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63/1

XELJANZ®
[tofacitinib citrate]

▼ This medicinal product issubject to additional monitoring.

In the treatment of adults with
MODERATE TO SEVERE RHEUMATOID ARTHRITIS (RA)
who have had an inadequate response or intolerance to methotrexate[3]

A MARK OF PROVEN EFFICACY[4-6]

XELJANZ® – provides proven, rapid, and sustained efficacy with AND without methotrexate.[4-6]

An estimated 208,000 patients worldwide have been treated with XELJANZ®[7,*]

Up to 9.5 years of Long Term Extension study on Safety and Efficacy[6,]**

*As of April 2019; **Efficacy data has reported up to 8 years

ACR: American Collage of Rheumatology; **JAK:** Janus kinase.

References: 1. Vyas D ve ark. Ann Pharmacother. 2013 Nov;47(11):1524-31. **2.** Paik J Deeks ED. Drugs. 2019 Apr;79(6):655-663. **3.** XELJANZ® SmPC. **4.** Fleischmann R ve ark. N Engl J Med 2012;367:495-507. **5.** Burmester GR ve ark. Lancet. 2013 Feb 9;381(9865):451-60. **6.** Wollenhaupt J ve ark. Arthritis Res Ther. 2019 Apr 5;21(1):89. **7.** Data on file.

XELJANZ® 5 mg Film-coated Tablets

▼ This medicinal product is subject to additional monitoring. This inverted triangle is dedicated to bringing new information related to safety. Health care providers are obligated to report suspected adverse reactions to TÜFAM.[1] **ACTIVE INGREDIENT:** Each 5 mg film-coated tablet, contains of 8.078 mg of tofacitinib citrate equivalent to 5 mg of tofacitinib free base active pharmaceutical ingredient. **INACTIVE INGREDIENT:** Each 5 mg film-coated tablet, also contains of 62.567 mg of lactose monohydrate. **CONTENT OF PACKAGE:** Foil blisters containing 56 film-coated tablets. **THERAPEUTIC INDICATIONS:** XELJANZ® is indicated for the treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to methotrexate. XELJANZ® may be used as monotherapy or in combination with methotrexate. Use of XELJANZ® in combination with biologic DMARDs or with potent immunosuppressants such as azathioprine and cyclosporine is not recommended. XELJANZ® is indicated for the treatment of adult patients with active psoriatic arthritis who have had an inadequate response or intolerance to DMARDs. **POSOLOGY/TIME AND INTERVAL OF ADMINISTRATION:** The recommended dosage is 5 mg BID for both indications. If patient develops a serious infection, the treatment should be interrupted until the infection is taken under control. Interruption of the treatment with XELJANZ® is also recommended for management of lymphopenia, neutropenia and anemia. XELJANZ® dosage should be reduced to 5 mg once daily in patients with severe renal insufficiency, with moderate hepatic impairment, receiving potent inhibitors of CYP3A4 (e.g. ketoconazole), receiving one or more concomitant medications that results in both moderate inhibition of CYP3A4 and potent inhibition of CYP2C19 (e.g. fluconazole). **METHOD OF ADMINISTRATION:** XELJANZ® is taken orally with or without food. **SPECIAL POPULATIONS: Renal failure:** No dose adjustment is required in patients with mild or moderate renal impairment. The daily dose of XELJANZ® should not exceed 5 mg in patients with severe renal impairment. In clinical trials, safety and efficacy of XELJANZ® is not evaluated in rheumatoid arthritis patients with baseline creatinine clearance values (estimated by the Cockcroft-Gault equation) less than 40 mL/min. **Hepatic failure:** No dose adjustment is required in patients with mild hepatic impairment. The daily dose should not exceed 5 mg in patients with moderate hepatic impairment. XELJANZ® should not be used in patients with severe hepatic impairment. **Pediatric population:** The safety and efficacy of XELJANZ® in children under 18 years have not yet been studied. No data is available. **Geriatric Use (≥65 years):** No dose adjustment is required in patients age ≥ 65. Limited data for patients age ≥ 75. **ADVERSE REACTIONS:** Safety profile of XELJANZ® is similar for both indications. The most commonly reported adverse reactions during the first 3 months in controlled clinical trials were headache, upper respiratory tract infections, nasopharyngitis, diarrhea, nausea and hypertension. **INFECTIONS:** The most common serious adverse reactions are serious infections. The most commonly reported severe infections with the use of XELJANZ® are pneumonia, cellulitis, herpes zoster, urinary tract infections, diverticulitis, appendicitis. Opportunistic infections have been reported with TB, and other mycobacterial infections, cryptococcal, histoplasmosis, esophageal candidiasis, multidermatomal herpes zoster, cytomegalovirus, BK virus infections and listeriosis upon the use of XELJANZ®. **LABORATORY PARAMETERS: Liver enzymes:** More than three times of the upper limit of normal (ULN) increase in liver enzymes (3xULN) is rarely observed. In patients with elevated liver enzymes, treatment modifications, such as reducing the concomitant DMARD dose, interrupting XELJANZ® therapy, or reducing the XELJANZ® dose, have resulted in a reduction or normalization of liver enzyme levels. **Lipids:** XELJANZ® treatment has been associated with increases in lipid parameters such as total cholesterol, low density lipoprotein (LDL) and high-density lipoprotein (HDL). Increases in lipid parameters (total cholesterol, LDL, HDL, triglycerides) were first assessed in the first month after the start of XELJANZ® in controlled double-blind clinical trials. Observed increases followed by stabilization. The increases in total and LDL cholesterol associated with XELJANZ® fall to pre-treatment levels with statin therapy. **INTERACTION WITH OTHER MEDICINAL PRODUCTS AND OTHER FORMS OF INTERACTION:** Since XELJANZ® is metabolized by CYP3A4, it is likely that it interacts with the metabolism of other drugs inhibiting or inducing CYP3A4. XELJANZ®'s exposure is increased when it is co-administered with potent CYP3A4 inhibitors (e.g. ketoconazole) or when administration of one or more concomitant medications resulting in moderate inhibition of CYP3A4 and potent inhibition of CYP2C19 (e.g. fluconazole) happens. XELJANZ®'s exposure is decreased when co-administered with potent CYP3A4 inducers (e.g. rifampin). Inhibitors of CYP2C19 or P-glycoprotein are unlikely to alter the pharmacokinetics of XELJANZ®. Concomitant administration of XELJANZ® with methotrexate (15-25 mg MTX once weekly) had no effect on the pharmacokinetics of XELJANZ®. **CONTRAINDICATIONS:** XELJANZ® is contraindicated in hypersensitivity to the active substance or to any of the ingredients listed or in case of severe liver failure, pregnancy and lactation period and severe infections like active tuberculosis, sepsis or opportunistic infections. **SPECIAL WARNINGS AND PRECAUTIONS: COMBINATION WITH OTHER RA TREATMENTS:** XELJANZ® has not been studied in combination with biological DMARDs and its use should be avoided in RA patients in combination with biological DMARDs such as TNF antagonists, IL-1R antagonists, IL-6R antagonists, anti-CD20 monoclonal antibodies and selective co-stimulation modulators and potent immunosuppressants such as azathioprine and cyclosporine because of the increased risk of immunosuppression and followed by an increased risk of infection. **SERIOUS INFECTIONS:** Serious and, in some cases, fatal infections due to bacterial, mycobacterial, invasive fungal, viral or other opportunistic pathogens have been reported in patients with rheumatoid arthritis treated with XELJANZ®. The risk of opportunistic infection is higher in Asian geography. XELJANZ® should not be started with active infections, including local infections. **Tuberculosis:** Patients should be evaluated and examined for latent or active infection before and during treatment with XELJANZ® periodically. **Viral reactivation:** Before treatment with XELJANZ® is initiated, screening for viral hepatitis should be performed in accordance with clinical guidelines. **MALIGNANCY AND LYMPHOPROLIFERATIVE DISEASE:** Lymphoma and other malignancies have been observed in patients treated with XELJANZ®. The effect of XELJANZ® on the development of lymphoma and malignancy is unknown. Non-melanoma skin cancers (NMSC) have been reported in patients treated with XELJANZ®. Periodic skin examination is recommended for patients who are at increased risk for skin cancer. **GASTROINTESTINAL PERFORATIONS:** Events of gastrointestinal perforation have been reported in clinical studies with XELJANZ® in rheumatoid arthritis patients, although the role of JAK inhibition in these events is not known. XELJANZ® should be used with caution in patients who are at increased risk for gastrointestinal perforation (e.g., patients with a history of diverticulitis). Patients presenting with new onset abdominal symptoms should be evaluated promptly for early identification of gastrointestinal perforation. **LABORATORY PARAMETERS: Lymphocytes:** XELJANZ® treatment has been associated with an increased incidence of lymphopenia compared with placebo. Lymphocyte counts below 750 cells/mm^3 were associated with an increased incidence of severe infection. XELJANZ® therapy should not be initiated or continued in patients who are confirmed to have a lymphocyte lower than 750 cells/mm^3. The lymphocytes level should be observed at beginning of treatment; after 4-8 weeks and then every three months. **Neutrophils:** XELJANZ® treatment has been associated with an increased incidence of neutropenia (<2000 cells/mm^3) when compared to placebo. It is not recommended to start XELJANZ® treatment in patients with ANC less than 1000 cells/mm^3. Neutrophil counts should be monitored at the beginning of treatment, after 4-8 weeks, and every 3 months thereafter. **Hemoglobin:** XELJANZ® treatment has been associated with a decrease in hemoglobin level. It is not recommended to start XELJANZ® treatment in patients with a hemoglobin level below 9 g/dL. The level of hemoglobin should be monitored at the beginning of treatment, 4-8 weeks after treatment and then every three months thereafter. **VACCINATION:** It is recommended that all patients vaccinations should be completed in accordance with current immunization guidelines before starting XELJANZ® treatment. Administration of live vaccines with XELJANZ® is not recommended. **OVERDOSE AND MANAGEMENT OF OVERDOSE:** There are currently no specific antidotes for treating overdose with XELJANZ®. Treatment should be symptomatic and supportive. **PREGNANCY AND LACTATION:** Pregnancy category is C. **Pregnancy period:** There is not enough data on the use of tofacitinib in pregnant women. Studies in rats and rabbits have shown that tofacitinib is teratogenic and also affects delivery and peri/ postnatal development. As a precautionary measure, the use of XELJANZ® is contraindicated in pregnancies. **Lactation period:** It is not known whether tofacitinib is excreted in human milk. But the risk of being harmful to the breastfeeding baby cannot be ignored. Tofacitinib has been passed on to suckling rats. As a precautionary measure, the use of XELJANZ® is contraindicated during the lactation period. **ABILITY TO DRIVE OR OPERATE MACHINERY:** Tofacitinib has no or negligible effect on the ability to drive a vehicle or operate machinery. **STORAGE:** Store XELJANZ® at room temperature, below 30°C and inside the original package. **SHELF-LIFE:** 24 months. **MARKETING AUTHORIZATION HOLDER:** Pfizer İlaçları Ltd. Şti. Muallim Naci Cad. No:55 34347 Ortaköy/İstanbul. **AUTHORIZATION DATE AND NUMBER:** First authorization date: 07/05/2015 2015/335. Sold by prescription. Currently reimbursed by Social Security Institution. For further information, please contact us. **VAT INCLUDED RETAIL PRICE AND APPROVAL DATE:** XELJANZ® 5 mg film-coated tablet: 2.917,84 TL (19.02.2020). **SmPC APPROVAL DATE:** 06.08.2019.

