concerning T1. Thus, a significant improvement in the clinical presentation of DS compared to the pre-intervention and post-intervention was evident, also between pre-intervention and six months after the discontinuation of the probiotics. Li et al. (12) used parameters such as grading of hyperemia using a card with four levels of red colors and pain assessment using a visual analog scale (VAS) to assess the clinical presentation of oral candidiasis among the study participants before, during, and after the intervention. The probiotic group showed significantly decreased VAS scores after two weeks of treatment. But there was no significant difference in the reduction of hyperemia in the probiotic group.

Prophylaxis against denture stomatitis

Oral candidiasis has a chronic course and often recurs in elderly individuals, as they use dentures and have a weak immune status. Therefore, there is a need for therapeutic agents with relatively low toxicity and side effects without compromising their effectiveness against *Candida*. Various systemic conditions, including diabetes, immune deficiencies, xerostomia, and various local factors such as ill-fitting dentures, and poor oral and denture hygiene, predispose to candidal infections (23). Patients using removable dentures are more prone to DS associated with *Candida*. The denture base materials serve as a substrate for the growth and colonization of microorganisms. It has been reported that 11-67% of complete denture wearers suffer from DS (24). Hence, there is a strong need for therapeutic agents that can prevent *Candida* with relatively lesser toxicity. Topical application of a lyophilized capsule containing *Lactobacillus rhamnosus HS111*, *Lactobacillus acidophilus HS101*, and *Bifidobacterium bifidum* over the tissue contacting area of maxillary dentures for 5 weeks showed successful elimination of *Candida* in samples taken from the palatal mucosa (25). Miyazima et al. (14) reported a significant reduction of *Candida* in the mouthwash samples of complete denture wearers who consumed probiotic cheese containing either *Lactobacillus acidophilus NCFM* or *Lactobacillus rhamnosus Lr-32* daily for 8 weeks. Hence, daily consumption of probiotic supplements in any convenient form such as cheese or milk can help in the reduction of the candidal load, thereby providing prophylaxis against oral candidal infections like DS in highly susceptible individuals.

Table 1. Characteristics of the included studies

Study	Probiotic used	Form of intervention	Treatment regimen	Duration of intervention
Li et al. (12)	*Mixture of Bifidobacterium longum, Lactobacillus bulgaricus and Streptococcus thermophilus*	Lozenges	1. Mouth gargling using 2% sodium bicarbonate solution for 30 seconds, 2. Application of 2% Nystatin paste, 10 minutes after mouth gargling, 3. After one hour, four probiotic lozenges were held in mouths (Procedure followed three times/ day).	4 weeks
Ishikawa et al. (25)	*Lactobacillus rhamnosus HS111, Lactobacillus acidophillus HS101, Bifidobacterium bifidum*	Lipophilized capsules	Local application by pouring the capsule content into the palatal region of the previously cleaned maxillary denture (1 capsule/day).	5 weeks
Kraft-Bodi et al. (16)	*Lactobacillus reuteri DSM 17938, Lactobacillus reuteri ATCC PTA 5289*	Lozenges	One lozenge in the morning, One lozenge in the early evening (2 lozenges/day).	12 weeks
Miyazima et al. (14)	*Lactobacillus acidophilus NCFM, Lactobacillus rhamnosus Lr-32*	Supplementation with cheese	20 g of fresh white cheese will be given every 2 weeks (Daily consumption).	8 weeks
Hu et al. (20)	*Streptococcus salivarius K12*	Lozenges	One lozenge of probiotic BID One Nystatin tablet (500,000 U) TID-Topical application.	4 weeks at 1-week interval
Lee et al. (22)	*Lactobacillus rhamnosus SP1*	Supplementation with milk	Oral hygiene training before the start of the study was given, 200 mL of probiotic milk for 5 days a week.	6 months

Table 2. Pre and post-interventional status with regard to the mycological load/cure

Study	Mycological load/cure	
	Before the intervention/baseline	**Post-intervention**
Li et al. (12)	Detection of *Candida* in samples: 100%	Detection of candida in samples: 8.21%
Ishikawa et al. (25)	*Candida* was detected in samples in all 30 participants in the probiotic group.	*Candida* was detected in samples in only 5 participants.
Kraft-Bodi et al. (16)	*Candida* in saliva: 72% *Candida* in plaque: 67%	*Candida* in saliva: 51% *Candida* in plaque: 50%
Miyazima et al. (14)	3.5 $\log_{10}$ CFU/mL (before intervention with *L. acidophilus* NCFM)	2.5 $\log_{10}$ CFU/mL (after intervention with *L. acidophilus* NCFM)
Hu et al. (20)	Mycological cure rate: -	Mycological cure rate: 90.48%
Lee et al. (22)	Viable cells: 1.98e+03 CFU/mL	Viable cells: 1.32e+03 CFU/mL

CFU: Colony forming units, mL: Milliliter

Adverse effects

While most studies have not reported any side effects, Hu et al. (20) reported xerostomia, numbness, burning sensation in the oral cavity, borborygmus and pharyngeal discomfort, light dizziness, and headache in a few participants who received *Streptococcus salivarius K12* with nystatin. However, there is evidence in the literature that the fermentation and enzymatic reactions of *Streptococcus salivarius K12* probiotics do not show harmful effects on humans (26).

There were no reports of major side effects after the intake of probiotic supplements in most studies. In the study by Kraft-Bodi et al. (16), a few participants reported a feeling of intense taste of tablets. Two participants in the probiotic group and three in the placebo group reported gastric complaints within the first week of the intervention, which may not be directly related to the probiotic. Instead, it may also be linked to the chemical composition of the lozenges. Probiotic-supplemented milk and cheese are not recommended for lactose-intolerant individuals.

Advantages of treatment with probiotics

Therapeutic and prophylactic use of probiotics in oral candidal infections can reduce the use of conventional antifungals and their side effects. They also have the added advantage of preventing the development of drug resistance by certain *Candida* species against azoles which are the most commonly used anti-fungal therapies in oral candidiasis.

Conclusion

All the studies included in our review compared the pre-interventional and post-interventional candidal status of the patients and probiotics were found efficient in both prophylaxis and management of oral candidal infections. From the available literature, it is evident that probiotics can be alternative to conventional antifungal therapy in oral candidal infections. It should also be noted that the effect of probiotics depends on the dosage, duration, and type of probiotic strain used. Therefore, it is necessary to select an appropriate probiotic strain for a successful treatment outcome.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.F.A., Concept: M.F.A., S.B., Design: M.F.A., S.B.G., Data Collection or Processing: M.F.A., Analysis or Interpretation: S.B.G., Literature Search: M.F.A., S.B., Writing: M.F.A.

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Charcoal-containing toothpastes

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ABSTRACT

The importance of having whiter smiles has increased with the increase in people's aesthetic expectations. Patients who wish to have whiter teeth consult their dentist. Whitening treatment or the use of various kinds of toothpaste is recommended according to the condition of the teeth. However, most people seek professional applications and products that they can apply at home to produce whiter teeth. The most important and most common of these products are the whitening kinds of toothpaste. They are used both for the maintenance phase of tooth whitening treatment and to provide whitening with toothpaste. Manufacturers have developed new formulations to meet consumer satisfaction. With newer formulations, new kinds of toothpaste take their place in the market. This article reviews the general content of toothpaste and the recently popular charcoal-containing types of toothpaste.

Introduction

Toothpaste has long been a part of our daily lives. In 500 BC, Indochina provided a recipe for a tooth-cleaning paste (1). Both France and the United States invented flexible lead or tin tubes in 1846, and toothpaste was first sold in bendable tubes in the US and Germany in 1896. In 1873, Colgate began manufacturing toothpaste in jars in the United States. The first fluoride toothpaste was introduced in the United States in 1955 (1).

Toothpaste has always been offered to consumers as changing and developing oral hygiene products. The most common and simplest method for providing oral dental care is mechanical cleaning of the teeth using toothbrushes and interface brushes. Toothpaste is used to increase the mechanical cleaning efficiency of toothbrushes and to obtain additional effects such as whitening. Because of this state, it can be ensured that the teeth are protected from plaque and tooth decay (2).

The oral hygiene products market aims to continuously improve the content of existing products and to develop new products to satisfy the expectations of consumers. Although toothpaste contains all basic active ingredients that protect from plaque and decay, various substances have recently been added to them (3).

The most common issue that makes patients disturbed is the discoloration of the teeth. Therefore, the ingredients added to toothpaste are formed according to consumer demands.

Due to these demands for tooth-whitening products and many new-toothpaste formations have been created to remove or prevent extrinsic stains (4).

Manufacturers claim that toothpaste and powders containing charcoal, which have recently become popular, have teeth-whitening properties to prevent the recurrence of discoloration (5).

This review aimed to provide an overview of toothpaste and to bring together studies related to the promised properties of charcoal toothpaste (their effects on tooth structure are also included), which have become popular recently.

Content of the toothpaste

1. Abrasives

Abrasives contained in toothpaste physically maintain the removal of tooth extrinsic stains (6). The ideal toothpaste should have low abrasive properties and obstructive tubules (7). The corrosive system must be insoluble in liquid, inert and non-toxic. Calcium carbonate, dicalcium phosphate dihydrate, alumina, silica, and sodium bicarbonate are the most frequently used abrasives in toothpaste production (8).

The abrasiveness of toothpaste depends on the characteristics of abrasives and tooth brushing parameters. Such as particle shape, size, and hardness, as well as the tooth brushing technique, the hardness of the toothbrush, the direction, and the number of brush strokes (9). Additionally, the use of abrasives, detergents, and both add different abrasive properties (6).

2. Humectants

Toothpaste is in the form of a mixture of powder and water. When the tube opens, toothpaste hardens in a short time regardless of water content (9). To regulate this process, humectants are added. Glycerin, sorbitol, propylene glycol, and mannitol are used as humectants (8,9). Among these, glycerin and sorbitol are the most common (10).

3. Surfactant

Toothbrush bristles remove debris and plaque, loose material is removed with the help of the foaming effect of soaps. Soaps left their place with surfactants due to their various disadvantages. Sodium lauryl sulfate (SLS) is the most widely used surfactant today. SLS anti-plaque effect is provided by killing microorganisms, reducing surface energy, and denaturing protein (10).

4. Viscosity and rheology modifiers

They are responsible for ensuring the stability and consistency of the toothpaste. They are responsible for pasting the toothpaste and its stability on the toothbrush (10). Frequently used binders are carrageenans alginate, sodium carboxymethyl cellulose, magnesium aluminum silicate, sodium magnesium silicate, and colloidal silicate (8).

5. Sweetening agents

One of the sweetening agents added to toothpaste is soluble saccharin. Also, mint spectrum, anise, lemon, eucalyptus, and others can be used (8). Several sweetening agents such as menthol show an antimicrobial effect. The concentration of menthol in toothpaste is between 0.1-0.5%. It is a kind of alcohol widely contained in many foods, cosmetics, soaps, and toothpaste. It has been reported that menthol can cause asthma and urticaria (10).

6. Therapeutic agents

Toothpaste is the most suitable tool that can be used to ensure oral health. Different therapeutic agents can be added to it. These agents can be classified as caries prevention agents, agents that prevent plaque formation, agents with antibacterial properties, agents that reduce calculus formation, agents that relieve tooth sensitivity, and whitening agents (8).

a) Anti-caries agents in toothpaste

Fluoride toothpaste helps prevent caries, according to a systematic review (11). Different types of fluoride have been used in toothpaste formulations, including amine fluoride, stannous fluoride, sodium fluoride, and sodium monofluorophosphate. Although there has been substantial discussion about the relative efficiency of these distinct fluoride salts (12), a systematic evaluation revealed that they were all equally beneficial (11).

Toothpaste with fluoride concentrations greater than 1.500 ppm is designated as prescription medication and should only be used to treat those over the age of 10 who are at high risk of caries, such as those who have a dry mouth or have root surface caries. Calcium, phosphorous (phosphates; trimetaphosphates, pyrophosphates, glycerophosphates), metals (zinc, tin, aluminum, iron, manganese, molybdenum), and different anti-microbials are all non-fluoride caries inhibitors in toothpaste formulations. A dental plaque containing calcium carbonate has pH-raising characteristics and may help with remineralization by increasing plaque calcium levels (13).

b) Anti-plaque agents in toothpaste

Most oral anti-plaque agents are antiseptics or antimicrobials that are used to prevent biofilm adhesion, reduce bacterial development, or eliminate and/or modify the pathogenicity of tooth plaque. Anti-plaque agents with adsorption and long-term retention on oral surfaces are the most effective. They should have a broad anti-bacterial range with low toxicity, as well as be compatible with other toothpaste constituents (13).

Triclosan (2,4,4’ trichlor-2’-hydroxydiphenyl ether) is a non-ionic chlorinated bisphenol that is commonly used in personal care products such as deodorants and soaps. It is safe, effective, and well-tolerated. It’s safe to use with toothpaste fluoride and the environment. Active chemicals are present, as well as anti-inflammatory properties. Trichlorosan, on the other hand, has only modest persistence and does not last long enough to have a major anti-bacterial impact. Its oral retention is greatly improved when coupled with a co-polymer, polyvinylmethyl ether maleic acid (PVM/MA or Gantrez) (13).

Concerning the sensitive bacteria, triclosan inhibits the enoyl-reductase enzymes of type 2 fatty acid synthases, causing damage to the cytoplasmic membrane and leaking. It offers a broad anti-microbial spectrum and a considerable anti-plaque impact without staining the teeth. Systematic reviews of six-month clinical studies found that triclosan and copolymer formulations significantly improved plaque control and periodontal health (14,15). Plaque control and gingival health can also be improved with toothpaste-containing triclosan and zinc citrate (13). According to a systematic review, stannous fluoride toothpaste enhanced plaque control and gingivitis (16). Herbal toothpaste has a much higher anti-plaque activity than conventional toothpaste occasionally (17). Both plaque and gingivitis have been observed to be inhibited by a zinc citrate/bromochlorophene/triglyceride composition (18).

c) Anti-calculus toothpaste

Supragingival calculus is a mineralized plaque and inhibiting mineralization with crystal growth inhibitors is one way to regulate it. Inhibitors include pyrophosphates, phosphonates, zinc salts, and substances such as a copolymer of methyl vinyl ether and maleic anhydride (13). In a clinical experiment, using toothpaste with zinc citrate trihydrate and triclosan on a 1450 ppm F silica base reduced calculus (19). Several studies have found that dentifrices containing 3.3 percent-soluble pyrophosphates considerably reduce calculus considerably (20,21). It has been demonstrated that adding copolymer (polyvinyl ether and MA) to pyrophosphate-containing formulations improves their efficacy in reducing calculus (22). Studies have shown that triclosan/copolymer toothpaste is effective in reducing calculus (23).

d) Desensitizing toothpaste

Gingival recession exposes root surfaces, which are a major risk factor for dentin root sensitivity, and is often aggravated following periodontal treatment. To treat sensitivity, two types of products are used: those that interfere with neural impulse transmission and those that block and occlude the dentinal tubules. In several countries, potassium nitrate (5%), potassium chloride (3.75%), and potassium citrate (5.5%) are frequently used since each of these salts contains 2 percent potassium ions, which impedes neuronal transmission. Potassium-based toothpaste should be used twice daily for at least two weeks to obtain measurable sensitivity decreases, according to clinical trials. Compared to conventional fluoride toothpaste, clinical data show that all three types of potassium help lower sensitivity (24,25). The idea of tubule closure is present in both strontium chloride and stannous fluoride. After four weeks of twice-daily use, these products show significant reductions in hypersensitivity. A new formulation has recently been released that works by occluding dentinal tubules. Toothpaste contains 8% arginine, calcium carbonate, and sodium monofluorophosphate (1450-ppm fluoride). This product's effectiveness has been proven in several clinical investigations (26).

e) Whitening toothpaste

Whitening toothpaste has content that offers physical-chemical cleaning that allows the removal of extrinsic stains on the tooth surfaces with daily brushing (3,5,27,28). In the literature (28), these stain-removal ingredients are stated as abrasives, surfactants, calcium chelators, enzymes, and polymers. Traditional whitening toothpaste contains substances such as silica, hydrogen peroxide, or carbamide peroxide that remove stains from the teeth and make them whiter (2,29-31). The whitening ingredients in toothpaste contain herbal origin substances mostly including papaya (papain enzyme), menthol, meswak, clove, salt, and citrus fruits (2).

Toothpaste containing blue covarine provides a whitening effect by depositing covarine on the tooth surface, where it changes the optical properties of the teeth, their appearance is perceived as whiter and measurably whiter. It has also been shown that this toothpaste does not have excessive abrasiveness on enamel or dentin compared to other commercial products (32).

Today, toothpaste containing charcoal is available on the market. The ability of coal powder to absorb teeth stains and unhealthy gums has enabled the intraoral use of coal throughout history. The charcoal used can be made from carbon-rich materials such as wood, nutshell, coconut shells, and bamboo. Coal powders have different degrees of abrasiveness based on their production methods (32).

Effects of charcoal toothpaste

Charcoal or activated carbon is an ingredient found in some toothpaste products (33). Laboratory studies have been conducted on the potential toxicity of charcoal. Teraoka et al. (34) observed that bamboo charcoal could inhibit more HeLa cell proliferation than fetal lung fibroblasts. In studies on bentonite clay in toothpaste containing coal, inhalation of crystalline silica, a mineral in this substance, has a carcinogen effect, but its use and safety in toothpaste have not been reported (35). Additionally, an in vitro study conducted in 2016 showed that bentonite clay induced lung adenocarcinoma cell line proliferation (36).

Since activated charcoal has the ability/capacity to absorb stains, it has attracted attention today (37). Charcoal-containing toothpaste has properties similar to conventional toothpaste in terms of functions. Indeed, the type of toothbrush, brushing technique, and the time spent are considered more important than the ingredients and consistency of the toothpaste. It is stated that activated charcoal is recommended in the product information provided with these charcoal-based toothpaste because it binds to all tooth surface deposits. In formulations

containing this binder clay, it is probably supported by bentonite clay, plaque, bacteria, and extrinsic stains claimed to adhere to the pores of the charcoal (and clay), clearing the tooth surfaces after brushing (32). The charcoal preparation for intraoral use contains various inorganic compounds, flavoring agents, and herbal substances to increase the acceptability of these preparations and to help fight bad breath (32). Because the absorption capacity of charcoal, fluoride, and other active ions, which have diverse effects on teeth, may not show the expected effects in toothpaste containing activated charcoal (38). Due to the absorbent capacity of activated charcoal, it can be used, to extract fluoride from drinking water in communities with a water source with an extremely high fluoride content (38,39). Thus, coal-based toothpaste may have a limited capacity to remineralize the mineral, despite containing fluoride. Therefore, switching from the regular use of fluoride-containing toothpaste to fluoride-free charcoal toothpaste may increase the risk of cavities. Brooks et al. (40) stated that considering the high absorbency of charcoal, any free radical bleaching agent that can chemically reduce the internal staining present in enamel and dentin cannot be found enough. Such toothpaste has the potential to clean hard-to-reach areas and absorb pigments, because of microcoal particles. In addition to these, the shape and size of the coal powders in the paste can become abrasive, increasing the roughness of the enamel (32). Another study by Pertiwi et al. (41) supports this information. However, the ideal toothpaste offers the cleanest teeth with the least abrasiveness (41).

Due to its absorption ability, toothpaste containing activated charcoal may limit the halitosis effect of sweeteners, essential oils, and other ingredients (32). Coal powders that cause gray/black discoloration may occur in patients with periodontal defects and pockets (32). Toothpaste containing activated charcoal provides gradual cleaning of the teeth owing to the absorption ability of the chromophores of the charcoal. Brooks et al. (40) stated that although the possible whitening agent is not supported by scientific evidence, 96% of the pastes containing charcoal claim that they whiten the teeth quite effectively (37).

Vaz et al. (37) showed that all whitening toothpaste (containing activated charcoal, blue covarine, hydrogen peroxide, micro-abrasive, and optimized abrasive) are effective for tooth whitening compared to a toothpaste without a whitening agent (TA) added. Although the whitening effect of all toothpaste increased with continuous use, the best whitening was provided by the paste containing micro-abrasive, followed by the toothpaste-containing hydrogen peroxide and blue covarine. Franco et al. (42) concluded that coal-based tooth powder has a certain degree of a whitening effect, but is not as effective as teeth whitening. Study results show that charcoal may not have any teeth-whitening properties. After 28 days of use, toothpaste with activated charcoal outperformed hydrogen peroxide, blue covariate, and ordinary toothpaste in another trial by Aydın et al. (43). Additionally, toothpaste containing coal has been found to delay the restoration of tooth color when used to delay the recurrence of surface staining on healthy teeth following professional cleaning (40).

Patients wishing to whiten their teeth through brushing have been advised to use one of the well-known brands of regular toothpaste developed for its bleach effect and effectively brush their teeth to remove plaque and external stains, thereby giving their teeth a whiter appearance (32). In their study to compare the color stability and gloss of ceramic stains and glazes with the use of pastes with different amounts of dentin abrasion, Sulaiman et al. (44) concluded that the long-term color stability and gloss retention of colored ceramic restorations remains a clinical concern even in traditional home care products and that charcoal-based toothpaste may be more abrasive than conventional toothpaste. In their study to examine the effectiveness of toothpaste on resin-based CAD/CAM blocks, Aydın et al. (45) reported that there was no statistically significant difference between toothpaste containing activated carbon and other whitening pastes in terms of color improvement.

In another study by Koc Vural et al. (46), in which they compared the effects of different charcoal-based whitening toothpaste on the color, surface roughness, and microhardness of human enamel, there was no difference in the color change between groups after 12 weeks of brushing, and all tested pastes did not show clinically acceptable whitening performance. In another study by Torso et al. (47) to evaluate the effect of charcoal-based toothpaste on the discoloration and surface wear of resin composites, resin composites exposed to charcoal-based toothpaste showed significantly higher discoloration and surface wear than conventional toothpaste. In the study by Dionysopoulos et al. (48) that investigated the effectiveness of charcoal-containing whitening toothpaste and mouthwash on tooth discoloration and enamel changes that may occur after brushing for 90 days, charcoal-containing toothpaste showed a higher whitening effect on teeth than normal toothpaste. However, they concluded that using charcoal-containing mouthwash with whitening toothpaste did not improve discoloration. Additionally, while the use of toothpaste during brushing affected the surface morphology of the enamel differently, whitening mouthwash did not affect these morphological changes (48). Palandi et al. (49) compared the effects of carbamide peroxide and activated charcoal powder mixed with traditional or whitening toothpaste on enamel color and surface. When mixed with traditional and whitening toothpaste, the activated charcoal powder did not increase the color change; however, low-concentration carbamide peroxide induced more color changes than charcoal powder, and charcoal powder alone increased enamel surface roughness.

The charcoal in the paste tends to stick to deposits/contamination and stains on the teeth, build up in gum pockets and change the color of the brush. Also, the tongue tends to darken and must be removed using tongue scrapers. Additionally, coal particles can accumulate along the cavosurface edges of restorations in any marginal defects and defects and complex anatomical structures such as deep cracks. In this situation, it may be harmful to the aesthetic properties of the restorations (32).

Conclusion

This new generation of oral hygiene products, launched in today's fashion, has shown deficient clinical and laboratory data to confirm safety and efficacy claims, according to research, despite its increasing use. Large-scale, redesigned, and comprehensive studies are needed to establish reliable evidence. Its effect on restorations, whitening teeth, and periodontal tissues should be supported by studies. Because of these studies, safer products can be introduced to the market by developing new formulations if necessary. Thus, more reliable kinds of toothpaste are available to patients.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.T.A, E.A.O., S.K., Design: E.T.A, E.A.O., S.K., Literature Search: E.T.A, E.A.O., S.K., Writing: E.T.A, E.A.O.

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Effects of COVID-19 on axial spondyloarthritis disease flare

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ABSTRACT

Aims: Rheumatological disease flares may occur after many infections. However, our knowledge of the post-Coronavirus disease-2019 (COVID-19) axial spondyloarthritis (SpA) flares and related factors is limited.

Methods: We retrospectively assessed the axial SpA patients who had COVID-19. Demographic and clinical data were collected from the medical records. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was applied via telephone for pre- and post-COVID-19 SpA symptoms. An increase of ≥2 points in the BASDAI score or any new extra-articular manifestations were defined as SpA flares and SpA patients were grouped as flares and no-flare. Factors predicting SpA flare were also analyzed.

Results: A total of 48 axial SpA patients were included in the study [age, mean±standard deviation (SD): 42.3±8.6 years; male: 65%]. Post-COVID-19 SpA flare was identified in 19 patients (40%), and new extra-articular manifestations were recorded in 6 patients (13%). Although the diagnosis of inflammatory bowel disease was more common in the flare group, the difference was not significant compared with that of the no-flare group. Other features of SpA and COVID-19 disease severity were similar between the flare and no-flare groups. In the flare group, the frequency of back pain (84% vs. 62%, p=0.091) and diarrhea (53% vs. 28%, p=0.080), and headache (84% vs. 52%, p=0.021) were higher than the no-flare group. No risk factor for a post-COVID-19 SpA flare could be identified.

Conclusions: Post-COVID-19 flare was common in the axial SpA, and even new extra-articular manifestations could be reported. Although some clinical manifestations of COVID-19 were more common in patients with a flare, any predictive factor could not be identified among the study variables.

Introduction

The coronavirus disease-2019 (COVID-19) outbreak has become a major global health problem since December 2019. COVID-19 has heterogeneous clinical features ranging from an asymptomatic course to multi-organ failure. It is also a major cause of morbidity and mortality in some patients. In this context, patients with chronic diseases are more susceptible to these effects. The cascade of inflammatory mediators in COVID-19 may lead to many systemic symptoms (1,2). Although direct involvement of the skeletal muscles by viral agents has not been shown, approximately 15% of the cases could have arthralgia and myalgia at several sites (3).

Spondyloarthritis (SpA) refers to a group of chronic inflammatory arthritis, which can be further classified according to the distribution of joint involvement as predominantly axial (SpA) or peripheral SpA. Axial forms of SpA, which consist of ankylosing spondylitis and non-radiographic axial SpA are the most frequent types and usually present with chronic lower back pain, peripheral arthritis, enthesitis, dactylitis, or in association with extra-articular manifestations include psoriasis, uveitis, and inflammatory bowel disease (4,5). Non-steroidal anti-inflammatory drugs (NSAID) are frequently used in axial SpA patients along with other immunosuppressive and biological agents (6). Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), often used in daily practice, is a useful clinical scale

for both disease activation and flare determination of SpA patients (5,6).

Viral infections may cause arthritis, but the spectrum of these musculoskeletal symptoms is wide, ranging from arthralgia to peripheral chronic arthritis. Additionally, viral infections have also been linked to SpA-like disease (7). Furthermore, chronic inflammatory arthritis can be triggered by the Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection, thus indicating that viral antigens can trigger systemic autoimmunity (8,9). Although there are some studies investigating the course of COVID-19 in various rheumatologic disorders, knowledge about the disease activation is scarce. Among the reasons leading to disease activity in these studies are disruptions in health services and drug use caused by the pandemic, and patients' delayed medical treatments due to fear of COVID-19 (10-12). So little is known about the post-COVID-19 disease activity of axial SpA patients. In this study, we evaluated the disease activity in patients with axial SpA following a COVID-19 diagnosis.

Methods

We conducted this retrospective, single-center, cross-sectional study at the Ankara City Hospital Rheumatology Clinic. Between 20 January 2021 and 10 January 2022, patients with a record of SARS-CoV-2 polymerized chain reaction (PCR) test results on nasopharyngeal swabs between 11 March 2021 and 01 January 2022 were screened using the Public Health Management System. All cases with a PCR test were registered in the database during the pandemic in the country. Among the SpA patients who were followed up, those who had a COVID-19 history were included in the study. Patients with a change for treating SpA in the last 6 months, coexisting rheumatic diseases, incomplete clinical data, patients older than 18 years, and pregnant were excluded from the study. Ethical approval for this study was obtained from the Ankara City Hospital Ethics Committee (approval number: E1-21-2154, date: 15.12.2021). The study protocol conforms to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The Turkish Ministry of Health, General Directorate of Health Services, approved the study protocol (2021-11-26T16_36_49).

A total of 361 axial spondyloarthropathy patients with COVID-19 were initially identified. The number of excluded subjects was 224 due to the following reasons; changes in the SpA-specific therapy in the last 6 months (n=130), coexisting rheumatic diseases (n=45), incomplete data (n=37), age than 18 years (n=6) and pregnancy (n=6).

Upon verbal consent, patients were interviewed via telephone for the post-COVID-19 symptoms, pre- and post-COVID-19 SpA treatment, and SpA symptoms. The demographics, disease characteristics, comorbidities, and medical therapies were confirmed through electronic medical records. Multimorbidity was defined as the presence of at least 2 or more comorbidities (13). The BASDAI was used in the clinical evaluation of SpA patients, which consists of 6 questions (14). The patient responds to the questions by considering events during the past week and scores the questions between 0 and 10, with 0 corresponding to "absent" and 10 corresponding to "very severe" (14). The BASDAI score is calculated by summing the average of the scores obtained from the fifth and sixth questions and the scores obtained from the first four questions and dividing the latter score by five. The validity and reliability of the Turkish version of the scale were previously reported (15). BASDAI was fulfilled for both pre-COVID and post-COVID SpA symptoms considering the situation two weeks before and after the infection. ≥2 points increase in the overall BASDAI score after COVID-19 was considered a disease flare (16). Additionally, the development of extra-articular manifestations was defined as a post-COVID flare. Axial SpA patients with and without post-COVID SpA flares were grouped as the "Flare group" and "No-flare group". All data were collected using a standardized case-report form by the same physician (BA).