[1] Türkiye Farmakovijilans Merkezi: Turkish Pharmacovigilance Center.

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Gülhane Medical Journal (Gulhane Med J) is the official, scientific, peer-reviewed, international journal of Gülhane Faculty of Medicine, University of Health Sciences Turkey. The journal accepts submissions on all aspects of general medicine. Featured article types include original articles, case reports, reviews, and letters to the editor. The history of the Gülhane Medical Journal dates back to 1871 and it was named with its current title in 1999.

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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

As the result of rigorous work at the GMJ, the second issue of the year includes exciting research articles, review articles, and case reports.

The number of submissions to GMJ has been increasing. Besides, we have been receiving manuscripts from diverse disciplines, which requires a broader Editorial Board coverage. In this respect, as of January 2021, we welcomed new colleagues in our Editorial Board;

Adıgüzel, Emre, Assoc. Prof. M.D.

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Çınar, Muhammet, Assoc. Prof. M.D.

Pakdemirli, Ahu, Assoc. Prof. M.D., Ph.D.

With their involvement, I believe the journal will continue to grow by delivering the most quality publications to our readers.

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

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

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New approaches in studying biological mechanisms in psychiatric diseases

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ABSTRACT

Special biological markers are needed in psychiatric diseases for understanding the organic etiology. Such kind of biological markers are required in differential diagnosis of psychiatric diseases and in following up of their response to treatment. While the etiology of most psychiatric disorders remains unclear and unknown, there are a lot of supporting evidences that hereditary factors are important in the pathogenesis of psychiatric diseases. Finding the roles of genetic factors in the etiology of diseases probably enhance the understanding of these illnesses better. We can recognize them earlier. Today, we are faced with new scientific developments in this field. Whole genome studies developed in these days have revealed many "hot spot" regions on human genome which could be related to psychiatric disorders. By using new cell culture techniques, we had a chance to have new biological markers in these diseases. Organoids, which resemble original cerebral tissues, were obtained in "*in vivo*" conditions. Induced pluripotent stem cells were used in organoid techniques for obtaining different cell types. Disease specific animals-genetically modified animals were created with known gene defects of psychiatric diseases in laboratories. New brain banks, which allow us to postmortem studies on human brain, are established in different cities around the world. In the light of the recent developments in cell biology and molecular biology/genetics, this review discusses organic structural findings, taking place in psychiatric disease progression. It is a fact that as the findings increase rapidly in this area, the etiologies of psychiatric diseases will be understood and clarified more easily. By using these technologies, important biological markers will be obtained in the diagnosis. The new treatment protocols can be revealed.

Introduction

General biology and neural system biology in particular have become powerful scientific disciplines in this century. Despite these improvements in biology and neural system biology, no satisfactory pathologic mechanism has been clarified in a psychiatric disease. Achieving a biological mechanism would create a paradigm shift for understanding the psychiatric diseases such as schizophrenia, bipolar disorder, anxiety disorders or obsessive-compulsive disorder (1,2).

Biological markers are needed in psychiatric diseases for understanding the etiology of these diseases. All psychiatric diseases have confusingly similar symptoms, such as long-run sadness, long term irritability, extremely high and low mood in normal conditions, and excessive anxiety or worry (1,2). A biological marker has a potential of revealing the diagnosis of an undiagnosed psychiatric case. Specialists can use these markers in differential diagnosis and in monitoring the achievement of treatment (3,4). Despite the fact that the mechanisms of psychiatric disorders remain obscure, there is strong evidence that hereditary factors are important in the progression of major psychiatric illnesses such as schizophrenia and bipolar disorder (5). Twins studies revealed hereditary predisposition in large series in literature. Big meta-analyses studies in literature presented the importance of inheritance in psychiatric illnesses (6). Genomix (functional genome analyses) and proteomix

(functional protein analyses) probably point out the significance the hereditary factors. These advances would bring up new approaches to the treatment of psychiatric diseases, an area that has been inert condition for the last thirty years (Table 1) (2).

In this review, the new approaches in cell biology and molecular biology/genetics are mentioned in understanding the pathology of psychiatric diseases. The new culture techniques used in the development of cerebral organoids are explained. Also, the advantages of a genetically engineered mouse model in the uses of experimental studies are tried to be explained. Finally, the roles of brain banks in postmortem studies are discussed (Table 1) (7).

Cell Biology Studies and Findings

Cell biology studies the structures and functions of cells. New microscopes and staining techniques give an opportunity to see more details within different cells. There are several main subfields in cell biology, including developmental biology and system biology. In developmental biology, cell growth, differentiation and morphogenesis are studied especially *in utero* conditions. Systems biology is the computational and mathematical analysis of cells. It includes modeling of complex biological systems. System biologists try to explain the interrelationship of gene control mechanisms in entire human genome. On computer simulations, they try to clarify intercellular and extracellular networks (Table 1) (8).

The generation and the maturation of neuronal synapses is important in brain developmant. Neuronal network development is also associated with the elimination process of synaptic structures in brain in gestational period. The elimination of synaptic structure in brain development is known as synaptic pruning. Neuron degeneration (neuronal cell death/neuronal apoptosis) is essential in pruning. Only axon/dendrite degeneration can be seen in a neuron. In neuron degeneration, apoptotic mechanisms are important (9). It is believed that the aims of synaptic pruning are to remove unnecessary neuronal structures from the brain and to reorganize the neuronal/glial structures. At the end of gestation period, simpler neuron structures are obtained in organogenesis period. Dysregulation of pruning in normal brain development seems important in the etiology of autism spectrum disorder (10). In recent years, over proliferation of synapses has been determined after pruning in "*in utero*" brain development. Such kinds of findings have represented that synaptic reorganizations are important in normal human brain development (11).

Glial cells like neuronal cells also have important role on synaptic pruning in all parts of the nervous system. Glias are the special types of cells in brain structure, which do not generate electrical impulse. Glia cells maintain homeostasis, form myelin, and provide support and protection for neurons. Glia and neuron cells use sets of common signaling pathways in pruning process

Table 1. Three major research areas are studied in understanding of etiology of psychiatric diseases

Research areas	Main laboratory studies	Main laboratory techniques
Genotyping	Sequencing and bioinformatics findings	Whole genome analyses and a single gene sequencing techniques w/o bioinformatics analyses
	Cell culture studies	2-D and 3-D cell culture techniques, induced pluripotent stem cell, and stem cell studies, organoid studies etc.
	Animal studies	Invertebrate models; e.g. C. elegans, fruit fly-drosophila melongester screens*, Mouse models for schizophrenia; e.g. disrupted in schizophrenia 1 (DISC1) and 22q11.2 deletion syndrome, and for autism; methyl CpG binding protein 2 (MECP2) and 15q11-13 deletion/duplication syndromes**, Zebrafish***
Molecular biology/ pathology	Genome-Wide Association Studies (GWAS), brain tissue studies, etc	Metabolomics, genomics, proteomics, transcriptomics etc.
	Cell culture studies	2-D and 3-D cell culture techniques, induced pluripotent stem cell, and stem cell studies, organoid studies etc.
	Animal studies	Brain tissue studies of model animals, obtained intervertebrate, vertebrate animal models.
	Human brain tissue studies	Brain tissue studies obtained from brain bank.
New therapies	Chemical screen	Brain tissue studies of model animals and humans.
	Cell culture studies	2-D and 3-D cell culture techniques, induced pluripotent stem cell, and stem cell studies, organoid studies etc.
	Animal studies	Brain tissue studies of model animals

*C. elegans is useful for interference (RNAi) screens. Invertebrate genes exhibit some evolutionary conservation with human genes. Homologues of ~50% and 75% of human disease genes can be identified in C. elegans and D. melanogaster respectively.
**Mouse is useful for single (or few) gene connections to phenotype, for biomarker studies and for new therapeutic approaches studies.
***Zebrafish is useful for genetic interactions and variant analysis, for biomarker analyses and for new therapeutic approaches studies.

(12). Local cytoskeleton disassembly occurs before axon/dendrite degeneration in pruning. Recent evidences suggest that microtubule disassembly is the local trigger mechanism in pruning (13,14). Dendritic spine formation is a critical process for many synaptic functions in human brain development. The formations and modifications of spines are accepted as vital process for learning and memory abilities of human brain. As known, dendritic spine formation occurs especially in early postnatal period in humans. Like the formation of spines, the removal of dendritic spines is also a normal process in human life. Despite this, we do not know clearly the causes of spine formation and removal in dendrites. The role of dendritic spines' disruption is explained in many neuro-developmental disorders (15). Normally, the strength of synapses can be increased or dicreased in different conditions. Strong action potential is associated with active synapses. Weak synapses are less active and generate weak action potentials. Synaptic plasticity in long term period forms the memory storage conditions in human brain. Long term synaptic depression which is characterized by weak action potentials seems important in the etiology of autism spectrum disorders (16).

As a complex psychiatric disorder, understanding the etiology of schizophrenia is very hard. Schizophrenia pathology is linked to a large number of genes in human genome with highly heritable condition. New technologies improved in cell and molecular biology are tried to be used to find the functions of these genes in disease etiology. Gene abnormalities cause developmental problems in cells such as neuronal migration and axon degeneration (17). Cell movement or migration is the dynamics of cellular processes which include microtubule and adhesion complex dynamics, contraction of actin/myosin filaments (Figure 1) (18). Hockemeyer and Jaenisch (19) have mentioned in a manuscript that schizophrenia patient-derived olfactory cells are more mobile than control-derived cells. Focal adhesion kinase signaling was found as the main factor in the motility of olfactory cells in this experiment.

Stem Cell and Cell Culture Studies

Stem cells are special cells that can differentiate into other types of cells. They can also divide in self-renewal condition to produce more of the same type of stem cells. Induced pluripotent stem cells (iPS cells or iPSCs) are human generated stem cells, differentiated from terminally differentiated cells. They can divide and differentiate into every other cell type in the body (such as neuron, fat, muscle, and pancreatic cells). Pluripotent stem cells hold promise in the field of regenerative medicine because of these specificities (Table 1) (20).

Cell culture in two dimensions (2D culture) has been routinely used in laboratories worldwide for many years. 2D culture is a simple and easy technique. Despite these advantages, it has very important disadvantages, e.g. it does not show the anatomy or physiology of a tissue. Cell culture in three dimensions (3D culture) is an artificially created environment. In 3D cell cultures, biological cells are grown and interacted with their surroundings like in tissues (21). Progress in stem cell and iPSC technologies make it possible to use 3D culture systems in laboratory conditions. Today, having an organoid structure is possible using these techniques (22,23). A cerebral organoid is an artificially grown model organ resembling the brain tissues. It can be created for studying a psychiatric disease in a simpler condition. Human brain consists of very complex and heterogeneous structure. The complexity of this tissue makes it difficult to understand how it works in neuroscience (Table 1) (24).

In schizophrenia, a genetically induced vulnerability in brain development was reported. iPSCs obtained from patients with schizophrenia indicate functional problems in neural progenitor cells. Neuronal progenitor cells differentiate into all glial and neuronal cell types, observed in central nerves system. Neural progenitor cells which have functional impairments disturb neocorticogenesis in an organoid model (23). On an organoid in a culture flask, a disorder that has been difficult to study such as microcephaly can be studied also (24).

Autism spectrum disorder is a disorder of neurobiological origin that affects children up to the age of 3. Developmental, cognitive, and behavioral problems occur in an autistic child. Gross motor skills and fine motor skills are also affected due to brain dysfunction. Connectivity problems due to dysfunction in neuronal migration directed by mitochondria are accused of as reliable reason in disease etiology. Intrauterine hyperglycemia and hyperinsulinemia also cause neuronal migration problems which may be important in autism pathology. Today, we know that prolonged neonatal hypoglycemia may cause mitochondrial dysfunction (25). Neuronal migration like neuronal differentiation is a fundamental process occurring in embryonic time period. Today, the effects of many genes on cell migration are put forward in literature. These genes also have similar effects on neural migration. Gene knockout studies in mice have revealed that specific mutations which affect neuron migration proceed severe brain malformations. It seems that such kinds of mutations can cause complex and heterogeneous developmental neuronal migration disorders (26). Cell movement or migration can be achieved by microtubule dynamics, contraction of actin and myosin filaments (Table 1, Figure 1) (18).

Molecular Biology/Genetic Studies and Findings

Today, we know that a potential genetic predisposition observed in a family is important in the occurrence of many psychiatric disorders such as autism, attention deficit hyperactivity disorder, bipolar disorder, major depression and schizophrenia (27). Cytogenetic analyses have revealed many chromosomal alterations on various kinds of psychiatric illnesses in so many

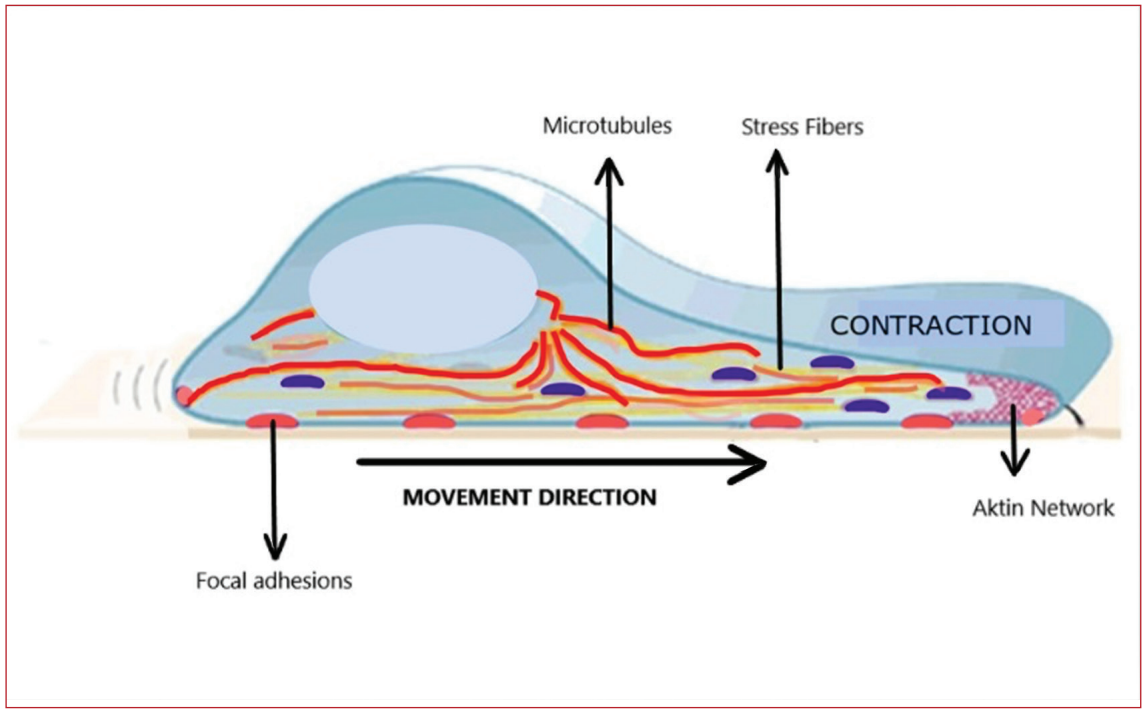

Figure 1. Microtubules and adhesion complexes are important on cell movement and migration. Microtubules and adhesion complexes may probably have roles on the migrations of neuron and glias in embryonic period during the brain development (the figure was modified and drawn again by the author from reference 18)

years. In linkage studies, “hot spots” probably susceptible in psychiatric disorders have been reported (28).

In 1990, a large Scottish family with a (1;11)(q42;q14.3) translocation was published, which had a significant correlation to specific clinical phenotypes, including schizophrenia and affective disorders (29). Ten years later, Millar et al. (30) described a translocation breakpoint region on chromosome 1q42. The same group identified two novel genes directly disrupted by this translocation (‘disrupted in schizophrenia’ 1-DISC1 and ‘disrupted in schizophrenia’ 2-DISC2). By using new techniques, genetically modified mice were obtained in recent years. This type of mouse is a genetically modified mouse in which researchers have inactivated a target gene. They are important animal models for studying the roles of genes which have been sequenced but whose functions have not been determined. Up to now, several DISC1 gene knockout mouse models have been generated for use in experimental studies. All these mouse models display neuro-anatomical and behavioral abnormalities relevant to schizophrenia (Table 1) (31).

In a separate study, the gene DIBD1 has been found to be disrupted by a translocation involving 11q23 chromosome region in a family for bipolar disorder (32). Bipolar disorder is a chronic neuropsychiatric condition characterized by pathological changes in mood of patients from mania to depression. So, bipolar disorders can be accepted as a multifactorial and polygenic genetic disorder. Today, we know that bipolar disorder is the most heritable form of mental illnesses. Despite these, clarifying the hereditary factors in this disease is not easy. Neuro-developmental factors which affect nervous system development and cell migration were found as altered in bipolar disorders in genome-wide association studies (GWAS) (33). Each GWAS marker appears to confer little risk in bipolar disorder. Even so, common variants together account for 25% of the heritability of bipolar disorder (34). In these studies, 16p11.2 gene region had been identified with a higher-risk association. If we found appropriate genetic markers for bipolar disorders, they can be used in the diagnosis of this illness easily (35). Twins studies also have elucidated a high heritability in autistic disorder (36). Large scale meta-analyses in GWAS have reliably identified huge numbers of genetic polymorphisms associated with neuropsychiatric disorders. In most of bipolar disorder, single nucleotide polymorphism (SNPs) located in non-coding genomic regions is correlated with the disease etiology. The molecular importance of SNPs in bipolar disorders is not fully understood. Today, studying the functional properties of disease-associated variants is important for finding the role of these variants in disease etiology in gene expression levels (35). In a manuscript written by Bryzgalov et al. (34), fourteen regulatory SNPs are selected as potential gene locus related to neuropsychiatric disorders. The functions of targeted genes are related to some important processes in cell life cycle such as “posttranscriptional regulation”, “neuron differentiation” and “neuron development”. On a similar large panel, the psychiatric GWAS consortium found regulatory SNP (rSNP)- targeted genes. This group explained

the reason of expression differences among patients and control groups in brain region (NRAS-in schizophrenia cohort, CDC25B, DDX21 and NUCKS1-in bipolar disorder cohort) (36). So, new genetic findings observed in psychiatric diseases are important for genetic counseling and genetic testing (Table 1) (37).

Animal Models Obtained by Using New Gene Technologies for Psychiatric Diseases

Genome/gene editing technology gives opportunity to scientists the ability to change a genome of an organism. In this technology, genetic material can be added, removed, or altered at targeted locations in the genome. Today, generally CRISPR-Cas9 methodology is used in the laboratories widely because it is faster, cheaper and efficient (38,39). By using CRISPR-Cas9 technology, genetically engineered mouse can be obtained such as *DTNBP1* gene disrupted mouse (40). *DTNBP1* gene encodes a protein that plays a role in organelle (e.g. lysosome) biogenesis in a cell. Also, this protein has been explained as a causative factor in schizophrenia etiology. We know that it regulates prefrontal brain functions in fetal and adult period. DTNBP1 gene expresses in the dorso-lateral prefrontal cortex and hippocampus. Decreased levels of dysbindin-1 mRNA were found in brain tissues in schizophrenia cases. Scientists claim a strong connection among dysbindin-1 function and schizophrenia pathology (41). In a genetically engineered mouse, which had functionally disturbed dysbindin-1 gene, similar behavioral activities can be observed like in schizophrenic patients. Huang et al. (42) found that this gene has important roles for in the regulations of amygdala functions (Table 1).

Reelin protein is located in the extracellular matrix. It is involved in the development of cortical neural connectivity at embryonic phase. Reelin protein generally regulates synaptic plasticity of neurons at postnatal stages. Epigenetic changes in reelin gene in schizophrenic patients probably cause an inhibition in reelin expression in human brain (43,44). Proper cortical development is important in brain structure. In reelin gene mutant mouse (named as a reeler mouse), dysfunction of cortical region was found. The absence of reelin expression in genetically engineered mouse caused migration defects in neurons and developmental defects in dendrites (45).

Injection of human progenitor cells into an immunodeficient mouse gives chance to have “humanized mouse”. By using this technology, it can be possible to obtain a brain in the mouse which has “human-like” neuronal structures (46). Capps explained in a manuscript that oligodendrocyte and astrocyte development was found as altered in schizophrenia in chimeric mice experiments. Also, cell-cell interactions were found as important in the pathogenesis of early-onset schizophrenia (47).

Zebra fish represents a good model among complexity and simplicity of system. Studying on zebra fish gives us a good opportunity for finding the mechanisms of brain functions in brain illnesses. Zebra fish can be used as a model for finding the effects of psychotic drugs. Fear and anxiety problems can be analyzed with zebra fish studies easily (Table 1) (48).