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY). Descriptive data are presented as means with standard deviation (SD) and median with interquartile range (IQR). The normality of the distribution was tested by visual (histogram and probability graphs) and analytical (Kolmogorov-Smirnov and Shapiro-Wilk) tests. The chi-square test and Fisher's Exact test were used to compare the categorical variables between the groups. Where appropriate, Student's t-test and Mann-Whitney U test were used to compare the scale variables. Multivariable logistic regression analysis was performed to identify the factors independently associated with a post-COVID SpA flare. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. All tests of significance were two-sided and for statistical significance, a total type-1 error level of 5% was used.

Results

A total of 48 axial SpA patients were included in the study (age, mean±SD: 42.3±8.6 years, male: 65%). Demographic characteristics, comorbidities, and medical treatments for axial SpA are shown in Table 1. The percentage of patients with only non-steroidal anti-inflammatory drug treatment was 27% (n=13). Immunosuppressive treatment [sulfasalazine 29% (n=14), biological agents 38% (n=18), and sulfasalazine + biological agent 6% (n=3)] was identified in 73% (n=35) of the subjects.

During the post-COVID period, 29% (n=14) of the patients interrupted and/or discontinued their treatment, and 46% (n=22) had to increase their daily NSAID dosage. The distribution of the treatment agents in patients who interrupted and/or discontinued

their treatment was as follows: 13% (n=8) NSAID alone, 6% (n=3) sulfasalazine, 6% (n=3) biological agent, and 4% (n=2) sulfasalazine + biological agent. COVID-19 pneumonia was recorded by 10% (n=5), while 8% (n=8) of the sample was hospitalized and 6% (n=3) required oxygen support (Table 1). No patient was transferred to intensive care unit or died. The median±SD length of hospital stay was 6.0±0.7 days.

Nineteen (40%) of the patients had a flare of SpA symptoms in the post-COVID period. The pre-COVID median (IQR) BASDAI score was 4.7 (7.5), while the post-COVID score was 6.2 (p<0.001) (8). The comparison of the pre-COVID and post-COVID median scores of each question in the BASDAI between no-flare and flare groups is shown in Figure 1. There was no difference in terms of age, gender, disease duration, extra-articular involvement, peripheral arthritis, comorbidities, smoking, and SpA or COVID-19-specific medical treatment between patients with and without disease flare (Table 1). COVID-19 severity was also similar between the groups. When COVID-19 symptoms were compared, although back pain (84% vs 62%, p=0.091) and diarrhea (53% vs. 28%, p=0.080) were more common in the flare group, only headache was statistically higher in the flare group than the no-flare group. All the other COVID-19 symptoms between no-flare and flare groups were similar (Figure 2). In the post-COVID period, new extra-articular manifestations were observed in a total of 6 (13%) SpA patients including uveitis in 3, inflammatory bowel disease in

Table 1. Demographic and clinical characteristics, and medical treatment history of spondyloarthritis patients with and without disease flare

	Overall, n=48	No-flare group, n=29	Flare group, n=19	p
Age, years, mean±SD	42.3±8.6	41.2±9.5	43.8±6.7	0.297
Male, n (%)	31 (65)	21 (72)	10 (53)	0.161
Disease duration, years, median (IQR)	10.3 (9.0)	10.2 (9.7)	9.8 (8.5)	0.690
Smoking, n (%)	15 (31)	11 (38)	4 (21)	0.341
Psoriasis, n (%)	2 (4)	1 (3)	1 (5)	1
Uveitis, n (%)	9 (19)	6 (21)	3 (16)	0.726
Inflammatory bowel disease, n (%)	5 (10)	1 (3)	4 (21)	0.072
Peripheral arthritis, n (%)	17 (35)	10 (35)	7 (37)	0.854
Comorbidity, n (%)	29 (60)	16 (55)	13 (68)	0.359
Multimorbidity, n (%)	17 (35)	12 (41)	5 (26)	0.286
Hypertension, n (%)	7 (15)	4 (14)	3 (16)	0.580
Diabetes mellitus, n (%)	6 (13)	4 (14)	2 (11)	1
Obesity, n (%)	13 (27)	9 (31)	3 (21)	0.522
Hyperlipidemia, n (%)	3 (6)	2 (7)	1 (5)	0.657
Coronary artery disease, n (%)	5 (10)	4 (14)	1 (5)	0.635
Chronic obstructive pulmonary disease, n (%)	6 (13)	6 (21)	0	0.068
Spondyloarthritis specific treatments				
Biological agent, n (%)	21 (44)	12 (41)	9 (47)	0.683
NSAIDs, n (%)	23 (48)	15 (52)	8 (42)	0.514
Sulfasalazine, n (%)	15 (31)	9 (31)	6 (32)	0.968
Corticosteroid, n (%)	3 (6)	2 (7)	1 (5)	0.208
COVID-19 specific treatments				
Hydroxychloroquine, n (%)	11 (23)	7 (24)	4 (21)	0.708
Favipiravir, n (%)	36 (75)	21 (72)	15 (79)	0.609
COVID-19 severity				
Pneumonia, n (%)	5 (10)	3 (10)	2 (11)	0.376
Hospitalization, n (%)	4 (8)	1 (3)	3 (17)	0.343
Oxygen support, n (%)	3 (6)	1 (3)	2 (11)	0.254
Patients post-COVID features				
Post-COVID treatment continuation, n (%)	34 (71)	20 (69)	14 (74)	0.797
Post-COVID increased NSAID need, n (%)	22 (46)	9 (31)	13 (68)	**0.011**

SD: Standard deviation, IQR: Interquartile range, NSAID: Non-steroidal anti-inflammatory drugs, COVID-19: Coronavirus disease-2019

2, and psoriasis in 1. Although post-COVID AxSpA treatment continuation was similar between the two groups, post-COVID NSAID need was higher in the flare group than in the no-flare group (p=0.011).

In logistic regression analysis, male gender, presence of multimorbidity and history of inflammatory bowel disease, and COVID-19 symptoms such as fever, joint pain, back pain, and diarrhea were not independently associated with axial SpA flare (Table 2).

Discussion

In this study, 40% of patients with axial SpA had SpA flare in the post-COVID period, and 13% of the patients had newly emerged extra-articular manifestations. Although inflammatory bowel disease was more common in the flare group, the difference did not reach statistical significance. Other SpA features and COVID-19 disease severity were similar between the flame and no-flare groups. In comparison with the no-flare group, the frequency of the back headache, pain, and diarrhea was higher in the flare group; but, only the headache variable showed a statistically significant difference. Logistic regression analysis did not show any independent predictor of a post-COVID SpA flare.

In a study, which evaluated treatment adherence of 304 SpA patients during the COVID-19 pandemic, disease activity was observed in about 40% of patients (11). Except for the non-adherence to treatment, it was linked to the psychological effects of the COVID-19 pandemic and the disruption of daily physical activity (11). In another study that evaluated 287 SpA patients, it was observed that there was no significant increase in disease activity during the COVID-19 pandemic (10). The above-mentioned studies used a different methodology from our study, but viral agents may trigger SpA group diseases or cause flares (17,18). To the best of our knowledge, there is only one study in the literature that has evaluated SpA disease activity after COVID-19 (7). This study included 18 patients with psoriatic arthritis (PsA) and COVID-19 was associated with the PsA flares (7). Sporadic reports of cases with post-COVID SpA have commonly concluded that axial SpA clinical findings may be exacerbated, mostly in the form of reactive arthritis (8,19-21). Our study confirms the knowledge in the literature, as 40%

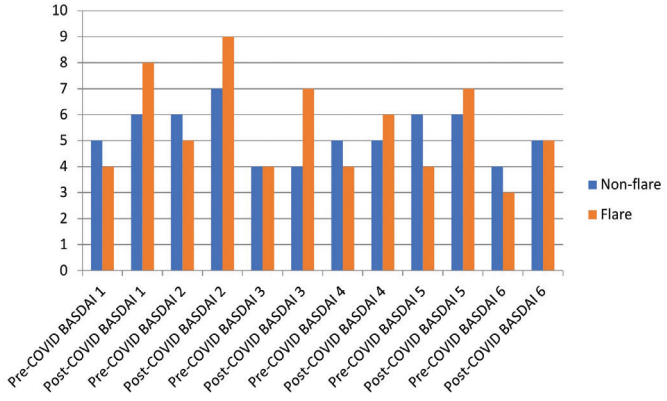

Figure 1. The comparison of the pre-COVID-19 and post-COVID-19 median scores (minimum-maximum: 0-10) of each BASDAI question between non-flare and flare groups

COVID-19: Coronavirus disease-2019, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

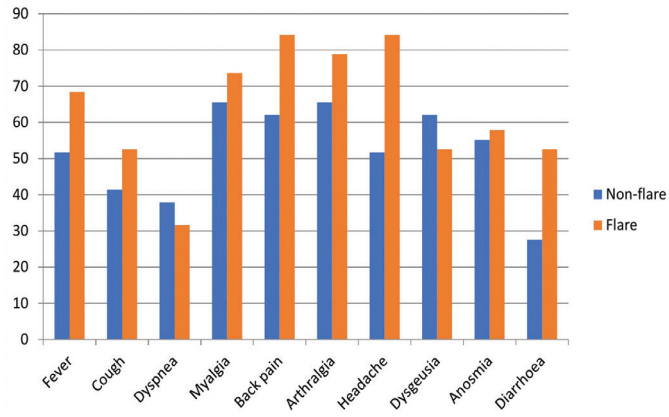

Figure 2. The comparison of all COVID-19 symptoms percentages between "non-flare" and "flare groups"

COVID-19: Coronavirus disease-2019

Table 2. Results of logistic regression analysis (outcome variable: spondyloarthritis flare)

	Univariate analysis		
Variables	**OR**	**95% CI**	**p**
Male gender	0.306	0.073-1.274	0.104
Multimorbidity	0.400	0.089-1.787	0.230
Inflammatory bowel disease	5.531	0.480-63.774	0.170
Fever	1.247	0.273-5.686	0.776
Arthralgia	0.575	0.049-6.794	0.660
Back pain	2.690	0.287-25.238	0.386
Diarrhea	2.405	0.486-11.912	0.282

OR: Odds ratio, CI: Confidence interval

of axial SpA patients had SpA flare after COVID-19. Moreover, age, extra-articular findings, presence of peripheral arthritis, and the medical treatment options used did not differ between the patients with and without a flare history.

COVID-19 may trigger several autoimmune pathways. Beyond the disease flares, patients with autoimmune rheumatic disease may also develop novel autoimmune characteristics such as psoriasis, vasculitis, and autoimmune colitis (22-24). Consistent with this, we found in our study that 13% of our patients had newly-emerging extra-articular SpA symptoms in the post-COVID period. Thus, it can be concluded that COVID-19 may cause new extra-articular manifestations in patients with axial SpA other than a classical disease flare.

Predisposing factors in the development of any joint involvement due to COVID-19 are still unknown. Prolonged exposure to the virus or more severe disease may lead to the spread of the virus to the respiratory and gastrointestinal tract, triggering autoimmunity (25). In the current sample of patients, headache, back pain, and diarrhea were more frequent in the SpA flare group. Apart from inflammatory load due to both axial SpA and COVID-19, upper respiratory tract obstruction due to COVID-19 and psychological factors could play a major role in the development of headache. Nevertheless, the mechanism of increased headache complaints in SpA patients with flare needs to be further studied.

Many reasons, such as treatment non-compliance, viral infections, physiological factors, and psychological stress, may cause flares in SpA patients (7,17,18). In logistic regression analysis, some conditions that may be associated with a post-COVID SpA flare were evaluated, but we could not find any predictor factor. The small number of patients in our study may be a reason why we could not find conditions related to disease flare in previous studies. Therefore, studies involving a larger number of patients could help us obtain clearer results in this regard.

Study Limitations

There are limitations to our study. Firstly, this was a retrospective study and the number of evaluable patients was low due to the inclusion criteria of only the patients with stable medical treatment in the last 6 months. We did not have stress, depression, or anxiety measures in this study, suggesting the presence of unmeasured confounding. Although the BASDAI score is useful for identifying the disease activations or flare, it is a patient-reported measure of disease activity and is mostly subjective. Another limitation is that NSAID use could not be defined and categorized objectively. The BASDAI questionnaire was completed via phone calls, which may have caused over or underestimation of the scores since physical examination data and the level of acute phase reactants were lacking. Lastly, some COVID-19 symptoms could be mixed with some questions in BASDAI. In order to better distinguish post-COVID SpA flare from the COVID-19 clinical findings, we carried out a BASDAI questionnaire considering 2 weeks previously and later. In the original BASDAI questionnaire, each question evaluates the previous week, so this condition precludes the generalizability of our results as exact SpA disease flares.

Conclusion

In conclusion, post-COVID flare is common in the axial SpA, and even new extra-articular manifestations may be observed. COVID-19 may induce disease flares in axial SpA patients, regardless of the ongoing rheumatological treatment. Diarrhea, back pain, and headache during COVID-19 may be the symptoms suggestive of a post-COVID flare in axial SpA patients.

Ethics

Ethics Committee Approval: This study was approved by the Ankara City Hospital Ethics Committee (protocol number: E1-21-2154, date: 15.12.2021). The Turkish Ministry of Health, General Directorate of Health Services, approved the study protocol (2021-11-26T16_36_49).

Informed Consent: Retrospective study.

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Authorship statement

Concept: B.A., O.K., A.E., Design: B.A., S.C.G., İ.D., Data Collection, or Processing: B.A., E.A., B.Ö., Ö.K., Analysis, or Interpretation: B.A., E.K.E., Literature Search: B.A., E.A., B.Ö., Ö.K., E.K.E., S.C.G., İ.D., O.K., Writing: B.A., A.E.

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Effects of surface treatment methods on shear bond strength of ceramic to cast, milled and laser-sintered titanium frameworks

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ABSTRACT

Aims: This study determined the effect of three different surface treatment methods [sandblasting (SB), tribochemical silica coating (TSC), and ytterbium fiber laser (YFL) roughening] on surface roughness and titanium-ceramic shear bond strength using specimens obtained using casting (CST), milling (ML) and selective laser melting (SLM).

Methods: In this *in vitro* study, we obtained 32 cylindrical titanium specimens for each fabrication method and subjected them to each surface treatment method. Nine experiments (n=11) were conducted. One specimen was randomly selected from each group for scanning electron microscope analysis. Surface roughness was examined using a profilometer device (n=10). Ceramic was applied to titanium specimens. A universal testing machine was used to determine shear bond strength in megapascal (MPa).

Results: Surface roughness of CST/YFL (1.254±0.058 µm), SLM/SB (1.294±0.054 µm), and SLM/YFL (1.208±0.057 µm) groups were significantly higher than other groups (CST/SB, CST/TSC, ML/SB, ML/TSC, ML/YFL, and SLM/TSC, $p<0.01$). Shear bond strengths of CST/YFL (20.28±6.97 MPa), SLM/SB (21.9±8.06 MPa), and SLM/YFL (29.92±5.67 MPa) were significantly lower than other groups ($p<0.01$). Shear bond strength of the ML/SB group (42.40±7.52 MPa) was highest but there were no significant differences between ML/SB and CST/SB (33.04±7.62, $p=0.101$), CST/TSC (35.38±4.15, $p=0.426$), ML/TSC (40.03±6.42, $p=0.998$), ML/YFL (39.43±9.24, $p=0.991$) and SLM/TSC (37.05±7.84, $p=0.766$).