Establishing Brain Banks for Postmortem Brain Studies

For postmortem brain studies, storing and banking of brain tissue samples is an important step. Generally, the brain tissues are stored in liquid nitrogen at -195 °C for a long time. Brain banks are given an opportunity to research on the etiologies of different psychiatric diseases. The recent advances in neuro-imaging and molecular biology increased the interests to human brain studies in these days (Table 1) (49).

Generally, brain banks collect human brain tissues from donors who had neurological and/or psychiatric disorders. Also, normal human brain tissues are stored in brain banks obtained from healthy donors. The brain tissues stored in brain banks are shared to other scientific researchers around the world. In this way, experiments about understanding the molecular mechanisms in psychiatric diseases can be done on brain samples in all laboratories (50). GWAS are achieving huge successes in identifying disease-associated variants in specific human brain tissues as seen in data in Psychiatric Genomics Consortium (PGC; http://www.med.unc.edu/pgc) (36). The disease-associated variants in psychiatric disease etiology are generally SNPs located in non-coding regions. The effect of each SNP seems in small size in disease etiology. Today, the scientists believe that the cumulative effects of SNPs are stimulated the initiation factors in psychiatric diseases. So, the interpretation of functions in disease associated gene variants can be hidden. In population-based human variation studies, DNA methylation and histone modification were found as important. Gene variant rs199347 is located on an non coding intron region in a protein coding gene named as GPNMB (Glycoprotein Nmb). This variant affects the expression of GPNMB only in human brain. We know that the effect of variant rs199347 is associated with Parkinson disease etiology. This kind of effect in rs199347 variant can not be observed in other tissues in organisms (51). Such kinds of findings represent that the human brain tissues obtained from brain banks can be used in comprehensive molecular studies for advanced researches in neuropsychiatric disorders (50). For that reason, the achievement of brain bank projects is generally dependent on these kinds of complex studies. First of all, the patients and their families should be convinced in the donation of the patient’s tissues in postmortem period. And then, the cooperation among scientists and clinicians should be established for more complex studies (49).

Conclusion

So, developments in cell biology and molecular biology/genetics techniques reveal new approaches in understanding of

etiology in psychiatric diseases. The improvements may affect the diagnosis and treatment of these cases in immediate future.

Ethics

Peer-review: Externally peer-reviewed.

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Innovative teaching methods in dental education

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ABSTRACT

Health professionals have often acknowledged that their profession does not prepare them for teaching. Hence the teaching style has been predominantly the traditional lecture based system. In this review, we get a gist of the innovative teaching methods, which have gained acceptance in the recent years, and how the implementation of these has become crucial for effective teaching learning process. Problem based learning, case based team based learning, microteaching and flipped classroom are some of the innovations discussed. The dental faculty also need to undergo a series of training to be well equipped with these methods, and also the institutes wanting to implement the above methods should have the infrastructure, so that the students are also well equipped and motivated to utilize the innovations which in turn will help them think and acquire knowledge in an effective, systematic manner.

Introduction

The faculty of dental education in India has been adapting newer and innovative methods in imparting lessons to the undergraduate students. Some of these methods have gained acceptance and are being utilized as a part of the curriculum. As a result of multitude of changes such as modifying social determinants of health, an exponential increase in technology, and a steep rise in health care, dental education also has a changed scenario (1). Dental education has developed from a self-learnt and self-proclaimed profession in the past to a one involving formal education and recognized competencies (2). Health professionals have acknowledged that their education does not equip them for teaching. However, due to an increasing expectation of the society from the health professionals, it necessitates them to be well versed in teaching techniques/ skills and be qualified adequately (3).

Problem Based Learning

In 1990, The Faculty of Odontology in Malma, Sweden was the first to introduce problem based learning (PBL) in the field of dental education (3). PBL has been described as a method where a problem aids as a catalyst for learning. In this approach, the students describe the problem and develop learning targets which are needed to promote their perception of the problem. The method involves miniature batches of students engaged together and in collaboration with facilitators in order to accomplish learning (4,5).

A prime necessity for a PBL curriculum is that “the problem always comes first”. This maybe conferred as one of the ground guidelines of the program and has a substantial significance on the format of the curriculum (4). Primarily, the syllabus is formulated based on problems rather than disciplines, with emphasis on combined learning, instead of segregation into basic and clinical sciences. In the next step, it is led by

circumstances which makes learning simple, for instance small-group teaching, a student focused approach, effective study and self-reliant learning. Next, it is decided by outcomes, such as enhanced practical knowledge, skill advancement and impetus required for constant learning along with the development of self-appraisal techniques (6).

The advantages of PBL, when compared to traditional methods, comprise of an enhanced assimilation of basic as well as clinical techniques, mainly refined communication, team work, independent learning, along with an enhanced inclination and satisfaction of participating together on a problem. In health education, instructors have an advantage when associating clinical cases with basic sciences, by equipping students to figure out clinical problems which depend on key fundamentals and prior knowledge. Moreover, the techniques acquired and accomplished in the course, teamwork, delegation along with the use of relevant literature are paramount when it comes to professional tasks following graduation (7).

The utilization of problems to impart the curriculum content via PBL pedagogy demands the organizing group of the curriculum to primarily identify the predetermined program outcomes and later develop an array of problems providing the content, which is required to accomplish the fixed objectives. With regard to this, the establishment as well as sequencing of problems is crucial to cater to a comprehensive set of learning outcomes along with a systematic advancement of the establishment of the competency. A PBL pedagogy constitutes of three important factors, which need to be incorporated to allow the promotion of an inquiry-driven learning zone. These constituents – the problems, the small group learning, and the student centered environment-should be present to achieve a fruitful conclusion (4).

PBL has been incorporated in dental institutes all over the world in the past decade. This is an emerging concept and its implementation has been increasing. Dental institutes, which are looking forward to this concept, should be aware of the modifications needed in the infrastructure, and the significance of faculty development, new curriculum materials and students admission procedure (4).

Microteaching

Microteaching is a mini teaching confrontation which is aimed to create recent techniques and improvise old ones (8). Allen(9) developed this concept in the 1960s at the Stanford Teacher Education Program. It is an approach for students who aspire to be teachers to improvise upon their techniques in an adjuvant peer learning set up. The pupils will observe that their associates experience similar struggles, weaknesses and strengths. New techniques will be recalled and learnt by watching their associates and consulting with their group leader (10).

Students improvise upon their descriptive skills by converting cumbersome chapters into learnable entities with the help of leading coordinators, associating the lecture utilizing topics spanning the psychomotor and cognitive learning realms by employing questioning and pauses. This method asks for a change in focus from, “What am I going to cover” to “What are my students going to learn?” (10).

Microteaching comprises of several stages such as briefing phase, preparation phase, teaching stage, an evaluation by the class and overseer followed by either preparing for the upcoming class or learning of the same technique (11). In the briefing phase, exercises are included to help the student face the camera and help them increase their confidence. The preparation stage requires the students to devise their own teaching layout, and the scenarios of which they would want to present in class with an established basic skill. The teaching phase will have the students to impart their lectures in a scaled-down session for a time frame between five and twenty-five minutes. Viewing along with evaluation commenced with self-appraisal on positive and negative points seen, improvements to be made and if the preparations were of a particular set standard (10,12). The physical, psychological and emotional dimensions of the teaching process were analysed (12). The final process of Debriefing takes place after each student had accomplished a skill. This is usually held on the last day of the class, where a final acclamation was made of each student (10,12).

Case Based Team Based Learning

In the recent years, case based – team based learning (TBL) models has been utilized in teaching strategies (13). According to studies, interactive teaching learning methods such as these can pass on sustainable knowledge along with a change in performance leading to immense satisfaction among students when compared to traditional lecture classes (14). Lecture classes are not considered as the optimal method for establishing in-depth and long lasting understanding of the subject matter learned by the students (13).

In the late 1970s, Larry Michaelson at Oklahoma University originally developed TBL. As the strength of his class grew from 40-120 students, it necessitated him to design a new pedagogic paradigm, which was learning in miniature groups (15). It is efficient in enabling students to help each other during small-group discussions in a larger class along with preparation prior to a class. All group activities within a larger class can be conducted by one teaching personnel. This is in contrary to PBL, which has small-group activities and usually requires independent tutors for each group to monitor the progress (16). Compartmentalized space for group activities is not needed, as they continue in an open space allowing effective interaction among the groups (17). Literature states that TBL for dental education has been followed in Periodontics (18), Oral medicine (19), Oral Radiology

(13), Removable (20) and Fixed Prosthodontics (17). All the above studies reported higher student satisfaction with better performance when compared to conventional lectures.

Case based learning (CBL) is an interactive, student-centered, instructor-led learning approach, which was first begun in a Medical school in Newfoundland, Canada. This methodology encourages active learning by employing clinical case scenarios, which mirror real life experiences that students will encounter during the clinical stage of their medical education (21). Cases are generally presented as problems which equip the students with patient details such as case history and laboratory results. Active learning ensues when students have the opportunity to have more interaction with the case. This empowers them to generate knowledge rather than only receive, enhancing their skills for sharing with other pupils in a group (22-24). Active learning is a student oriented process, which makes the learners to be liable for their learning in a self – directed, peer-assisted seeking of new information (25). Self-directed learning is one of the advantages of CBL. It also strengthens the logic, collaborative as well as communication skills in students (22-24).

Flipped Classroom

At its basic level, flipped classroom is a method of assigning learner's didactic material, conventionally covered in lectures, to be learnt prior class, along with utilizing classroom hour for interactive and active learning (26). This methodology was noticed when a YouTube video, "The Flipped Classroom" was posted by Sams and Bergmann (27) on 16th December 2010. This requires the facilitators to previously record the lectures and posting in an online portal for pupils to observe on their own, thus dedicating the class hours for learning activities. This also makes the students responsible enough to have a basic understanding of the topic, which makes them participate well in discussions and other exercises. The faculty organizes content, establishing interactive participation, which requires the pupils to understand along with providing expert insight and evaluation (28). Park and Howell (29), in 2015, described the model of a flipped classroom in a predoctoral dental program; second year Dental Anatomy for thirty-six pupils. There was reluctance from the faculty with regard to the implementation, especially when they were challenged with a heightened task in view of the duration and struggle required for interactive activities. Although faculty was hesitant, positive feedback was obtained after implementation. Students found this format as entertaining, interactive as well as collaborative in comparison to the conventional lecture format. Along with technological issues, they also felt that the group discussions, at times, were chaotic. Shapiro et al. (30) reported that interactive online modules were effective in imparting to the students, regarding identification as well as reporting of child abuse in pediatric dentistry. Though the module was effective, they would not opt for it as an alternative to the lecture based approach. It was also reported to be effective in educating students on periodontal diagnosis and treatment planning as per a study conducted by Lee and Kim (31). This approach involves a more advanced commitment by faculty as well as the pupils with technology. It creates a platform for involving faculty members of a department in the era of online teaching and learning in a relaxed and healthy environment (32).

Flipped classroom has been initiated at all educational levels from Kindergarten to University and have been implemented in a variety of ways. Generally, video lectures made by the instructor or third parties are utilized to convey the content, even if online collaborative discussions, digital research and text readings could be used. There has been a significant change in the quantity of online educational videos over the last 15 years. Podcasts, YouTube, the Khan Academy and such in order to make the introduction of a flipped classroom simpler and economical (33).

Some of the active learning techniques include problem solving cases, small group learning, mini case studies, videos, clinical recreations, role playing exercises, student presentations, test based learning, debates, lab work, peer-review etc (34).

Though this method has advantages, it has also faced several challenges. Some students may have limited access to videos. For the facilitator, preparing videos is often an arduous task. There will be students who still want the traditional lecture system. They should be motivated enough to watch the videos (34).

Conclusion

Teaching in all fields has progressed a lot from the conventional lecture based system. Though the conventional lecture based system continues to be the main method in many institutes, others have adopted innovative methods for the benefits of students as well as the teachers. Teaching in dental education has also seen a wide variety of changes. As dental education continues to embrace new methodologies, it is necessary to broaden research to analyze the impact on students as well as faculty. Prior to implementing a method, the institutions should see that they have sufficient infrastructure required for the particular method. This is very important for the implemented innovation to progress in a smooth manner. Appropriate training should be given to the faculty, as well as the students, prior to implementing a teaching method. Also, efforts should be taken to determine that the implemented innovative methods are being conducted effectively, and not just for the sake of implementation. Constant monitoring should be done if the students are effectively benefitted from these methods. Feedback should be collected from the students as well as the

teachers. This is crucial in determining the effectiveness of the system, and also to address the challenges, if any.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.B., A.M., G.S.B., Design: S.B., Literature Search: S.B., A.M., G.S.B., Writing: S.B., A.M.

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Assessment of mandibular buccal shelf for an ideal miniscrew implantation site using cone-beam computed Tomography

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Keywords: Miniscrew implant, cortical bone thickness, buccal shelf area

ABSTRACT

Aims: The aim of the study was to evaluate the cortical bone thickness and bone width of the buccal shelf area and to determine the optimal sites for mini screw-implant placement.

Methods: In this retrospective study, cortical bone thickness and buccal shelf bone width of 20 patients were measured at 3 sites on both right and left sides using cone-beam computed tomography images. The three sites were the measurements were done were areas buccal to the distobuccal cusps of mandibular first molar (6D), and buccal to the mesiobuccal (7M) and distobuccal (7D) cusps of mandibular second molar. Also, buccal shelf bone width was measured at 8 and 4 mm from the cementoenamel junction (CEJ).

Results: The study group included 10 males with the mean age of 24.5 years and 10 females with the mean age of 26.5 years. There was statistically significant difference between the cortical bone thickness, between the left and the right sides in the mesiobuccal cusp of mandibular 2nd molar (MB7) and the distobuccal cusp of mandibular 2nd molar (DB7). The values obtained at MB7 on the right and left sides were 4.74±0.94 and 4.16±0.91 with p value of 0.006, respectively. Similarly, data obtained at DB7 on the right and left sides were 4.13±0.94 and 5.08±1.12 with p value of 15<0.001, respectively.

Conclusions: Maximum bone thickness was found at distal region of the mandibular second molar at 8 mm from the CEJ, hence this is the ideal site for miniscrew placement in buccal shelf area (horizontal bone level at DB7=6.01 mm and vertical bone level at DB7=4.06 mm).

Introduction

The introduction of absolute anchorage in orthodontics has changed the way we see treatment planning of an orthodontic case. It has increased the envelope of tooth movement by fixed orthodontic appliances. The position of these miniscrews is based on the mechanical requirement for a particular case (1-4). The most commonly used sites are the maxillary alveolar process, retromolar area, palate, mandibular and maxillary buccal alveolar processes (2-6).

Thickness of the cortical bone is an important factor to be considered for the stability of miniscrew implant. Inadequate bone thickness leads to diminished primary stability of the miniscrew, which will later lead to its failure during the orthodontic treatment. Less than 1 mm cortical bone thickness can lead to higher rate of failure as compared to 1 mm or more.

Cortical bone with thickness of less than 1 mm is vulnerable to stresses, which further causes bone loss in the nearby area (7-14). The two most important factors for the initial stability of the miniscrew are the quantity and quality of bone. These two factors also have the effect on the long term stability. Stationary anchorage failure occurs mostly due to diminished bone density and the

incidences further increase in case of reduced cortical bone thickness (7-14). The thickness of the bone can be studied with the help of cone-beam computed tomography (CBCT). Hence, it is better to use CBCT in bone screw cases rather than panoramic radiograph and lateral cephalogram as it fails to provide full information about the subject.

Recently, the introduction of bone screws in orthodontics has made full arch distalization and camouflage treatment easier. The buccal shelf area is a site for the placement of such a bone screw for the treatment of class 3 cases (15-18). Despite several CBCT studies, there is inconsistency in the placement site of these implants. Varied bone thickness in these areas has made the standardization difficult as the bone width and thickness change every millimeter. As we go away from the alveolar crestal region, the bone thickness and density increase.

Importance should also be given to the soft tissue and nerve relation with roots of teeth in the neighboring areas (19).

The purpose behind the study was to evaluate the thickness of the cortical bone and bone width of the buccal shelf area and thus to determine the ideal sites for the placement of miniscrew implant in the buccal shelf area of the mandible using CBCT.

Methods

This retrospective study included full field of view (FOV) CBCT records of 20 patients in the age group of 19 to 33 years, including 10 males and 10 females who were admitted to the department of Orthodontics for orthodontic treatment. Full FOV CBCT images were taken for evaluating impacted canines, for assessing sites for zygomatic implants and for the purpose of orthognathic surgeries. Informed consent was also taken from all subjects. Ethical clearance for the study was obtained from AB Shetty Memorial Institute of Dental Sciences Institutional Ethical Committee (Cert. no. ABSM/EC02/2016). The information for each image analyzed was retrieved from the records maintained at the department of oral radiology at the institute. Subjects who had full complement of teeth except for third molars with class 1 molar relation were included in the study. Subjects who had severe skeletal abnormalities and facial anomalies, periodontal disease, systemic illness and endocrinal disorders were not included in the study. The records were analyzed by a single observer.

The CBCT images were taken using Planmeca ProMax™ Machine (230-240 V, 50 Hz, 16 A) manufactured by PLANMECA OY (Helsinki Finland). Full FOV images were retrieved from already existing CBCT images. The images were analyzed using Planmeca Romexis Viewer (version 4.1.2). 2 mm cross sections of the mandible were obtained. The 3D images were reconstructed around the axial plane. These measurements were made from 4 mm and 8 mm from the cementoenamel junction (CEJ). The CEJ was customized by tracing the CEJ of each tooth individually on the coronal view.

Thickness of the cortical bone and the width of the buccal shelf bone were measured at 3 sites on both right and left sides. Measurements were done at areas buccal to the distobuccal cusps of mandibular first molar (6D), and buccal to the mesiobuccal (7M) and distobuccal (7D) cusps of mandibular second molar. Cortical bone thickness was measured (parallel to buccal root surface) from the midpoint of the osseous ledge buccal to first and second mandibular molars (buccal shelf area) (Figure 1).

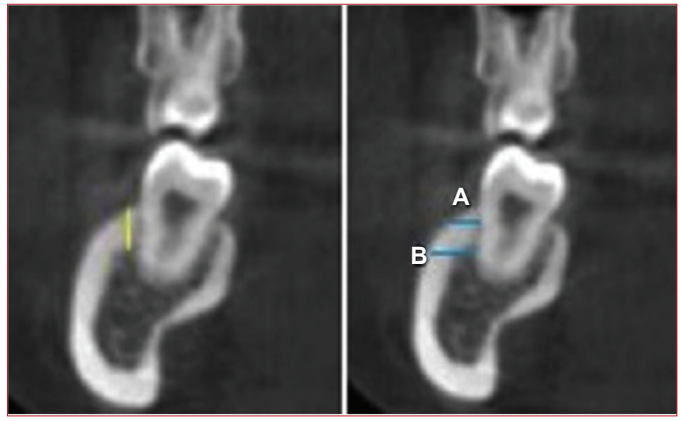

Figure 1. Measurement of horizontal and vertical cortical bone thickness

Buccal shelf bone width was the total bone width present in buccolingual direction (parallel to occlusal plane) from the root of the mandibular molars to the most buccal point of the alveolar bone at 8 and 4 mm from the CEJ. These measurements were recorded at 3 sites at 4 mm and 8 mm: buccal to the distobuccal cusp of mandibular first molar (6D4, 6D8), and buccal to the mesiobuccal (7M4, 7M8) and distobuccal (7D4, 7D8) cusps of mandibular second molar (Figure 1).

All measurements were made on the reconstructed axial plane using the “Measure Length” Tool. (Planmeca Romexis V. 4.1.2™).

Statistical Analysis

The data collected were entered in Microsoft Excel work sheet and analyzed using IBM SPSS version 22. The descriptive statistics were represented in the form of mean and standard deviation.

A paired Student’s t-test was used for additional preliminary data analysis to test the differences between the left and right sides in the cortical bone width. Analysis of variance was used to evaluate the importance of the site and measurement level on cortical bone thickness and buccal shelf bone width. The post-hoc Tukey test was used to further evaluate interactions of the different variables. Intraclass correlation was also evaluated.

Results

This retrospective study included 20 patients comprising of 10 males with the mean age of 24.5 years and 10 females with

the mean age of 26.5 years (Table 1). The horizontal and vertical bone levels between right and left sides at different sites were compared (Table 2) and there was no statistically significant difference between the left and the right sides of the cortical bone width at 4 and 8 mm from CEJ. There was statistically significant difference between the cortical bone thickness between the left and the right sides in MB7 and DB7. The values obtained at MB7 on the right and left sides were 4.74±0.94 and 4.16±0.91 with p value of 0.006, respectively. Similarly, data obtained at DB7 on the right and left sides were 4.13±0.94 and 5.08±1.12 with p value of 101<0.001, respectively.

The horizontal bone levels (cortical bone width) between different sites at 4 mm were compared (Tables 3, 4) and statistically significant difference was found among DB6, MB7 and DB7.

The maximum bone width was seen at DB7 (with average thickness of 4.79 mm) followed by MB7 (3.80 mm) and minimum levels at DB6 (1.81 mm).