Conclusions: This study showed that the production and surface treatment method impact shear bond strength. Excessive roughness affects the bonding strength. The highest shear bond strength was identified in the ML group.

Introduction

Metal-ceramic restorations (MCRs) have been used to combine the high resistance of metal with the high aesthetic features of ceramic since 1960s (1). Many types of metals and metal alloys have been tried in the fabrication of MCR frameworks in the last five decades. Due to costs, base metal alloys have become the most frequent alternatives (2). However, base metal alloys, especially nickel-chrome in MCRs, may have some biological effects (2-4). Commercially, pure titanium and titanium alloys (Ti) are alternative MCR frameworks because of their excellent biocompatibility with lower density and thermal conductivity, good corrosion, and fatigue resistance (5).

Ti has a strong tendency to oxidize. When Ti contacts with oxygen, a thick titanium oxide (TiO or TiO_2) layer, known as α-case, is formed on its surface. The surface TiO_2 layer shields the alloy to corrode (6). However, some disadvantages of Ti casting (CST) such as a strong tendency to oxidize and increased chemical reactivity at high temperatures, limit its use

(7,8). Porosity and incomplete CST, especially in the margins of the restoration, can also occur (7). Therefore, using an arc melting pressure and a high-speed centrifugal CST machine with an argon atmosphere are recommended to improve the castability of Ti (9). Also, stable oxides like magnesia, alumina, and zirconia, refractory materials of investment, are used to control the thickness of the surface TiO_2 layer, which weakens the Ti-ceramic bond (10,11). Because of CST difficulties of Ti, milling (ML), spark erosion, laser welding, and selective laser melting (SLM) emerged as alternative techniques for the fabrication of more predictable Ti frameworks than CST (6,12-14).

ML is a subtractive method that reduces the oxide formation on the surface of Ti frameworks. However, disadvantages of this procedure, such as a significant amount of wasted material, time spent on production, limited production of complex specimens and the need for manual finishing after fabrication limit its use (15). Laser sintering is a relatively new alternative additive technique developed for the fabrication of metal frameworks. A high-power laser beam melts the alloy powder to form a thin solid layer (0.02-0.06-mm thickness) on a metal bed. The fabrication is completed by repeating the path layer by layer (16). This procedure enables a high degree of accuracy of the framework's manual finishing or wasted material (17).

However, the production of mechanically and chemically consistent frameworks does not guarantee the clinical success of MCRs. The clinical success of MCR is related to the bond strength between the framework and the ceramic (17). The thickness of the TiO_2 layer on the Ti surface and the mismatch of coefficients of thermal expansion Ti and ceramics reduce the bond strength (18). To minimize the mismatch of coefficients of thermal expansion, veneering Ti substructures with low-fusing ceramics were recommended (15).

To improve the bonding strength between Ti-low fused ceramic, surface treatment techniques such as airborne particle abrasion, acid etching, pre-oxidation, tribochemical silica coating (TSC), and laser etching (LE) applied on the adherent surface is recommended for clinical success (19,20). Sandblasting (SB) is the most common ST. During the procedure, the size of Al_2O_3 particles and applied pressure affect Ti-ceramic bond strength. Al_2O_3 particle size from 110 µm to 250 µm can be used to achieve an adequate bond strength between Ti and ceramic (21). However, surface contamination with Al_2O_3 can reduce Ti-ceramic bond strength (22,23).

LE is an acceptable alternative without the risk of Al_2O_3 contamination. It easily modifies the surface properties of materials and increases the metal-ceramic bond strength (24). It was reported that LE with Nd: Yg improved Ti-low fused ceramic bonds as strongly as SB (25,26) and was better than acid etching (25). Another method used to improve Ti-ceramic bond strength is TSC. The scientific basis of TSC is that SB with silica-coated alumina powder forms a silicate layer on an adherent surface (27). It was reported that TSC improved Ti-ceramic bond strength (28,29).

Although several studies have evaluated Ti-ceramic bond strength, to the best of our knowledge, limited studies have evaluated the effect of the production and surface treatment methods on Ti-ceramic bond strength. Therefore, this study aimed to examine the effects of different production methods such as CST, ML, and SLM and surface treatment methods such as SB, TSC, and ytterbium fiber laser (YFL) etching methods on Ti-low-fused ceramic bond strength. The null hypothesis was that various production methods and surface treatments do not affect the surface roughness of Ti specimens and the Ti-ceramic bond strength.

Methods

Specimen preparation

A total of 99 Ti specimens, cylindrical in shape (10 mm in diameter and 15 mm in height) were prepared: CST (n=33), ML (n=33) and SLM (n=33).

CST specimens were manufactured using the lost-wax technique. A cylindrical metal mold of 10 mm diameter and 15 mm height was prepared. Inlay wax (774 Inlay wax, Dental Direct, Spenge, Germany) was melted and poured into the mold. Then, the wax specimen was positioned in the silicone CST ring. Phosphate-bonded investment (Rematitan Plus, Dentaurum, Germany) was vacuum mixed according to the manufacturer's recommendations (liquid/powder ration: 40 mL/250 gr) and poured into a silicone CST ring. The ring was placed in a pre-heating furnace and wax was eliminated (900 °C for 50 min.). Grade 1 commercially pure titanium (Tritan, Dentaurum, Germany) (Lot no: 161) was melted (1668 °C for 40 sec.) and CST was prepared using a CST device (Rematitan, Dentaurum, Germany) according to the manufacturer's instructions. After the removal of the investment, CST specimens were trimmed with a carbide bur. The α-case layer was removed using a universal grinding machine (FV-315-V/2, Tak-San, Turkey) in all CST specimens.

For the production of Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) specimens, 10 mm in diameter and 15 mm in height, were designed with CAD software (RapidForm XOR3; 3D Systems Inc). The stereolithography file was transferred to the 5 Axis Milling Machine (Deckel Maho HSC 20 Linear, Pfronten, Germany) for the ML groups and an SLM unit (M2, Concept Laser; Hoffmann Innovation Group) for the SLM groups. ML specimens were prepared using grade 5 Ti blank (CopraTi-5, Whitepeaks Dental Solutions GmbH & Co. KG, Wessel, Germany) (98.3 mm in diameter and 15 mm in height, lot no: 0483). A 200 W fiber laser beam melted and fused-grade 5 Ti powder (CL 41 Ti Eli, Concept Laser GmbH, Lichtenfels, Germany) (Lot no: UK1058) into 40 µ layers until the

completion of the production. According to the manufacturer's instructions, the SLM specimens were transferred to a sintering furnace (Protherm Furnaces ACF, Ostim, Ankara, Turkey). The furnace temperature was adjusted to 850 °C for four hours. The specimens were exposed to this temperature for two more hours.

Ti specimens were subjected to SB, TSC, and YFL roughening. A total of 9 experimental groups (n=11) were assigned according to production and ST (groups CST/SB, CST/TSC, CST/YFL, ML/SB, ML/TSC, ML/YFL, SLM/SB, SLM/TSC, and SLM/YFL). Airborne particle abrasion with 250 µm aluminum oxide (Al_2O_3) particles (Korox® BEGO, Bremer Goldschlägerei Wilh. Herbst GmbH & Co. Bremen, Germany) was applied to bonding surfaces of CST/SB, ML/SB, and SLM/SB for 10 seconds at 0.2 MPa pressure and from a distance of 10 mm from the surface using an airborne particle abrasion device (Meliodent, Heraeus Kulzer GmbH, Hanau, Germany). For TSC, the bond surfaces of CST/TSC, ML/TSC, and SLM/TSC specimens were cleaned and activated by blasting 110 µ pure aluminum sand (Rocatec Pre, 3M ESPE, St. Paul, MN, USA) with a sanding device (Junior Blasting Module 3M ESPE, St. Paul, MN, USA) at 0.2 MPa pressure from a distance of 10 mm for 10 secs. Then, the TSC was applied to the bonding surfaces of the specimens by sanding with 30 µm silica-coated Al_2O_3 (Rocatec soft, 3M ESPE, St. Paul, MN, USA). TSC procedure was performed according to manufacturer instructions. CST/YFL, ML/YFL, and SLM/YFL specimens were treated using a YFL (SCANLAB, Puchheim, Germany) (7 W, 140 mJ with 50-Hz frequency with 300 µsecs pulse duration) (YFL). The bonding surfaces of the specimens were irradiated for 10 seconds by the linear movement of a glass fiber of the YFL laser, positioned 17 cm away from the bonding surface. After applying surface treatments, all the specimens were ultrasonically cleaned in an ultrasonic bath (Mercury Ultrasonic Cleaner, Sozer Machine Co, Turkey) with distilled water for 10 mins.

Determination of bonding surface property

One specimen for each group was randomly selected and the microstructural analysis was performed using a scanning electron microscope (SEM) (Carl Zeiss SMT, EVO® 40 Series, Oberkochen, Germany), an area of 10 µm^2 was examined at x1000 magnification. The surface roughness values (Ra) for all specimens (n=10) were measured using a profilometer device (time TR 100, Surface Roughness Tester, PHYNIX GmbH & Co. KG, Germany). Roughness measurements (0.25-mm cut-off length, 0.05-10.0 µm measuring range, and 6 mm tracing length) on two perpendicular measuring lines of radius length on the treated surface of each specimen were made. A mean of measurement was calculated and the Ra for each specimen was obtained.

Veneering procedure

A silicone mold was prepared at 10 mm in diameter and 15 mm in height with openings of 4 mm in height and 6mm in diameter to standardize the size of the ceramic with the manual layering technique. Low-fusing ceramic (Vita Titankeramik, Vita Zahnfabrick, Bad Säckingen, Germany) was applied to the Ti specimens with the use of a silicone mold. Binder, opaque, dentin, and glaze layers were fired using a ceramic furnace (Programat P 300 Ivoclar Vivadent AG Schaan, Liechtenstein) according to the manufacturer's instructions (Table 1). After firing procedures, a micro-measuring device (Alpha-Tools Digital Caliper, CA, USA) with a minimum reading of ±0.01 was used to measure the exact size of bonded ceramic before the shear bond strength (SBS) test.

SBS test

An SBS test was performed using a universal testing machine (Instron 1195, Instron Corp, Canton, MA) at a crosshead speed of 1 mm/min. The loading jig was positioned 1mm away from the ceramic-metal joint line at an angle of 10 degrees. SBS at failure was measured at Newton. The measured values were divided by the bonding surface area of the specimens to calculate the SBS in megapascal (MPa).

Fracture modes were classified as cohesive failure within the veneering porcelain, adhesive failure between titanium and porcelain, or a combination of both (13) and were identified by a trinocular invert metal microscope (SOIF XJP-6A Boeco, Hamburg, Germany) at x40 magnification.

Table 1. Firing schedules of the veneering porcelain according to manufacturers' instructions

		Start temperature (°C)	Rate of temperature rise (°C/min)	End of temperature (°C)	Vacuum
Titankeramic	Bonder	600	65	795	+
	1st opaque	600	65	795	+
	2nd opaque	600	65	785	+
	1st dentin	600	55	755	+
	2nd dentin	600	55	755	+
	Glaze	600	55	755	-

min: Minimum

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 22.0 software package (IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was used to analyze the effects of production methods and surface treatment methods on surface roughness and SBS. A one-way ANOVA was used to analyze the effects of surface treatment methods on surface roughness and SBS of production method groups. Comparisons among the roughness and SBS values of production method groups were made using the post-hoc Tukey's honestly significant difference test. A p-value less than 0.05 was considered statistically significant.

Results

The SEM images of the specimens after applying surface treatments are presented in Figure 1. In SB specimens, heterogeneous wide craters were imaged on bonding surfaces in parallel with the size of the Al_2O_3 particles (Figure 1A-1C). Surface contamination with Al_2O_3 particles was seen in the SB groups. On TSC surfaces, homogeneous, narrower, and shallow craters were imaged with partially silicated micropits (Figure 1D-1F). In the YFL groups, molten Ti marks compatible and smooth surfaces extending linearly between them were observed (Figure 1G-1I).

Roughness results of the two-way ANOVA determined that there were significant differences between production methods and surface treatment techniques ($F_{(2,81)}$=196.538, p<0.01). The mean Ra and standard deviations are summarized in Table 2. For the CST groups, there was no significant difference between CST/SB and CST/YFL groups (p=0.176) that was significantly higher than the CST/TSC group (p<0.01). For the ML groups, there were significant differences between all groups (p<0.01). For SLM groups, there was no significant difference between SLM/SB and SLM/YFL groups (p=0.062) which was significantly higher than the SLM/TSC group (p<0.01).

SBS results of the two-way ANOVA determined that there were significant differences between production methods and surface treatment techniques ($F_{(2,81)}$=9.594, p<0.01). The mean SBS values and standard deviations are summarized in Table 3. For the CST groups, no difference was found between the mean SBS of CST/SB and CST/TSC (p=0.697) which were significantly higher than the mean SBS of CST/YFL (p<0.01). For the SLM groups, the mean SBS of SLM/SB and SLM/YFL was significantly lower than SLM/TSC (p<0.01). No difference

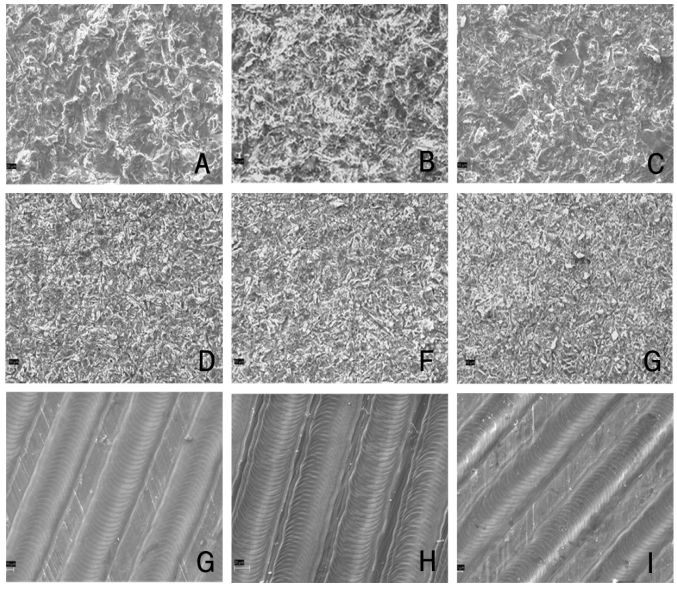

Figure 1. SEM images of titanium surfaces at x1000 magnification: CST/SB group (a), CST/TSC group (b), CST/YFL group (c), ML/SB group (d), ML/TSC group (e), ML/YFL etching group (f), SLM/SB group (g), SLM/TSC group (h), SLM/YFL etching group (i)

SB: Sandblasting, CST: Casting, ML: Milling, SLM: Selective laser melting, YFL: Ytterbium fiber laser, TSC: Tribochemical silica coating

Table 2. Results of surface roughness values (Ra) of test groups

	Sandblasting	Tribochemical silica coating	Laser etching	F	p
Casting, µm, mean (SD)	1.069 (0.05)	0.628 (0.058)	1.254 (0.058)	336.24	**<0.01***
Miling, µm, mean (SD)	1.149 (0.058)	0.687 (0.056)	0.916 (0.05)	191.40	**<0.01***
Selective laser melting, µm, mean (SD)	1.294 (0.054)	0.917 (0.05)	1.208 (0.057)	135.144	**<0.01***

*Indicates significant changes for intragroup comparison (one-way ANOVA).
µm: Micrometer, SD: Standard deviation

Table 3. Shear bond strengths of test groups

	Sandblasting	Tribochemical silica coating	Laser etching	F	p
Casting, MPa, mean (SD)	33.04 (7.62)	35.38 (4.15)	20.28 (6.97)	15.997	**<0.01***
Miling, MPa, mean (SD)	42.40 (7.52)	40.03 (6.42)	39.43 (9.24)	0.404	0.671
Selective laser melting, MPa, mean (SD)	21.90 (8.06)	37.05 (7.84)	29.92 (5.67)	11.512	**<0.01***

*Indicates significant changes for intragroup comparison (one-way ANOVA).
MPa: Megapascal, SD: Standard deviation

was found between SLM/TSC and SLM/YFL (p=0.09). However, surface treatment methods on ML specimens did not affect the mean SBS (p>0.05).