Table 1. Basic data of participants

Gender	Number	Mean age (years)
Males	10	24.5
Females	10	26.5

It was also seen that when the horizontal bone levels between different sites at 8 mm (Tables 3, 4) were compared, there was statistically significant difference among the DB6, MB7 and DB7. The maximum bone width was seen at DB7 (with average thickness of 6.01 mm) followed by MB7 (4.85 mm) and minimum levels at DB6 (2.27 mm).

The vertical bone levels between different sites (Tables 3, 4) were also compared and statistically significant difference was found among the DB6, MB7 and DB7. The maximum bone width was seen at DB7 (with average thickness of 4.60 mm) followed by MB7 (4.45 mm) and minimum levels at DB6 (3.42 mm). Intraclass correlation was also evaluated for horizontal and vertical bone levels at different sites (Tables 5, 6).

Discussion

The decision for placing the miniscrew is dependent on the bio-mechanics and the local anatomy of that particular region. Local anatomy varies with different individuals, but some sites are more reliable and show more bone thickness than others (1,5,20). The two most important factors for the primary stability of the implant are the quality and quantity of bone. CBCT may be used to study this, though the gold standard for studying the quality of bone is biopsy.

Table 2. Comparison of horizontal and vertical bone levels between right and left side at different sites

Site			N	Mean	Standard deviation	Mean difference	95% confidence interval of the difference		t	df	p value
							Lower	Upper			
DB6	H-4 mm	Right	20	1.78	0.46	-0.07	-0.36	0.22	-0.50	19	0.63 (NS)
		Left	20	1.85	0.50						
	H-8 mm	Right	20	2.29	0.76	0.03	-0.49	0.55	0.13	19	0.90 (NS)
		Left	20	2.25	0.90						
	Vertical	Right	20	3.53	0.98	0.22	-0.27	0.71	0.93	19	0.36 (NS)
		Left	20	3.31	0.58						
MB7	H-4 mm	Right	20	3.58	1.15	-0.44	-1.04	0.15	-1.57	19	0.13 (NS)
		Left	20	4.02	1.45						
	H-8 mm	Right	20	4.85	1.09	0.01	-0.90	0.93	0.03	19	0.98 (NS)
		Left	20	4.84	1.84						
	Vertical	Right	20	4.74	0.94	0.57	0.18	0.96	3.07	19	0.006*
		Left	20	4.16	0.91						
DB7	H-4 mm	Right	20	4.92	0.94	0.26	-0.36	0.87	0.88	19	0.39 (NS)
		Left	20	4.66	1.51						
	H-8 mm	Right	20	5.89	0.88	-0.24	-0.78	0.30	-0.91	19	0.37 (NS)
		Left	20	6.13	1.29						
	Vertical	Right	20	4.13	0.94	-0.95	-1.40	-0.51	-4.46	19	<0.001*
		Left	20	5.08	1.12						

Paired t-test, *p<0.05 statistically significant, p>0.05.
NS: Non-significant

Table 3. Comparison of horizontal bone levels between different sites at 4 mm and 8 mm, comparison of vertical bone levels between different sites

4 mm	Sites	N	Mean	Standard deviation	ANOVA	
					F	p value
Right	DB6	20	1.78	0.46	61.82	<0.001*
	MB7	20	3.58	1.15		
	DB7	20	4.92	0.94		
Left	DB6	20	1.85	0.50	28.32	<0.001*
	MB7	20	4.02	1.45		
	DB7	20	4.66	1.51		
Average	DB6	20	1.81	0.36	53.37	<0.001*
	MB7	20	3.80	1.14		
	DB7	20	4.79	1.07		
8 mm	**Sites**	**N**	**Mean**	**Standard deviation**	**ANOVA**	
					F	**p value**
Right	DB6	20	2.29	0.76	81.35	<0.001*
	MB7	20	4.85	1.09		
	DB7	20	5.89	0.88		
Left	DB6	20	2.25	0.90	39.98	<0.001*
	MB7	20	4.84	1.84		
	DB7	20	6.13	1.29		
Average	DB6	20	2.27	0.62	84.62	<0.001*
	MB7	20	4.85	1.16		
	DB7	20	6.01	0.94		
Vertical	**Sites**	**N**	**Mean**	**Standard deviation**	**ANOVA**	
					F	**p value**
Right	DB6	20	3.53	0.98	7.97	0.001*
	MB7	20	4.74	0.94		
	DB7	20	4.13	0.94		
Left	DB6	20	3.31	0.58	19.44	<0.001*
	MB7	20	4.16	0.91		
	DB7	20	5.08	1.12		
Average	DB6	20	3.42	0.61	13.10	<0.001*
	MB7	20	4.45	0.83		
	DB7	20	4.60	0.91		

*p<0.05 statistically significant, p>0.05.
NS: Non-significant

Mandibular buccal shelf area is the area between the buccal frenum and anterior border of masseter muscle. It extends medially from the crest of the ridge, laterally to the external oblique ridge and distally up to the retromolar pad. There is significant amount of bone present in this area and thus permits clinicians to place miniscrews in a direction parallel to the long axes of the molar roots. With this mode of insertion, screw-to-root contact can be avoided during the procedure of insertion and also during retraction of the posterior tooth (21). Buccal shelf area is one of the areas which are most favorable insertion sites for the placement of miniscrew especially in class 3 cases.

The purpose of this study was to investigate the cortical bone thickness and cortical bone width at 3 sites (DB6 MD7 DB7) and two levels (4 mm and 8 mm) from the CEJ using CBCT technology in Indian population.

The findings of this study show different bone thickness and bone width in different region of buccal shelf area. The width of bone increased from the first molar region to the second molar region, which was statistically significant (p=0.006). The minimum bone width was measured with respect to first molar region (at 4 mm site=1.86 mm and at 8 mm site=2.27 mm), hence it is not suitable for miniscrew placement. The maximum

Table 4. Pairwise comparison of horizontal bone levels between different sites at 4 mm and 8 mm, pairwise comparison of vertical bone levels between different sites

4 mm	(I) Site	(J) Site	Mean difference (I-J)	Standard error	p value	95% confidence interval	
						Lower bound	Upper bound
Right	DB6	MB7	-1.80	0.28	<0.001*	-2.48	-1.12
		DB7	-3.14	0.28	<0.001*	-3.82	-2.46
	MB7	DB7	-1.34	0.28	<0.001*	-2.02	-0.66
Left	DB6	MB7	-2.18	0.39	<0.001*	-3.12	-1.23
		DB7	-2.81	0.39	<0.001*	-3.76	-1.87
	MB7	DB7	-0.64	0.39	0.24 (NS)	-1.58	0.31
Average	DB6	MB7	-1.99	0.29	<0.001*	-2.69	-1.28
		DB7	-2.98	0.29	<0.001*	-3.68	-2.27
	MB7	DB7	-0.99	0.29	0.004*	-1.69	-0.28
8 mm	**(I) Site**	**(J) Site**	**Mean difference (I-J)**	**Standard error**	**p value**	**95% confidence interval**	
						Lower bound	**Upper bound**
Right	DB6	MB7	-2.57	0.29	<0.001*	-3.27	-1.87
		DB7	-3.61	0.29	<0.001*	-4.31	-2.91
	MB7	DB7	-1.04	0.29	0.002*	-1.74	-0.34
Left	DB6	MB7	-2.59	0.44	<0.001*	-3.65	-1.53
		DB7	-3.88	0.44	<0.001*	-4.94	-2.81
	MB7	DB7	-1.29	0.44	0.01*	-2.35	-0.23
Average	DB6	MB7	-2.58	0.29	<0.001*	-3.29	-1.87
		DB7	-3.74	0.29	<0.001*	-4.45	-3.03
	MB7	DB7	-1.16	0.29	0.001*	-1.87	-0.46
Vertical	**(I) Site**	**(J) Site**	**Mean difference (I-J)**	**Standard error**	**p value**	**95% confidence interval**	
						Lower bound	**Upper bound**
Right	DB6	MB7	-1.21	0.30	0.001*	-1.93	-0.48
		DB7	-0.60	0.30	0.13 (NS)	-1.32	0.13
	MB7	DB7	0.61	0.30	0.12 (NS)	-0.12	1.33
Left	DB6	MB7	-0.85	0.28	0.01*	-1.54	-0.17
		DB7	-1.77	0.28	<0.001*	-2.45	-1.09
	MB7	DB7	-0.91	0.28	0.006*	-1.60	-0.23
Average	DB6	MB7	-1.03	0.25	<0.001*	-1.63	-0.42
		DB7	-1.18	0.25	<0.001*	-1.79	-0.58
	MB7	DB7	-0.15	0.25	0.82 (NS)	-0.76	0.45

Tukey post-hoc test, *p<0.05 statistically significant, p>0.05.
NS: Non-significant

width of bone was witnessed with respect to distobuccal cusp of second molar (at 4 mm site=4.79 mm and at 8 mm site=6.01 mm) hence it is the best region for the placement of miniscrew.

There was variation in the width of bone seen from 4 mm from CEJ to 8 mm from CEJ. The width of the bone was more on the 8 mm site compared to 4 mm site. The result of the bone width was in correlation with the studies performed by Elshebiny et al. (19) and Nucera et al (22). Bone thickness is one of the essential factors for primary stability of miniscrew and it correlates directly to the placement torque, which in turn influences the stability of the screw. Baumgaertel (23,24) confirmed the importance of cortical bone thickness in implant site preparation. Lim et al. (25) also mentioned the significant role of cortical bone thickness in the evaluation of maximum insertion torque value as it determines the stability of the implant. Various studies have proven that extreme values of cortical bone thickness can affect the insertion outcome. Areas of very thin bone will lead to stress in the bone and later lead to implant failure whereas very thick bone will have good initial stability but later will lead to implant failure due to compression of the bone. In case where there is excessive bone thickness, predrilling is recommended as it will produce lesser heat at the implant bone surface. The thickness

Table 5. Intraclass correlation coefficient-horizontal bone level

Tooth	mm	Side	Intraclass correlation	95% confidence interval	
				Lower bound	Upper bound
DB6	4	Left	0.99	0.99	1.00
		Right	0.99	0.99	1.00
	8	Left	1.00	0.99	1.00
		Right	1.00	0.99	1.00
MB7	4	Left	1.00	1.00	1.00
		Right	1.00	0.99	1.00
	8	Left	1.00	1.00	1.00
		Right	1.00	1.00	1.00
DB7	4	Left	1.00	1.00	1.00
		Right	1.00	0.99	1.00
	8	Left	1.00	1.00	1.00
		Right	1.00	0.99	1.00

Tooth	mm	Intraclass correlation Lower bound	95% confidence interval	
			Upper bound	
DB6	4	0.99	0.99	1.00
	8	1.00	0.99	1.00
MB7	4	1.00	1.00	1.00
	8	1.00	1.00	1.00
DB7	4	1.00	1.00	1.00
	8	1.00	1.00	1.00

Table 6. Intraclass correlation coefficient-vertical bone level

Tooth	mm	Side	Intraclass correlation	95% confidence interval	
				Lower bound	Upper bound
DB6	4	Left	0.99	0.99	1.00
		Right	1.00	0.99	1.00
MB7	4	Left	1.00	0.99	1.00
		Right	1.00	1.00	1.00
DB7	4	Left	1.00	0.99	1.00
		Right	1.00	0.99	1.00

Tooth	mm	Intraclass correlation Lower bound	95% confidence interval	
			Upper bound	
DB6	4	1.00	0.99	1.00
MB7	4	1.00	1.00	1.00
DB7	4	1.00	1.00	1.00

of the bone increases from mesial site to the distal as shown by other studies as well. The maximum thickness was found with respect to the distobuccal cusp of the second molar and there was a statistically significant difference among the three sites. It was in accordance with studies done by Elshebiny et al. (19) and Nucera et al. (22). The torque values used in the buccal shelf area is generally higher than other areas as the cortical bone thickness is more, but care should be taken not to exceed the recommended torque levels as it might fracture the implant. In case of excessive bone thickness, drilling should be done and then the implant should be placed.