The distribution of failure modes of groups is provided in Table 4. No cohesive-type fracture was observed in any specimen. In the SLM/SB group, all failures were in the combined mode. In the CST/SB, CST/TSC, ML/YFL, and SLM/YFL groups, all failures were in the adhesive mode.

Discussion

The main purpose of the study was to evaluate the various production (CST, ML, and SLM) and surface treatments (SB, TSC, and YFL) on surface roughness and the bond strength of ceramic to the Ti specimens. Surface roughness and the bond strength results revealed that the production and surface treatment methods affected both roughness and SBS values; therefore, the null hypothesis was rejected.

Metal-ceramic bonding is a crucial factor that affects the clinical performance of MCRs. According to ISO 9693-2019 (30), a minimum acceptable value of 25 MPa calculated using a three-point bending test was defined for reliable clinical MCRs. However, the stress distribution at the interface between the ceramic and metal makes the 3-point bending test method difficult for interpreting the bond strength measurement (25). Smaller variations in the SBS test were identified rather than in the three-point bending test on measuring bond strength (23). Therefore, the SBS test was used to evaluate bond strength between Ti and low-fusing ceramic in this study.

The mismatch of thermal expansion coefficients of Ti and ceramics (18) and the strong tendency of Ti alloys to oxidize limited bonding strength between Ti and ceramics. Low-fusing ceramics with compatible thermal expansion coefficients were recommended for the veneering of Ti frameworks (18). The silicon dioxide (SiO_2) concentration on the Ti-ceramic interface also affects the Ti-ceramic bond strength (31,32). To obtain the highest concentration of SiO_2, a ceramic border was applied to the Ti-ceramic interface. The highest SiO_2 concentration was achieved with the Titankeramik border (32). In this study, low fused ceramic with a Ti border was used to eliminate the incompatibility of the thermal expansion coefficient, to prevent the oxidation of the Ti-ceramic interface during firing cycles, and to obtain the highest SiO_2 concentration on the Ti-ceramic interface.

Ti-ceramic bonding strength also depends on various factors such as the production methods of Ti specimens (7) and surface treatment procedures (19). In research (13,20,33,34) including the presented research, different SBS values were obtained in Ti specimens produced with different production methods. Mohsen (35) demonstrated that the composition differences in Ti alloys affect the bonding strength. It can be concluded that not only the production method and surface treatment procedures but also Ti composition (35) affects SBS values.

Ra demonstrated that surface treatment methods had different effects on the surface roughness of CST, ML, and SLM specimens. SLM induces rougher surfaces before surface treatment due to the “balling phenomenon”, which is a partial fusion of isolated powder particles during SLM (33). The partially melted particles loosely attached to the Ti specimens cause rougher surfaces (36). SB reduces the roughness of SLM specimens while increasing ML ones (37). The Ra of SLM specimens is also affected by material composition, powder particle size, layer thickness, laser type, and power (36). The Ra of the SLM/SB specimens differed from those of recent studies (34,37). The reason for this difference in results may be the differences in Ra measurement methods, as well as the factors that affect the Ra of SLM Ti specimens, as mentioned above.

It is generally considered that Ra is imported for mechanical locking on the metal-ceramic interface and enlarges the chemical bonding surface (19). Excessive roughness affects the bonding strength adversely by reducing the wetting of ceramics (37,38). In the SLM/SB group, the main factor for lesser bonding strength was due to excessive roughness. The surface contamination with Al_2O_3 particles can also be considered a factor for loosening bonding strength (22,23). The combined mode of fracture pattern

Table 4. Failure modes distribution

Production method of specimen	Surface treatment method	Adhesive failure	Combined failure
Casting	SB	10	-
	TSG	10	-
	YFL	9	1
Milling	SB	9	1
	TSC	9	1
	YFL	10	-
Selective laser melting	SB	-	10
	TSC	7	3
	YFL	10	-

SB: Sandblasting, TSC: Tribochemical silica coating, YFL: Ytterbium fiber laser etching

in all samples supports Al_2O_3 contamination. However, residual Al_2O_3 particles were observed on the surface in all SB groups in SEM imaging, and a dramatic decrease in SBS was observed only in the SLM/SB group. The main reason for the difference between the SBS values obtained in the SLM/SB group and the other SB groups can also be considered as due to the content of the Ti powder (35) and the production method (7).

LE with YFL (140 mJ, 50 Hz, 7 W, 300 µ secs) was also used as a surface treatment in this study. LE has become current for avoiding Al_2O_3 contamination on the Ti-ceramic interface with SB (17). It was mentioned that LE enhances the SBS between Ti and ceramics (17,26). In this study, however, the lowest SBS values were obtained in the YFL groups. The higher pulse energy of the YFL was evaluated to produce increased Ra and may cause material deterioration (25,35). The adhesive failures, that support the implication, were observed in almost all CST/YLF and SLM/YFL specimens. However, the element distribution on the Ti-ceramic interface was not examined for material deterioration in this study which could be considered a limitation. However, similar to the results of this study, Kim and Cho (25) reported that laser-etched ML specimens demonstrated no significant difference in the bond strength compared to ML/SB. So it can be concluded that the YFL procedure could be applied to milled Ti substructures to optimize the surface texture for the wetting ability of low-fused ceramics.

SB also causes different Ra and bonding strengths on commercially pure Ti. In previous studies (18,35,38), Ti surfaces were polished for standardization before SB treatment. However, in this study, to imitate clinical practice, only the α-case layer was removed and no polishing process was applied. SB decreased the roughness of the bonding interface of CST and other groups. Therefore, the obtained Ra differed from those of previous studies (35,38). The bond strength between sandblasted commercially pure Ti and low-fused ceramics with bonded (Titankeramik) was reported to be between 25.2 MPa (39) and 28.78 MPa (20). However, these values were obtained using the 3-point bending test. Iseri et al. (13) evaluated bonding strength with SBS between the same Ti alloy and ceramic materials and determined lower SBS values in the CST/SB group than in this study. The difference between these two studies is in the SB process. Although Iseri et al. (13) did not compare the Ra, it can be considered that this difference in SBS values may be due to the difference in sample roughness difference between the two studies.

Mohsen (35) and Fukuyama et al. (40) stated that TSC could significantly improve SBS between commercially pure Ti and ceramics. In this study, the mean SBS of CST/TSC was slightly higher than CST/SB. Contrary to Fukuyama et al. (40), no significant difference was observed between the mean SBS of CST/TSC and CST/SB. As in the CST group, the SBS values of ML/TSC do not statistically differ from those obtained using ML/SB. However, the highest SBS values in SLM groups were determined in the SLM/TSC group in this study. TSC seemed to be an effective method on SLM specimens to form a silicate layer like CST specimens (27). Considering the results of this study, effective SBS values can be obtained by treating SLM specimens with TSC. Although TSC is considered an effective method among the surface treatment processes applied on the Ti-ceramic interface, it was reported that a significant decrease in SBS values in TSC groups was determined in Ti specimens with artificial aging compared to SB groups (35). In this study, artificial aging was not applied.

Study Limitations

This study has several limitations. Only a single brand of ceramic was applied in this study. Therefore, the results of this study cannot be extrapolated to similar low-fused ceramics with a different chemical composition. Artificial aging, which may affect SBS values, was also applied. The relationship between the compositions of Ti alloys and ceramics requires further studies. And, clinical studies are necessary to assess the long-term performance of titanium-ceramic fixed dental prostheses.

Conclusion

Within the limitations of the study, the production and the surface treatment method affected the SBS between titanium and low-fusing ceramic. The highest SBS values could be achieved by ML. Excessive roughness affects the bonding strength adversely. The highest surface roughness was identified in the YFL etc. CST group and airborne particle abraded SLM group. In these groups, the fewest SBS values were identified.

Ethic

Ethics Committee Approval and Informed Consent: Since there's no data used referring to any living thing in this research, it is not necessary to provide an Ethics Committee Approval Form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., Concept: H.Y, C.O.S., Design: H.Y., C.O.S., Data Collection, or Processing: H.Y., Analysis, or Interpretation: H.Y., B.E., Literature Search: H.Y., B.E., Writing: H.Y., B.E., C.S.

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Fabry disease screening in kidney transplant patients: A single-center study in Türkiye

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ABSTRACT

Aims: This study aimed to screen for Fabry disease in adult kidney transplant patients at a nephrology clinic in Türkiye.

Methods: This cross-sectional, single-center study prospectively enrolled kidney transplant recipients regardless of the etiology of renal failure. α-galactosidase A (α-GLA) enzyme activity and α-GLA gene analysis were used to screen for Fabry disease. The screening was initiated by measuring enzyme activity in males, and those with <2.5 nmol/mL/hour activity underwent gene analysis. Females were screened directly by gene analysis, independent of the enzyme activity.

Results: We screened 125 patients (age: 48.9±10.1, male: 70.4%). Gene analysis was performed on a 68-year-old male patient with enzyme activity at the lower end of the reference range. No mutations associated with Fabry disease were detected. The enzyme activity test was considered false positive. A heterozygous c.937G>T (p. D313Y) mutation was detected in the gene analysis of a 29-year-old female patient. However, systemic evaluation did not reveal any clinical findings consistent with Fabry disease. Screening tests were within normal limits in other patients. Although there were abnormal screening findings in 2 patients, none was diagnosed with Fabry disease.

Conclusions: Screening studies for Fabry disease in kidney transplant patients may contribute to the determination of the true prevalence.

Introduction

Fabry disease, also called Anderson-Fabry disease, is a rare lysosomal storage disorder carried on the X chromosome. Pathogenic mutations diminish the activity of the enzyme α-galactosidase A (α-GAL-A) and lead to the accumulation of substrates, such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Endothelial deposits that progress over the years result in progressive organ destruction and failure, particularly in renal, cardiac, and cerebrovascular cells (1-3).

The prevalence of the disease is reported in the general population between 1: 40,000 and 1: 117,000 (1,4). Non-specific clinical findings and a relatively slow rate of progression make it difficult to identify the patients despite obvious clinical symptoms. Moreover, a study from Türkiye has shown that physicians were not adequately familiar with the clinical signs and symptoms of Fabry disease, which may cause delayed diagnosis (5). Thus, screening studies in high-risk groups may help identify undiagnosed patients.

The results of studies conducted on kidney transplant patients declare that Fabry disease is detected more frequently in this group than in the general population (6-11). In a few studies conducted in our society, the prevalence varies from 0.09% to 0.5% (8-10). Therefore, kidney transplant patients can

be considered a risky population. Screening these patients may enable the diagnosis of formerly undiagnosed cases and perhaps some patients among their family members. In all identified cases, with or without a kidney transplant, specific treatments such as enzyme replacement and chaperone treatments that can offset some multisystemic effects of the disease may come to the fore (12,13).

This study aimed to screen for Fabry disease in adult kidney transplant patients at a nephrology clinic in Türkiye.

Methods

Study design

This was a cross-sectional, prospective, single-center screening study designed to screen for Fabry disease in kidney transplant patients at the University of Health Sciences Türkiye, Diskapi Yildirim Beyazit Training and Research Hospital, Nephrology Clinic, Ankara, Türkiye. The Local Ethics Committee (protocol no: 64/10, date: 28.05.2019) approved the study protocol. All participants provided written informed consent. The study protocol followed the principles of the revised version of the Declaration of Helsinki.

All kidney transplant patients aged 18 years or older under follow-up with functional grafts in our institution were deemed eligible for the study. A male patient diagnosed with Fabry disease before the initiation of the study was excluded. Patients who agreed to participate were included in the study independent of signs, symptoms, or family history, even if a primary kidney disease had already been recorded. There was no intervention in the routine treatment and follow-up of the patients. Demographic data included age, gender, etiology of kidney disease, and the date of transplantation.

Screening protocol

α-GAL-A enzyme activity and α-GLA gene analysis were used to screen for Fabry disease. The screening was started by measuring enzyme activity in men, and gene analysis was planned for those with <2.5 nmol/mL/hour. Since the sensitivity and specificity of enzyme activity measurement results in females were below 50%, screening was performed with α-GLA gene analysis (4). A detailed clinical work-up that included cardiologic, neurologic, dermatologic and ophthalmologic examinations was prepared for the individuals diagnosed with Fabry disease by screening tests.

α-GAL-A enzyme activity analysis

α-GAL-A enzyme activity was measured in dry blood samples by the method described by Chamoles et al. (14). Peripheral blood samples taken during the routine examination of the patients were dropped immediately on filter paper [dry blood samples (DBS)], and the three circles on the paper were equally saturated. The paper was dried at room temperature for at least four h and stored at + 4 °C until reaching the Düzen Laboratories, Ankara, Türkiye, within five days. Later, DBS paper was processed by the fluorimetric method. 4-methylumbelliferyl-α-D-galactopyranoside (TRC, M334475) was used as the substrate, and N-acetyl-D-galactosamine (Sigma, A2795) was used as the inhibitor. 3 mm DBS punches were incubated with substrate and inhibitor at 37 °C for 17 h. Fluorescence was recorded in the fluorimeter. The results were examined by constructing a calibration curve with 4-methylumbelliferone (Sigma M1381). For α-GAL-A enzyme activity, values of 0.6 nmol/mL/hour were significant for deficiency, while values above 2.5 nmol/mL/hour were normal.

α-GAL-A gene mutation analysis

α-GAL-A gene mutation analyzes were performed on 3 cc venous blood samples collected in EDTA-containing tubes (stored at +4 ºC, and for a maximum of 5 days). All analyses were performed at the Intergen Genetic Diagnosis Center, Ankara, Türkiye, as described in the literature (15). DNA extracted from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen Inc.) was stored at -20 °C until the polymerase chain reaction (PCR) step. Using PCR primers designed with Primer© - Primer Designer v.2.0 (Scientific and Educational Software), all coding exons of the gene and their splice junctions were amplified. The PCR pool was purified using the NucleoFast® 96 PCR cleanup kit (Macherey-Nagel GmbH). The purified PCR pool was measured using a Nanodrop 1000 microvolume spectrophotometer (Thermo Inc.) and diluted before sequencing. α-GLA gene sequence analysis was performed using the MiSeq NGS (Next Generation Sequencing) platform (Illumina, San Diego, CA, USA). Data were visualized with IGV 2.3 (Broad Institute) software.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences Statistics for Windows, version 22.0 (Armonk, NY: IBM Corp., 2013). The distribution normality for continuous variables was tested using the Kolmogorov-Smirnov test and histograms. Normally distributed data were presented as mean±standard deviation. Categorical variables are shown as frequency and percentage.