The cortical bone thickness in the present retrospective study was done on full FOV rather than small FOV, which could provide a better image quality and smaller voxel size.

Ethnicity does play a role in the morphology of bone as the values found in this study were different from the values found in the study done by Elshebiny et al. (19) and Nucera et al. (22). This might be dependent on the facial type of patients and the skeletal malocclusion of the patient. Also, comparison of cortical bone thickness between adults and adolescent groups can be studied. Hence, it is recommended that further research in this area will give a better picture of the bone in the buccal shelf region.

Conclusion

The mandibular buccal shelf area is a suitable site for bone screw placement. With the limitation of the study, the bone buccal to the mandibular second molar region appears to be the most favorable site for the miniscrew placement. The maximum bone thickness was found at distal region of the mandibular second molar at 8 mm from the CEJ, hence this is the ideal site for miniscrew placement. The insertion of the miniscrew at the first molar region would require further investigation.

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Ethics

Ethics Committee Approval: Ethical clearance for the study was obtained from AB Shetty Memorial Institute of Dental Sciences Institutional Ethical Committee (Cert. no. ABSM/EC02/2016).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: V.K., Concept: M.P., Design: M.P., Data Collection or Processing: V.K., Analysis or Interpretation: R.S., C.R.S., S.A., Literature Search: V.K., R.S., C.R.S., S.A., Writing: V.K., S.A.

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A study of pericardial effusion in HIV positive patients and its correlation with the CD4 count

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ABSTRACT

Aims: Cardiovascular diseases in human immunodeficiency virus (HIV)-positive patients are becoming increasingly detected in developing countries and pericardial effusion is one of the common problems amongst them. The relation of CD4 count is also important, as it correlates with the severity of cardiac involvement. The present study was therefore undertaken to determine the clinical profile of pericardial effusion in HIV patients and its correlation with CD4 count.

Methods: This cross-sectional study was conducted in a tertiary care hospital from 2015 to 2017 and it included 500 HIV patients, 39 of whom had pericardial effusion. All patients were assessed clinically and had undergone electrocardiograph, echocardiography and CD4 count along with the routine investigations. The presence of pericardial effusion was determined by echocardiography. The pericardial fluid was analyzed for cells, biochemistry, Gram staining, AFB staining and cultures. An attempt was made to correlate the findings with CD4 count.

Results: Out of 500 HIV-positive patients, 39 (7.8%) were found to have pericardial effusion. Majority were male (64.1%) and middle-aged (40-65 y/o) (59%). Most of the effusions (61.54%) were quantified as large and the most common etiology was tuberculous (41%). Twenty-one patients (53.85%) were in the subgroup of CD4 count <50 cells/μL and 16 of them had severe pericardial effusion. Sixty-four percent of the patients underwent pericardial drainage and the remaining were managed medically.

Conclusions: In the present study, 7.8% of HIV-infected patients had pericardial effusion. Tuberculosis is the most common cause. The lower the CD4 count is, the larger the pericardial effusion will be.

Introduction

Pericardial disease was the most frequent clinical manifestation of cardiac disease in patients with human immunodeficiency virus (HIV) infection and, specifically, acquired immunodeficiency syndrome (AIDS). However, in developed countries with widespread access to antiretroviral therapy (ART), the incidence of symptomatic pericardial disease in HIV positive patients has declined significantly. Pericardial effusion was mainly observed in patients with AIDS (1,2). In resource-limited settings, the prevalence of pericardial disease in patients with HIV is similar to that reported from pre-ART studies (3). Nevertheless, cardiac involvement in HIV/AIDS was often underdiagnosed or attributed wrongly to other non-cardiac diseases. This is because symptoms like fatigue or reduced exercise tolerance are common in this population (1). Tuberculosis and viral infections are the most common causes of pericardial disease in developing countries (4). The normal CD4 count for most of the laboratories falls in a range of 800 to 1,050 cells/μL. A CD4 count of less than 200 cells/μL indicates the clinical stage of AIDS. Previous studies have shown that HIV related cardiac abnormalities are more frequently encountered in patients with low CD4 count. Improvement in CD4 count in response to ART has been shown to be the most important predictor of clinical outcome (5).

The present study was therefore undertaken to determine the clinical profile of pericardial effusion in HIV infection and its correlation with CD4 count.

Methods

This study was a cross-sectional study conducted in a training and research hospital in Manipal, India, for a period of one year and eight months (September 2015 - May 2017. This study included 500 HIV positive patients, 39 of whom had pericardial effusion. Institutional Ethics Committee, Kasturba Medical College (September 09, 2015, number: IEC 486/2015) approval was sought and granted. All the patients were informed in detail about the study and their written consent was obtained.

Inclusion criteria were HIV-positive patients aged more than 18 years with echocardiographic evidence of pericardial effusion. Development of pericardial effusion following cardiopulmonary surgery or post-myocardial infarction were excluded from the study.

The presenting symptoms of all the patients were recorded. All participants underwent detailed clinical examination with special attention to the cardiovascular system. Complete blood counts, liver & kidney function tests, CD4 count, chest radiograph, electrocardiograph (ECG) and 2D echocardiogram were performed for all the participants. The pericardial fluid was analyzed for Gram staining, Ziehl-Neelson (Z-N) staining, Gene-Xpert, cytology (cell count, differential counts and malignant cells), biochemistry (protein, albumin, glucose, lactic acid dehydrogenase, adenosine deaminase and pH), culture and antibiotic sensitivity. Miscellaneous investigations like thyroid function test (T3, T4 and thyroid stimulating hormone), antinuclear antibody (ANA) and anti-cyclic citrullinated peptide were done in a few selected patients. And finally, the association between the severity of pericardial effusion and CD4 count was assessed.

Statistical Analysis

All the collected data were entered in a Microsoft Excel sheet and then analyzed using SPSS software (version 15.0, SPSS South Asia Bangalore). Qualitative data were presented as frequency and percentages and analyzed using the chi-square test or Fisher's exact test (in case of 2x2 contingency tables). Quantitative data were presented as mean and standard deviation. Statistical significance was defined as $p<0.05$.

Results

Out of 500 HIV patients, 39 patients (7.8%) had pericardial effusion. The majority of the study population were males (64.1%) and belonged to the age group of 41-50 years (51.28%). Dyspnea or breathlessness is the most common presenting complaint among the study participants (92.3%) followed by palpitations (64.1%) and cough (48.7%). On cardiovascular examination, the common findings noted were tachycardia (61.5%) followed by elevated jugular venous pressure (JVP) (41%) and hypotension (25.6%). Chest X-ray showed cardiomegaly in 74.4%. Around one-half of the patients (51.3%) had sinus tachycardia followed by low voltage complexes (20.5%). Out of 39 patients, 24 (61.54%) had severe pericardial effusion, 9 (23.08%) had moderate and 6 (15.38%) patients had mild pericardial effusion. More than half (53.85%) of the study participants had a CD4 count of less than 50 cells/μL, 10 (25.64%) patients had CD4 count 50-200 cells/μL and 8 (20.51%) patients had CD4 count of more than 200 cells/μL (Table 1).

The most common etiology for pericardial effusion was tuberculosis (41%) followed by idiopathic/viral (15.4%), malignant (15.4%), purulent (10.3), chronic kidney disease (10.3), connective tissue disease (5.1) and hypothyroidism (2.6%) (Table 2). Sixty-four percent of the patients underwent pericardial drainage and the remaining were managed medically.

As evident from Table 3, out of 39 participants, 21 patients (53.85%) were in the subgroup of CD4 count less than 50 cells/μL and the majority of them presented with severe pericardial effusion (41.02%). Whereas only eight patients (20.51%) had CD4 count of more than 200/μL and half of them (four patients) had only mild effusion. The difference was statistically significant with a p-value of 0.029.

Discussion

Pericardial effusion was described as one of the common cardiac manifestations in HIV infected patients. But the number of HIV patients developing pericardial effusion is decreasing dramatically in developed countries after the introduction of Antiretroviral therapy (1). However, the incidence in resource-limited settings is similar to that reported in pre-ART studies (6). Sliwa et al. (7) conducted a study in South Africa, which included 518 HIV patients and reported a 25% prevalence of pericardial disease. However, our study showed that only 7.8% of patients with HIV had pericardial effusion, as all of them are on ART.

Symptoms range from asymptomatic to severe dyspnea of cardiac tamponade depending on the amount and rate of pericardial effusion. Among symptomatic patients, approximately one-third present with cardiac tamponade (8,9). Only a few cases of acute cardiac tamponade present with classical Beck's triad, which includes low blood pressure (hypotension), elevated JVP and muffled or absent heart sounds on auscultation. One more important finding of cardiac tamponade is pulsus paradoxus. It is defined as a fall in systolic blood pressure of more than 10 mm of Hg during inspiration, which is nothing but exaggerated normal physiology (10). In our study, the most common cardiac symptom was breathlessness (92.3%), followed by palpitations (64.1%) and cough (48.7%). On examination, the common cardiac manifestations included tachycardia (61.5%) followed by elevated JVP (41%) and hypotension (25.6%).