Results

The characteristics of the study population are presented in Table 1. We screened 125 kidney transplant patients with a mean age of 48.9±10.1 years and male predominance (n=88, 70.4%). The time after transplantation was 10.1±6.8 years. The underlying kidney disease was unknown in (56%) 70 patients. The remaining 24 (19.2%) subjects had hypertension, 8 (6.4%) had glomerulonephritis, 6 (4.8%) had diabetes mellitus, 6 (4.8%)

had vesicourethral reflux disease, 5 (4%) had pyelonephritis, 3 (2.4%) had kidney stones, 2 (1.6%) had polycystic kidney disease, and 1 (0.8%) had Alport's disease.

The characteristics of two cases with abnormal findings are presented in Table 2. In 1 of 88 male patients, α-GAL-A enzyme activity was detected at the lower end of the reference range (Case 1). α-GLA gene analysis was performed on this patient and no mutations related to Fabry disease were detected. Enzyme measurements of the other males were within the normal range. All female patients (n=37) were screened by gene mutation analysis. A heterozygous mutation of c.937G>T (p.D313Y) was detected in one female patient (Case 2). Gene analysis was normal in the remaining 36 female patients.

Case 1

A 68-year-old male patient had undergone ABO-compatible living kidney transplant 14 years ago. The cause of kidney disease was unknown. The serum creatinine level was approximately 1.3 mg/dL under triple immunosuppressive therapy comprising prednisolone, tacrolimus, and mycophenolate mofetil. Urinalysis was within normal limits. α-GLA gene analysis was performed because the result of enzyme activity, which was the first screening test of this patient, was at the lower limit of normal. No mutations associated with Fabry disease were detected. The enzyme activity test was considered false positive.

Case 2

A 29-year-old female patient with a kidney transplantation history from her mother at another center two months ago was admitted to our clinic for follow-up. She had C.937G>T (p.D313Y) heterozygous mutation on gene analysis. However, there were no clinical finding consistent with Fabry disease for further evaluation. Genetic analysis of her mother showed no mutations. It was considered the mutant allele inherited from her father who was not alive. Of her eight siblings, three had kidney disease of unknown origin. However, they were also all abroad. Although the findings suggested an unexplained familial kidney disease, they were inconsistent with Fabry disease. She is still followed up with stable kidney function and urinalysis.

Table 1. Characteristics of the study population

	Total patients (n=125)
Gender (male, n, %)	88 (70.4)
Age at screening (year)	48.9±10.1
Primary cause of kidney disease (n, %)	
Hypertensive nephropathy	24 (19.2)
Chronic glomerulonephritis	8 (6.4)
Diabetes mellitus	6 (4.8)
Vesicourethral reflux disease	6 (4.8)
Pyelonephritis	5 (4.0)
Nephrolithiasis	3 (2.4)
Polycystic kidney disease	2 (1.6)
Alport disease	1 (0.8)
Unknown	70 (56.0)
Time after transplantation (years)	10.1±6.8

Continuous variables were expressed as mean±standard deviation

Discussion

Nephropathy is one of the most frequent complications of Fabry disease. For this reason, screening chronic kidney disease patients as a relevant risk group for Fabry disease is sound to manage the potential complications earlier. In this study, we screened Fabry disease in kidney transplant patients followed up in our institution, and identified a heterozygous variant mutation in a female patient. However, we were unable to substantiate its clinical implication.

The frequency of Fabry disease in kidney transplant patients is unclear, and screening studies in this population are limited (6-11). In two screening studies, 1 male in 673 (6) and 5 males in 1,306 kidney transplant recipients were diagnosed with Fabry disease (7). Only a few studies have been conducted in Türkiye so far, which have indicated that the prevalence of Fabry disease among kidney transplant patients is above the general population figures. Concerning kidney transplant patients, 1 (0.09%) in 1,095 at Ege University (8), 1 (0.33%) in 301 at Ankara University (9), and 1 (0.5%) in 200 at Haydarpasa Numune Hospital (10) were the carriers of the specific mutation. Although the current study sample was small, it is one of the few studies that have screened Fabry disease in kidney transplant patients in the country, and we have identified no mutation that

Table 2. Characteristics of the cases with abnormal findings

	Case 1	Case 2
Age (year)	68	29
Gender	Male	Female
Donor	Non-relative male	Mother
Primary kidney disease	Unknown	Unknown
α-GAL-A enzyme activity	2.5 nmol/mL/hour	-
α-GLA gene mutation	No mutation	c.937G>T (p.D313Y) heterozygote

α-GLA: α-galactosidase, α-GAL-A: α-galactosidase A

would definitively confirm Fabry disease in any recipient. Existing knowledge suggests that physician awareness of Fabry disease is insufficient in Türkiye, which may delay the diagnosis (5). The patient we excluded from the analysis because Fabry disease was diagnosed before the current study may be an example of a late diagnosis. The patient had Fabry disease diagnosis 6 years after the transplantation. Using a screening program, he could have been accurately diagnosed with a screening test before experiencing a cerebrovascular accident. However, with limited data and practice, it may not be feasible to screen all transplant cases for Fabry disease. However, screening for Fabry disease can be feasible, particularly in kidney recipients with an unknown etiology of kidney damage and whose family members have kidney disease.

In this study, one of the two patients with an abnormal result was a male patient whose α-GLA gene analysis was performed because α-GAL-A enzyme activity was at the lower limit of normal. No pathological mutation was detected in the gene analysis of this patient. A similar finding was previously found in a screening study on hemodialysis patients, in which no gene mutations were detected despite low enzyme activity in 29 of 526 patients (16). The authors suggested that malnutrition and chronic inflammation, common in dialysis patients, have led to false positive test results by impairing protein synthesis (16). In our study, only one of 88 patients had a false-positive result. This patient was on antibiotic therapy for a urinary tract infection when the blood sample was collected, suggesting that acute inflammation affected the test result. Performing enzyme activity analyses in stable periods can prevent unnecessary loss of time and cost.

In this study, the c.937G>T (p.D313Y) mutation was detected in the genetic analysis of a female patient. This is a variant whose pathogenicity was questioned after a second mutation was detected in further analysis of the genetic material of the patient in which it was first identified (17). It has been shown that the activity of the enzyme in patients carrying this mutation is reduced at neutral pH (7.4) but stabilized at lysosomal pH (4.6). With this finding, it was interpreted that this mutation may cause a false deficiency in plasma α-GAL-A activity (18). These data are also supported by a meta-analysis of 35 recent clinical studies. High residual enzyme activities and normal lyso-Gb3 concentrations were detected in patients with the D313Y genotype without Gb3 deposits. A striking point is the higher prevalence of this variant in patients with neurological disorders (19). However, more studies are needed to clarify its relationship with neurological findings. The prevalence of D313Y in the general population is around 0.5% (18). In this study, it was detected in only one patient and its prevalence was calculated as 0.79%. However, in this study, male patients were initially screened with enzyme activity analysis. Considering that enzyme activity may not be decreased in D313Y carriers, male patients with mutations may have been overlooked. As a result, D313Y was classified as a neutral variant of unknown significance based on the available data. There were no additional findings consistent with Fabry disease in the patient we display in this study. Although a native kidney biopsy could not be performed, the obtained data did not support the diagnosis of Fabry disease.

Study Limitations

This study has some limitations. Since it was a single-center study, the number of participants was limited. Also, the family members of patient 2 remained unexamined except for the mother.

Conclusion

In conclusion, screening studies for Fabry disease in kidney transplant patients may contribute to the determination of the true prevalence and allow diagnosis before some complications develop.

Acknowledgments

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Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, Diskapi Yildirim Beyazit Training and Research Hospital Local Ethics Committee (protocol no: 64/10, date: 28.05.2019).

Informed Consent: All participants provided written informed consent.

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Authorship Contributions

Surgical and Medical Practices: Ş.M.A., G.U.O., T.S., K.G.A., M.D.A., Concept: Ş.M.A., G.U.O., M.D.A., Design: Ş.M.A., G.U.O., Data Collection or Processing: Ş.M.A., G.U.O., K.G.A., Analysis or Interpretation: Ş.M.A., G.U.O., Literature Search: Ş.M.A., G.U.O., T.S., Writing: Ş.M.A., G.U.O.

Conflict of Interest: All authors declare that they have no conflict of interest.

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The relationship between vitamin D level and echocardiographically detected pulmonary artery stiffness in young adult patients presenting with dyspnea

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ABSTRACT

Aims: Vitamin D plays a role in controlling the function of vascular smooth muscle and endothelial cells, even in the pulmonary artery. We hypothesized that pulmonary artery elasticity were comprimised in individuals with relatively low vitamin D levels.

Methods: Adult individuals with the complaint of shortness of breath were enrolled. They were divided into 2 groups according to vitamin D levels, with a cut-off of 20 ng/mL. Pulmonary artery stiffness (PAS) was calculated using the following formula: PAS (kHz/sec) = maximal frequency shift/pulmonary acceleration time. The six-minute walk distance (6MWD) was used to assess the functional exercise capability of subjects.

Results: A total of 71 individuals (male: 31%) were enrolled. Subjects with low vitamin D levels had lower 6MWD than subjects with higher vitamin D levels (443.58±56.20 m vs. 483.20±58.43 m, p=0.007). The PAS was significantly higher in individuals with vitamin D level <20 ng/mL compared with subjects with vitamin D level > 20 ng/mL (11.65±3.76 vs. 9.46±2.53, respectively, p=0.011). Multiple regression showed that vitamin D level was inversely associated with PAS (β=-0.280, p=0.009).

Conclusions: We found that PAS was associated with lower vitamin D levels. Vitamin D deficiency might involved in the dynamics of the pulmonary artery vasculature, even in the absence of significant pulmonary artery pressure elevation.

Introduction

Vitamin D plays a crucial role in developing skeletal function and integrity by controlling calcium homeostasis and bone mineralization. The deficiency of vitamin D is a foremost public health problem worldwide. Its overall incidence is about 30-50% (1), exceeding 50% in some studies (2). Vitamin D has functions other than bone metabolism, and vitamin D deficiency is involved in autoimmune disorders, infectious diseases, inflammatory diseases, metabolic diseases, and certain types of cancer types (3,4). Additionally, recent studies have shown that vitamin D levels are associated with cardiovascular risk factors (age, obesity, diabetes mellitus, chronic kidney disease) and, thereby, may contribute to the development of cardiovascular diseases (5). Nevertheless, low vitamin D levels were associated with significant deterioration in respiratory functions, exercise capacity, and related quality of life (6).

Pulmonary hypertension (PH) is a heterogeneous disorder described by a progressive increase in pulmonary artery pressure and pulmonary vascular resistance leading to right heart failure and reduced cardiac output (7). This hemodynamic definition of PH includes mean pulmonary artery pressure (mPAP) ≥25 mm high, pulmonary capillary wedge pressure ≤15 mm high, and pulmonary vascular resistance greater than or equal to 3 Wood units. Nevertheless, the 6th World Symposium, held in Nice, France, in 2018, suggested taking mPAP >20 mmHg for the definition and treatment of PH (8). While the clinical significance

of those with PAP >20 mmHg is obvious, it is unclear how the pathophysiology and clinical course are affected in those with shortness of breath and PAP <20 mmHg.

Pulmonary arterial stiffness (PAS) is a novel echo parameter used to guess the pulmonary arterial vasculature's elastic properties. In this technique, the elasticity of the pulmonary artery is computed using a method for defining aortic stiffness (9). Although there are a restricted number of studies investigating the relationship between vitamin D levels and respiratory functions in the literature, there is no study investigating the relationship between PAS and vitamin D levels.

Since vitamin D is involved in the regulation of vascular smooth muscle and endothelial cell functions, we assumed that the elasticity of the pulmonary artery might be disturbed in adults individuals with relatively low levels of vitamin D. Hence, this study aimed to investigate the PAS changes with varying degrees of vitamin D levels in adult individuals with dyspnea but no PH (mPAP <20 mmHg at rest).

Methods

Patient selection

Adult individuals admitted with complaints of shortness of breath and who agreed to participate in this study were enrolled. The baseline demographic, clinical, and biochemical data were obtained from the hospital registry system. Individuals with abnormal hemogram and biochemical parameters (such as anemia, kidney, and liver function abnormalities), coronary artery disease, congenital heart disease, heart failure (ejection fraction below 50%), prominent valvular heart disease, previous history of any heart surgery, atrial fibrillation, diabetes mellitus, hypertension, mPAP higher than 20 mmHg, chronic thromboembolic PH, chronic obstructive pulmonary disease, chronic liver and renal failure, bone mineral disorders, systemic inflammatory disease, and medication may affect vitamin D level were excluded from the study. Also, elderly patients were excluded from this article to avoid the effect of comorbidities and possible vascular aging outcomes on shortness of breath.

We divided 71 individuals into 2 groups according to their vitamin D levels. A 20 ng/mL cut-off of plasma 1,25-dihydroxy vitamin D3 levels was decided. Group 1 consisted of individuals with a 1,25-dihydroxy vitamin D3 <20 ng/mL, while group 2 consisted of individuals with a 1,25-dihydroxy vitamin D3 ≥20 ng/mL.

Echocardiography

We peroformed transthoracic echocardiography using a Vivid S70 ultrasound system (GE Healthcare) using a 3.5 MHz transducer in the left lateral decubitus position at the end of the expiration. Echocardiographic images were saved digitally and analyzed offline by two separate blinded investigators. Echocardiography measurements were obtained according to the standard criteria. Subsequently, the next steps were made to measure PAS. First, pulse-wave Doppler sample volume was placed just 1 cm distal to the pulmonary valve annulus at a speed of 100 mm/sec from the parasternal short axis view and the Doppler flow trace of the pulmonary artery was recorded. Second, Doppler frequency shift, acceleration time (AcT), maximum flow velocity (MFV), and velocity time integral of the pulmonary artery Doppler flow trace were measured. Later, PAS was computed according to the formerly defined formula as the ratio of MFV to pulmonary AcT: PAS (kHz/sec) = MFV/AcT (9).

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and Good Clinical Practice/ International Conference on Harmonization and was approved by the Non-Interventional Research Ethics Committee of the University of Health Sciences Türkiye (protocol number: 19/280, date: 25.06.2019). Informed consent was obtained from the participants before enrolling in the study.

Six-minute walk test

The six-minute walk test (6MWT) according to the instructions of the American Thoracic Society is measured to estimate the functional exercise capacity of individuals (10). A suitable hospital corridor was selected for a safe test. Subjects were warned to walk as fast as they could, and a six-minute walking distance (6MWD) was calculated at the end of the test. Also, pulse, respiratory rate, blood pressure, and perceived fatigue on Borg's scale were measured before the test and at the end of the test. Chest pain, severe shortness of breath, dizziness, and sudden pallor was determined as test interruption criteria.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) program for Mac, version 26.0 (Chicago, IL, USA) was used for all statistical analyses and calculations. All the data are transferred to a computer. Numeric variables are presented as means (standard deviation) and categorical variables as percentage values. The distribution of data was assessed graphically and statistically by the Kolmogorov-Smirnov test. For quantitative data with normal distribution Student's t-test was used and the Mann-Whitney U test was used for data without normal distribution. The chi-square test or Fisher's Exact test was used for categorical variables. Pearson's correlation analysis was used for univariate correlation with PAS and, subsequently, a multivariate linear regression model with backward selection was performed to ascertain independent predictors of PAS. A $p < 0.05$ agreed to be statistically significant.

Results

A total of 71 individuals (males: 31%) were enrolled in this study. Group 1 consisted of 46 individuals with a 1,25-dihydroxy vitamin D3 level <20 ng/mL (13 men, 28.3%),

and group 2 consisted of 25 individuals with a 1,25-dihydroxy vitamin D3 level >20 ng/mL (9 men, 36%). There were no significant differences between the study groups concerning the demographic characteristics and laboratory findings (Table 1). During the 6MWT, the average distance achieved by subjects with 1,25-dihydroxy vitamin D3 level <20 ng/mL was lower than subjects with a 1,25-dihydroxy vitamin D3 level >20 ng/mL (443.58±56.20 m vs. 483.20±58.43 m, p=0.007). When we evaluated the individuals according to their smoking status and gender, there was no significant difference between the groups concerning 1,25-dihydroxy vitamin D3 levels and 6DWM (p>0.05 for all).

There was no significant difference between the two groups in terms of conventional echocardiography variables except PAS (Table 2). PAS was considerably increased in individuals

Table 1. Baseline characteristics

	1,25-dihydroxy vit D3 <20 ng/mL (n=46)	1,25-dihydroxy vit D3 ≥20 ng/mL (n=25)	p value
Age, years, mean±SD	28.1±7.5	31.5±8.2	0.083
Male, n (%)	13 (28.3)	9 (36)	0.501
BMI, (kg/m^2), mean±SD	24.39±4.98	24.26±3.76	0.908
Smokers, n (%)	29 (63)	12 (48)	0.384
SBP (mmHg), mean±SD	116.60±12.99	115.64±8.91	0.741
DBP (mmHg), mean±SD	73.28±8.92	75.24±7.82	0.361
Fasting glucose, (mg/dL), mean±SD	92.32±36.14	88.92±14.18	0.653
LDL-C, (mg/dL), mean±SD	106.76±25.15	109.54±30.80	0.686
HDL-C, (mg/dL), mean±SD	50.93±11.91	50.29±10.71	0.825
Total-C, (mg/dL), mean±SD	169.80±46.49	178.45±41.25	0.446
Triglyceride, (mg/dL), mean±SD	118.84±76.50	105.29±44.21	0.427
eGFR (mL/min), mean±SD	101.50±13.54	96.71±25.65	0.306
Hemoglobin (g/dL), mean±SD	13.53±1.43	13.84±1.34	0.393
White blood cell count (10^3/µL), mean±SD	6.83±1.46	6.75±1.64	0.384
Platelet count (10^3/µL), mean±SD	261.58±52.56	243.52±59.86	0.831
1,25-dihydroxy vitamin D3 (ng/mL), mean±SD	11.24±4.77	37.85±16.71	**<0.001**
Calcium (mg/dL), mean±SD	9.40±0.32	9.43±0.43	0.732
Magnesium (mg/dL), mean±SD	1.99±0.14	1.98±0.14	0.785
6MWD (m), mean±SD	443.58±56.20	483.20±58.43	**0.007**

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, eGFR: Estimated glomerular filtration rate, 6MWD: Six-minute walk distance, SD: Standard deviation, vit D3: Vitamin D3, BMI: Body mass index

Table 2. Comparison of echocardiography variables

	1,25-dihydroxy vit D3 <20 ng/mL (n=46)	1,25-dihydroxy vit D3 ≥20 ng/mL (n=25)	p value
LVIDd (mm), mean±SD	42.34±4.87	43.08±4.55	0.539
LVEF (%)	67.52±4.49	66.48±2.98	0.302
IVSd (mm), mean±SD	8.39±1.27	7.88±1.12	0.098
LA (mm), mean±SD	29.45±3.31	29.64±3.38	0.826
Aod (mm), mean±SD	24.78±3.14	25.76±3.39	0.228
Mitral E/A, mean±SD	1.51±0.39	1.50±0.38	0.890
Mitral E/E', mean±SD	5.02±1.97	4.89±2.18	0.799
Tricuspid E/A, mean±SD	1.28±0.32	1.39±0.41	0.227
Tricuspid E/E', mean±SD	4.86±1.56	4.94±1.61	0.854
TAPSE (mm), mean±SD	23.45±2.41	22.40±2.51	0.087
PAS (kHz/sec), mean±SD	11.65±3.76	9.46±2.53	**0.011**

LVIDd: Left ventricular internal diameter end diastole, LVEF: Left ventricular ejection fraction, IVSd: Interventricular septal thickness in diastole, LA: Left atrium, Aod: Aortic root diameter, TAPSE: Tricuspid annular plane systolic excursion, PAS: Pulmonary artery stiffness, SD: Standard deviation, vit D3: Vitamin D3

with 1,25-dihydroxy vitamin D3 level <20 ng/mL compared with subjects with 1,25-dihydroxy vitamin D3 level <20 ng/mL (11.65±3.76 vs. 9.46±2.53, p=0.011) (Table 2). There was a significant correlation between 6MWD, 1,25-dihydroxy vitamin D3, fasting glucose level, hemoglobin level, serum calcium level, and PAS (for 6MWD and PAS r=-0.483, p<0.001; for 1,25-dihydroxy vitamin D3 and PAS r=-0.375, p=0.001; for fasting glucose level and PAS r=0.316, p=0.007; for hemoglobin level and PAS r=-0.266, p=0.025; for serum calcium level and PAS r=-0.303, p=0.010). Also, there was a statistically moderate correlation between 1,25-dihydroxy vitamin D3 level and 6MWD (r=0.339, p=0.004) (Figure 1A-1C). In multiple regression analysis using the backward method entering the independent variables likely to affect the PAS (age, gender, body mass index, systolic blood pressure, estimated glomerular filtration rate, 1,25-dihydroxy vitamin D3, fasting glucose, serum calcium, hemoglobin, 6MWD, mitral EA ratio, tricuspid EA ratio, systolic blood pressure), 1,25-dihydroxy vitamin D3 levels were inversely associated with PAS (β=-0.280, p=0.009). Also, predictors of increased PAS were higher fasting glucose levels (β=0.242, p=0.019) and lower 6MWD (β=-0.293, p=0.010) (Table 3).

Discussion

This study revealed that the pulmonary artery's elastic properties tend to be compromised (as initial vascular alterations) in individuals with low 1,25-dihydroxy vitamin D3 levels.

Vitamin D receptors are present all throughout the human body in several tissue types (11). Since the detection of vitamin D receptors in the cardiovascular system, such as vascular endothelial cells, vascular smooth muscle cells, and cardiac myocytes, there is increasing interest that vitamin D deficiency might be an independent risk factor for cardiovascular and pulmonary disease. Vitamin D may affect the cardiovascular system in a few means. Several pathophysiological pathways have been suggested to explain the relationship between low vitamin D levels and poor cardiovascular function. Vitamin D deficiency may have adverse effects on vascular functions by the expression of inflammatory mediators, such as IL-6, or nuclear factor kβ (12). Low levels of vitamin D have also been associated with endothelial dysfunction, accelerated cellular calcium influx and vascular calcification, smooth muscle proliferation, reduced production of matrix metalloproteinase 2 and 9, increased arterial stiffness and decreased vasoreactivity, expression of LDL receptors on vessels and atherosclerosis, increased oxidative stress, cytokine secretion, thromboembolism, decrease in vascular endothelial growth factor and left ventricular hypertrophy (13-23). Consequently, although it can be said that there is a relationship between vitamin D deficiency and vascular impairment, whether this association is a cause, or a result is still controversial.

Additionally, it has been suggested that vitamin D deficiency has an unfortunate consequence on the pulmonary vascular structure and function, aggravating endothelial dysfunction with reduced NO-dependent relaxation to acetylcholine and increased contractile response to 5-HT (24,25). This situation has been explained by various studies. Downregulation of TASK-1 channels known to be expressed in the lung in pulmonary artery smooth muscle cells, pulmonary endothelial, and epithelial cells is a key event in PH pathogenesis (26,27). Also, in their animal

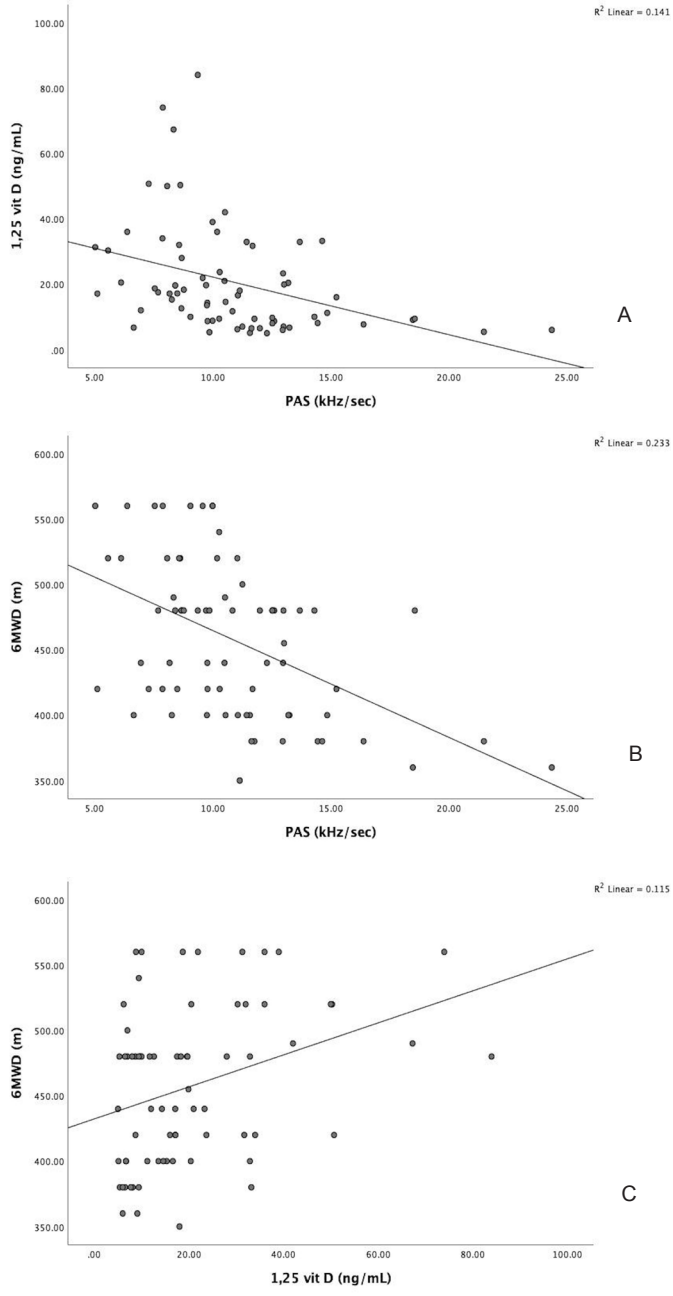

Figure 1. A) Correlations graphs between PAS and 1,25-dihydroxy vitamin D3 level. B) Between PAS and a six-minute walk distance. C) 1,25-dihydroxy vitamin D3 level and six-minute walk distance

PAS: Pulmonary artery stiffness, vit D: Vitamin D, 6MWD: Six-minute walk distance

Table 3. Univariate and multivariate regression analysis based on independent variables likely to affect the pulmonary artery stiffness

Independent variables	Univariate analysis		Multivariate analysis	
	β	p	β	p*
6MWD	-0.483	**<0.001**	-0.293	**0.010**
1,25-dihydroxy vitamin D3	-0.375	**0.001**	-0.280	**0.009**
Fasting glucose	0.316	**0.007**	0.242	**0.019**
Serum calcium	-0.303	**0.010**	-0.116	0.271
Hemoglobin	-0.266	**0.025**	-0.180	0.080

*p-value at the last step in which the independent variables remained in the model.
6MWD: Six-minute walk distance

study, Callejo et al. (24) showed that a vitamin D-free diet caused pulmonary artery muscularization, increased hyperreactivity to 5-HT, worsened endothelial function, reduced TASK-1 currents, decreased KCNK3 mRNA expression and thus a moderate but statically significant increase in mean PAP (24). Furthermore, vitamin D may also act as an endogenous inhibitor of renin biosynthesis, which has been reported as one of the important mechanisms of PH by affecting the concentration of calcium in juxtaglomerular cells (28).

Taken together, it can be considered that a low vitamin D level may induce/accelerate/deteriorate pulmonary artery elastic properties. To the best of our knowledge, there are no studies have examined the role of vitamin D levels in PAS, although there are a limited number of reports about the relationship between vitamin D deficiency and PH. Consequently, bearing in mind this evidence demonstrating the direct effect of vitamin D on the endothelial function and vascular smooth muscle cells, in our study, we examined the different levels of vitamin D on PAS in subjects without PH to delineate the effects of vitamin D on pulmonary vascular structure in detail. Our study is of significance in this aspect. We found that decreased vitamin D level was significantly negatively correlated with PAS in individuals without overt PH.

The description of vitamin D status is debatable, with different levels used throughout the literature. A wide variety of threshold values have been used for vitamin D deficiency, but no definite assumptions have been reached. Generally, a level of 20-30 ng/mL is acceptable to indicate relative vitamin D deficiency and a level >30 ng/L is acceptable to indicate adequate vitamin D (29). For this study, we have considered plasma vitamin D levels <20 ng/mL as a severe deficiency.

Vitamin D replacement therapy is a low-cost treatment method and has no notable adverse effects. While the exact doses and duration of vitamin D are still uncertain, it is important to maintain the circulating vitamin D value >30 ng/mL to observe the beneficial effects on arterial stiffness (16). Although limited data from small clinical studies have shown that vitamin D therapy improves vascular markers such as endothelial function and cardiovascular parameters, it is not exactly known whether it improves pulmonary functions. Higher vitamin D status has been found to be associated with good lung function through an improvement in inspiratory muscle strength and maximum oxygen uptake both in the general population and specifically in patients with chronic obstructive pulmonary disease in whom vitamin D deficiency is also common (30,31). Moreover, Mirdamadi and Moshkdar (6) showed that vitamin D replacement therapy was accompanied by significant improvement in right ventricular size, 6MWD, and mPAP. Although the positive effect of vitamin D treatment on pulmonary functions has been demonstrated, albeit limited, we did not investigate the effects of vitamin D treatment on PAS or other echo parameters in our study. But still, we can speculate that vitamin D deficiency might be considered a modifiable risk factor in individuals with shortness of breath.

Study Limitations

First, the relatively small number of subjects is the main limitation of our single-center study. These might be ambiguous regarding the effect of vitamin D on PAS. Since our study coincided with the Coronavirus disease-2019 outbreak, we could not include the planned number of patients in the study. However, we believe that our findings may inspire further studies to clarify the effect of vitamin D on PAS. Second, since we included adult individuals with atypical complaints, clinically relevant biochemical parameters such as parathyroid hormone level, brain natriuretic peptide level, and inflammatory markers were not studied. Third, there is no agreement about the average range of PAS by this technique. However, our results were consistent with those of previous studies.

Conclusion

In conclusion, in our study, we found that PAS measured echocardiographically might seem to be affected by the low level of vitamin D. The measurement of vitamin D might be used to gain insight into the mechanics of the pulmonary artery vasculature and might be an early marker of pulmonary artery wall's elastic disruption, even in the absence of significant pulmonary artery pressure elevation. Considering the high prevalence of vitamin D deficiency in the general population, care to screen and

treatment of vitamin D deficiency in individuals presenting with shortness of breath is important. Further prospective studies with long-term follow-up periods, preferably randomized controlled trials, must clarify whether vitamin D deficiency is a causal and reversible factor for increased PAS.

Ethics

Ethics Committee Approval: The study was approved by the Non-Interventional Research Ethics Committee of the University of Health Sciences Türkiye (protocol number: 19/280, date: 25.06.2019).

Informed Consent: Informed consent was obtained from all participants before enrolling in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.T., M.Ç., Design: H.T., E.M., Ö.E., S.E., M.Ç., Data Collection or Processing: H.T., M.S.K., O.K., F.B., S.A., Analysis or Interpretation: H.T., E.M., Ö.E., S.E., S.A., Literature Search: Ö.E., O.K., F.B., S.A., Writing: H.T., M.Ç.

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Giant ameloblastoma with intracranial invasion treated with a pure endoscopic approach

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ABSTRACT

Ameloblastoma is a benign but locally aggressive tumor that can invade the brain. A 75-year-old male patient admitted with massive exophthalmos and a tumor protruding from his nose. Magnetic resonance imaging revealed a giant mass lesion occupying the entire ventral skull base. The tumor was removed with endoscopic endonasal surgery. The patient was stable in the postoperative period, and there was no recurrence during the 5-year follow-up. Ameloblastoma may reach a huge size despite its benign character. Surgical treatment can prevent serious complications, and an endoscopic approach can be considered a treatment option.

Introduction

Ameloblastoma is a benign but locally aggressive odontogenic epithelial neoplasm that presents as a slowly growing painless swelling of the jaws and accounts for approximately 1% of all mandibular tumors and cysts (1). Although histologically benign, ameloblastoma can be locally destructive, spreading to the base of the skull, paranasal sinuses, infratemporal fossa, pterygopalatine fossa, parapharyngeal space, therefore causing severe facial deformity and functional impairment (2,3).

The treatment of ameloblastoma varies based on clinical, histopathologic, and radiographic characteristics (4). Surgery is the first line of treatment recommended to prevent recurrence and metastasis, and the goal should be complete surgical resection (5). Several surgical approaches have been used in its treatment, with varying success.

With the development of technology recently, new approaches have been added to surgical treatment techniques for ameloblastoma, and one of them is the endoscopic endonasal approach (EEA). The main advantage of EEAs is not only providing more direct access to the anterior and central skull base while avoiding craniofacial incisions commonly used in open surgical approaches but also increasing the range of the endoscopic visual surgical field with the angled lenses (6,7). EEA precisely determines the areas of tumor attachment by improving access, allowing complete tumor removal while minimizing the size of the maxillary defect and the associated morbidity as well as allowing brainstem and optic nerve decompression with less risk of damage to nervous and vascular structures (8,9).

The case described in this report is the second patient treated with a pure endoscopic approach. Additionally, it is one of the huge ameloblastomas published in the literature

Case Presentation

A 75-year-old male patient admitted with massive exophthalmos in the left eye and a tumor protruding from his nose (Figure 1A,1B). He had anosmia, headache, tingling sensation in the left half of his face, and swallowing difficulty with frequent choking for two months. His symptoms had rapidly progressed in the last two weeks. Neurological examination revealed a Glasgow Coma scale of 15, low visual acuity in the left eye, left facial hypesthesia, hearing impairment, and absence of vomiting reflex.

Magnetic resonance imaging (MRI) showed a slightly enhanced giant mass occupying the entire ventral skull base extending into cavernous sinuses, left pterygopalatine, and infratemporal fossae (Figure 1C-1E).

Computed tomography (CT) showed that all bony structures of the ventral skull base were invaded by the lesion (Figure 1F-1H).

CT angiography showed that the tumoral tissue was fed by both external carotid artery branches, more prominently on the right. Bilateral internal carotid arteries were surrounded by the tumor and displaced superiorly.

Initially, an excisional biopsy was performed. The endonasal part of the tumor was markedly removed for diagnostic purposes. Histological examination showed ameloblastoma, and the patient was referred to the neurosuregery diviison for further management where tumor excision was planned using the endoscopic skull base approach.

General anesthesia was performed by percutaneous tracheotomy due to the difficult intubation of the patient. After induction of anesthesia, head was fixed in a Mayfield clamp, head position was adapted to the tumor location and then registered with the navigation system (StealthStation System, Medtronic, USA). Initially, the tumor remaining in the nasal cavity was resected. After major decompression, with the help of endoscopic endonasal surgery, the tumor was removed from the clival, sellar, suprasellar, frontobasal regions, both cavernous sinuses, and the pterygopalatine and infratemporal fossa. After the hemostasis was obtained, skull base repair was performed with fascia lata and subcutaneous fat tissue obtained from the anterolateral of the left femoral region. Additionally, a lumbar drain was placed.

Histopathological examination was reported as plexiform type ameloblastoma **(**Figure 2A-2D).

The postoperative course was safe and stable. Neurological examination findings also remained stable. A CT scan on postoperative day 1 showed gross total resection of the tumor (Figure 1I-1K). Lumbar drain was removed on postoperative day five. A permanent dental prosthesis was placed in the dentistry service. During follow-up, annual MRI showed no signs of residual disease or recurrence for five years as of the submission of this report (Figure 1L-1P).

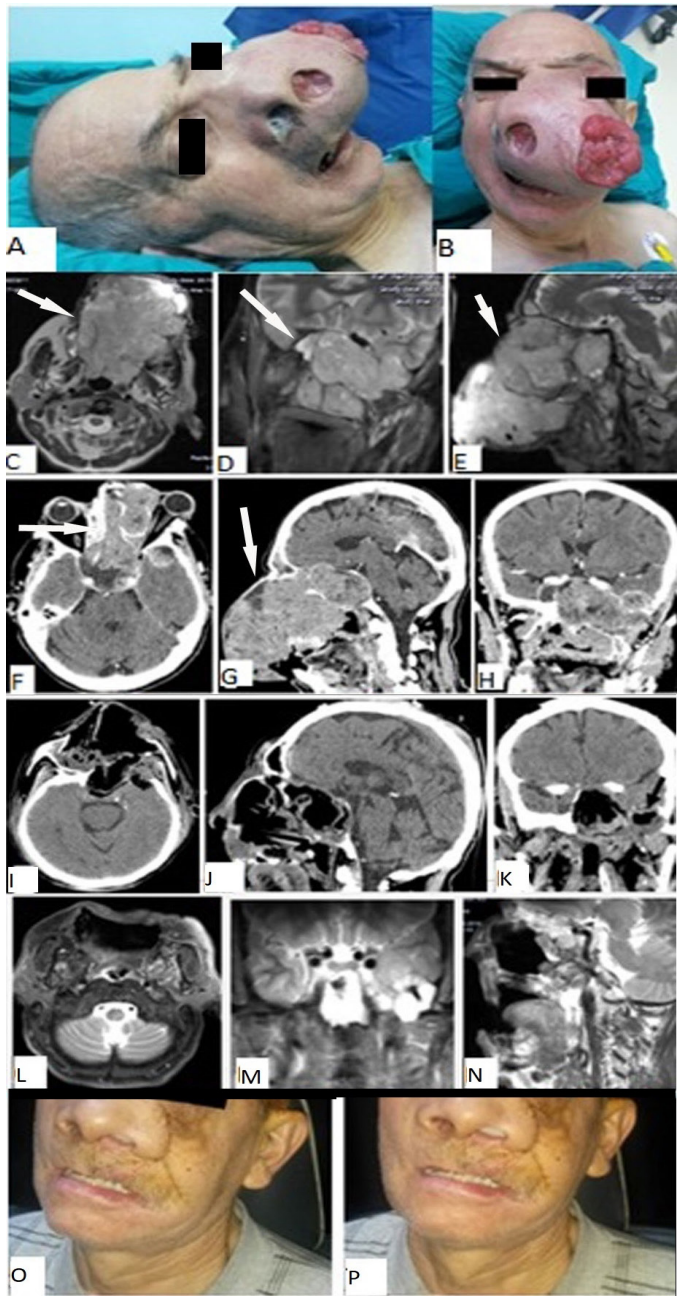

Figure 1. Preoperative (A, B) and postoperative photographs (O, P) of the patient. Preoperative axial (C), coronal (D), and sagittal (E) contrast-enhanced MRI showing a slightly enhancing, large, huge mass occupying the entire ventral skull base. The left side infratemporal fossa, pterygopalatine fossa, and base of the middle cranial fossa have been invaded by the tumor. Both cavernous was also involved by the tumor displacing both intracavernous internal carotid arteries superiorly and laterally. Axial (F), sagittal (G), and coronal (H) CT scans of the patient demonstrated that all ventral skull base bony landmarks were devastated by the expansile, lytic tumor. Axial (I), sagittal (J), and coronal (K) CT scans were obtained on the first postoperative day demonstrating the gross total resection of the tumor including the temporobasal component. Axial (L), coronal (M), and sagittal (N) contrast-enhanced MRI five years after the intervention, showing the continuous integrity of the skull base and no signs of recurrence. Both internal carotid arteries are in their normal anatomical location and are free from compression (white arrows=tumor)

MRI: Magnetic resonance imaging, CT: Computed tomography

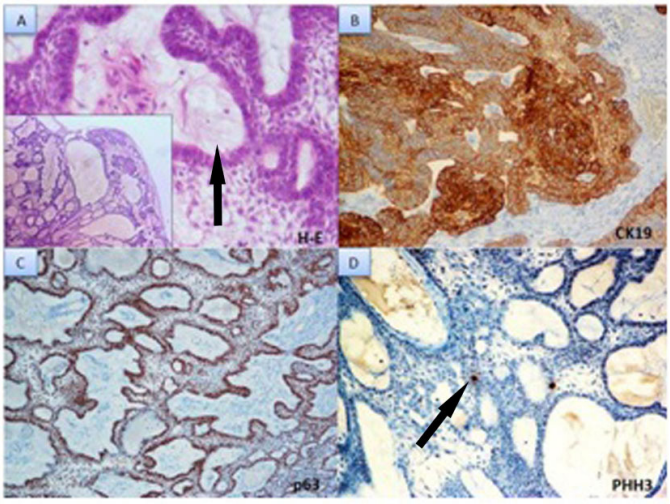

Figure 2. Solid/multicystic type, infiltrative ameloblastoma of the sinonasal tract: (A) cysts and follicular islands (black arrow) of odontogenic epithelium in the myxoid and edematous stroma, protruding to the nasal cavity as a polypoid mass (inlet) [hematoxylin-eosin (H-E), x20], (B) immunohistochemically, strong cytokeratin (CK) 19 expression (x40), (C) p63 expression of all cells (x20), and (D) a few mitoses (black arrow) with Phosphohistone H3 (PHH3) at the ameloblastic epithelium (x40)

Discussion

Ameloblastoma is a locally invasive benign epithelial origin tumor that may originate from the rest of the dental lamina, enamel apparatus, the epithelial lining of an odontogenic (dentigerous) cyst, or from the basal epithelial cells of the oral mucosa (10). They are rare neoplasms with a global incidence of 0.5 cases per million person-years most common between 30 to 60 years of age with an average of 36 years and a peak around the fifth decade (11).

The main approved treatment for ameloblastoma is surgery, and overall aim is complete resection. The literature describes two surgical therapy strategies to achieve wide surgical resection; 1) conservative surgical methods like decompression, enucleation, or curettage, and 2) radical procedures like marginal or segmental resection (4,12).

Recently, EEA has emerged as a new option for treating ameloblastoma, as the optics of endoscopes have improved, along with angled scope options. Its main advantages are direct visualization, enhanced magnification, avoidance of skin incisions, no external deformity, as well as less brain retraction, and less soft tissue damage resulting in cranial nerve deficits (13,14). Another advantage of the endoscopic surgical approach is that it reduces the operation time and has significant cosmetic benefits (15).

Conclusion

Ameloblastoma with intracranial invasion is very rare. To the best of our knowledge, the current patient is one of the few reported cases of ameloblastoma with intracranial involvement and was treated by pure endoscopic approach despite its hugeness. Endoscopic intervention may be an alternative to the conventional treatment of ameloblastoma.

Ethics

Informed Consent: Written informed consent was obtained from the patient to publish this case report anonymously.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.D., A.M.K., Concept: G.D., M.O.D., A.M.K., Design: M.O.D., M.C.E., A.M.K., Data Collection or Processing: D.E., Analysis or Interpretation: D.E., M.O.D., M.C.E., Literature Search: D.E., Writing: D.E., M.O.D., M.C.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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2022 Referee Index

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Ahmet Coşar
Ahmet Kasapkara
Ahmet Kokurcan
Ahmet Omma
Ajay Bhat
Ali Mohamed Ali Ismail
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Aniekan Monday Abasiattai
Arif Aslaner
Arzu Kaya Mumcu
Ayşe Saatçi Yaşar
Bahadır Ezmek
Berna Eren Fidancı
Bilal Katipoğlu
Burcu Buzkan
Burcu Erbaykent Tepedelen
Büşra Kibar Kurt
Cahit Kural
Can Hüzmeli
Canan Çulha
Cansu Köseoğlu Seçgin
Cem Haymana
Cemile Özsürekci
Cengiz Beyan
Cenkhan Bal
Drmariyam Zehra
Duray Şeker
Duygu Mert
Duygu Türker
Eda Ünal
Edis Çolak
Elif Güler Kazancı
Emine Öksüz
Emre Ata
Emre Tekgöz
Fatma İlknur Çınar
Fatma Yıldırım
Fatma Yurdakul
Ferhat Cüce
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Ömer Erşen
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Özlem Kılıç
Rahşan Ilıkçı Sağkan
Ramazan Acar
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Recep Alanlı
Sacide Duman
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Zafer Atbaşı

2022 Author Index

2022 Author Index

2022 Author Index

2022 Subject Index

2022 Subject Index

2022 Subject Index

NOTES

CONTENTS

REVIEWS

ORIGINAL ARTICLES

CASE REPORT