Table 1. Demographic, clinical and laboratory characteristics among the study population

Demographic characteristics	Frequency	Percentage (%)
Total number of patients	39	100
Age range (in years)	Mean: 44.32±SD 12.30	
Male	25	64.1
Female	14	35.9
Symptoms		
Breathlessness	36	92.3
Palpitations	25	64.1
Cough	19	48.7
Fever	18	46.1
Chest pain	9	23.1
Edema	6	15.4
Signs		
Tachycardia (HR >100 bpm)	24	61.5
Bradycardia (HR <60 bpm)	1	2.6
Elevated jugular venous pressure	16	41.0
Hypotension	10	25.6
Muffled cardiac sounds	9	23.1
Pericardial friction rub	2	5.1
Chest X-ray		
Normal chest X-ray	10	25.6
Cardiomegaly	29	74.4
Signs of pulmonary TB	2	5.1
Lung Malignancy	1	2.6
ECG findings		
Sinus tachycardia	20	51.3
Low voltage complexes	8	20.5
Electrical alternans	1	2.6
Left axis deviation	7	17.9
Echocardiography findings		
Mild pericardial effusion	6	15.38
Moderate pericardial effusion	9	23.08
Severe pericardial effusion	24	61.54
CD4 count (cells/µL)		
<50	21	53.85
50-200	10	25.64
>200	8	20.51

SD: Standard deviation, TB: Tuberculosis, ECG: Electrocardiograph, HR: Heart rate

Table 2. Etiological profile of pericardial effusion

Etiologies	Frequency	%
Tuberculosis	16	41.0
Idiopathic/viral	6	15.4
Malignancy	6	15.4
Purulent	4	10.3
CKD	4	10.3
CTD (SLE)	2	5.1
Hypothyroidism	1	2.6

CKD: Chronic kidney disease, CTD: Connective tissue disease, SLE: Systemic lupus erythematosus

Table 3. Comparison of pericardial effusion vs CD4 count amongst study population

CD4 count (cells/µL)	Pericardial effusion-frequency (%)			Total
	Mild	Moderate	Severe	
Less than 50	1 (2.56)	4 (10.25)	16 (41.02)	21 (53.85)
50-200	1 (2.56)	3 (7.69)	6 (15.38)	10 (25.64)
More than 200	4 (10.25)	2 (5.13)	2 (5.13)	8 (20.51)
Total	6 (15.38)	9 (23.08)	24 (61.54)	39 (100)

The chi-square statistic is 10.7998. The p-value is 0.029

The ECG changes seen in pericardial effusion are low voltage, diffuse ST-segment elevation and total electrical alternans. ST-segment elevation occurs because of the involvement of myocardial inflammation. Total electrical alternans refers to the alternating high and low voltages of all ECG waveforms between cardiac cycles within a given lead due to swinging of the heart in the pericardial fluid (11,12). Low voltage QRS complex is said to be present when the total amplitude of the QRS complex is less than 5 mm (0.5 mV) in limb leads (lead I, II, III, aVL, aVF, aVR) and QRS amplitude less than 10 mm (1 mV) in chest leads (V1 to V6). Pericardial effusion with cardiac tamponade should be suspected when there is a combination of low voltage complexes and sinus tachycardia in the ECG (13). In the present study, the most common abnormal ECG finding was sinus tachycardia (51.3%) followed by low voltage complexes (20.5%). One patient with massive pericardial effusion had total electrical alternans.

The findings on chest X-ray are inconsistent, based on the etiology and size of the effusion and underlying cardiac illness. Effusion volume less than 200 to 300 mL (small to moderate effusion) may not be visible in chest X-ray at all, while larger pericardial effusion is classically present with an enlarged cardiac silhouette. Lung fields are usually clear unless there is an associated heart failure. However, these findings on chest X-ray are neither sensitive nor specific for the diagnosis of pericardial effusion (14). In the present study, two-third of the study population was found to have cardiomegaly in chest X-ray.

The pericardial effusion is usually graded into mild, moderate and severe using echocardiography and it is measured in diastole. When the effusion is seen only posteriorly with a thickness of less than 10 mm, it is classified as mild effusion (50 to 100 mL). Moderate effusion (100 to 500 mL) is seen along the length of the posterior wall but not anteriorly and the thickness is 10 to 20 mm. If the effusion is seen circumferentially and the thickness greater than 20 mm, it is said to be large (>500 mL) (14). In the present study, 24 patients (61.54%) presented with large pericardial effusion.

Among asymptomatic patients, most of the time, the exact etiology of pericardial effusion is not known or not identified as they rarely require pericardiocentesis. Infection and neoplasm constitute approximately two-thirds of the causes among symptomatic patients (2). Infectious causes vary with geography. In developing countries, tuberculosis is the most common cause of pericardial effusion. By contrast, in resource-rich settings, less than 5 percent of pericarditis in HIV is due to tuberculosis (15,16). Other causes for pericardial effusion in HIV patients include *Staphylococcus aureus, Streptococcus pneumoniae, Listeria, Chlamydia species, Cryptococcus, Nocardia, Aspergillus* and neoplasms such as lymphoma and Kaposi's sarcoma (KS) (17,18). Even in our study, the most common etiology for pericardial effusion was tuberculosis (41%) followed by idiopathic/viral (15.4%), malignant (15.4%) and purulent (10.3%) effusion. Other rare causes we encountered were chronic kidney disease (10.3%), connective tissue disease (5.1%) and hypothyroidism (2.6%). Two patients with connective tissue disease had systemic lupus erythematosus and both of them had high titers of ANA and anti-ds DNA.

The most common cause of malignant pericardial effusion in HIV positive patients is AIDS-related KS. However, with the advent of potent ART, the incidence of KS has reduced significantly. Other common primary tumours involving the pericardium are lung, breast and esophageal malignancies (8,19). In the present study, out of 6 malignant pericardial effusions, three patients had lung cancer, two patients had breast cancer and the remaining one patient had AIDS-related KS.

The treatment of pericardial effusion in patients with HIV varies depending on the severity and etiology of the disease. Asymptomatic patients with small pericardial effusion without tamponade require no intervention, but they should be followed-up to look for any progression of the disease. By contrast, symptomatic patients with a large effusion require pericardiocentesis and further treatment. Cardiac tamponade is a medical emergency and requires immediate drainage (20,21). Further treatment is warranted based on the identified or suspected etiology. For example, anti-tubercular therapy is indicated in tubercular pericardial effusion (22,23). Purulent pericardial effusion is treated with intravenous antimicrobial therapy. The empirical antibiotic therapy should cover both Gram-positive and Gram-negative bacterial pathogens. The most commonly used antibiotics are Vancomycin plus 3rd generation cephalosporins like Ceftriaxone or a carbapenem such as imipenem. Intravenous antibiotic therapy should be continued for approximately two to four weeks until the resolution of all clinical signs of infection. Cardiac tamponade can be drained either by pericardiocentesis (i.e. echocardiography guided percutaneous drainage) or by surgical drainage. Both are highly effective in the removal of fluid and relief of symptoms related to hemodynamic compromise. According to the 2015 European Society of Cardiology guidelines on pericardial effusion, catheter pericardiocentesis is the preferred treatment in most patients. An indwelling catheter is usually placed in the pericardial space until the daily pericardial fluid drain is less than 25 mL. Whereas, neoplastic effusion requires an extended period of drainage (14). In the present study, 64% of the patients underwent pericardial drainage and the remaining cases were managed medically.

The occurrence of pericardial effusion in an HIV positive patient is generally considered as a poor prognostic sign. Previous studies have shown that HIV related cardiac abnormalities are more frequently encountered in patients with low CD4 count. There are several potential explanations existing for this observation. Low CD4 count suggests the state of severe immunosuppression and development of pericardial effusion may be a marker for undiagnosed opportunistic infections (5). Previous studies have reported that in patients with AIDS with pericardial effusion, CD4 counts were significantly lower than in those without effusion. The pericardial effusion with tamponade in HIV patients is associated with low CD4 count and may be a marker of end-stage infection (18,19,21). Even in our study, 21 patients had a CD4 count of less than 50 cells/μL and the majority of them (16 patients) presented with severe pericardial effusion.

The sample size of the present study is relatively small. The exact etiology of mild and few moderate pericardial effusions were uncertain as they were managed conservatively without pericardiocentesis. Larger follow-up studies are required for a better understanding of pericardial effusion complications and survival amongst HIV positive patients.

Conclusion

In the present study, 7.8% of HIV-infected patients had pericardial effusion. Tuberculosis is the most common cause. The management of pericardial effusion was directed at the etiology and one-third of them were managed conservatively. The lower the CD4 count is, the larger the pericardial effusion will be.

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Ethics

Ethics Committee Approval: Approval from the Institutional Ethics Committee, Kasturba Medical College (September 09, 2015, number: IEC 486/2015) was sought and granted.

Informed Consent: Written informed consent was obtained from all the participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.A.S., S.M.P., Design: G.V.S., Data Collection or Processing: C.T.R., G.V.S., Analysis or Interpretation: S.M.P., A.D., Literature Search: C.T.R., S.M.P., Writing: S.M.P., C.T.R.

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Importance of vitamin D among patients with recurrent acute otitis externa

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ABSTRACT

Aims: The aim of this study is to analyze blood serum 25-OH vitamin D levels among patients diagnosed with recurrent acute otitis externa (OE).

Methods: This research was conducted among a total of 147 patients having applied to Private Corlu Reyap Hospital and Private Kesan Hospital Otorhinolaryngology Outpatient Clinic. 59 patients within the age range of 18-45 years (mean age 32.3 years) with recurrent acute OE were selected. Patients whose one of the ears displayed recurrent OE at least three or more times in the last two years were included in the scope of this research for which two groups were formed. Patients were expected to have no vitamin D intake 30 days prior to diagnosis. Patients were examined to estimate serum blood levels of 25-OH vitamin D.

Results: 88 patients with no recurrent acute OE were placed into the control group (1) whereas 59 patients with recurrent acute OE story were placed into group (2). 25-OH vitamin D results of group (1) varied between 15 and 54 ng/mL and 25-OH vitamin D results in group (2) moved in between 3 and 42 ng/mL. In the control group (1), the mean ratio was 32.13, the mean standard error was 0.826. In group (2), the mean ratio was 20.54 and the mean standard error was 0.896. Compared to the control group, serum 25-OH vitamin D levels in recurrent OE group were measured to be in a statistically low level (p=0.000).

Conclusions: This study shows the relationship between vitamin D deficiency and recurrent acute OE.

Introduction

Meatus acusticus externus is an anatomic formation with an approximate length of 2.5 cm and under normal circumstances, it manifests an ability to enable external epithelial migration with itself cleaning capacity. About one-third of external canal consists of cartilage formation, fat layer, apocrine glands and pilar formations. Thanks to its antimicrobial lysozyme characteristic, apocrine glands produce a cerumen layer offering protection. Cerumen has a 6.9 of pH level which can stop microbial growth (1). Otitis externa (OE) is defined as an inflammation measured at different degrees on the external surface of auricula, meatus acusticus externus or tympanic membrane (2). It was defined in 1844 by Mayer (3) at first. OE is a disease that affects daily life. Just in one year, it can affect every four people out of 1000. Diagnosis is made upon a physical examination that involves autoscopy concomitant with patient history. Clinical indications are characterized by irritation, pain and erythematous meatus acusticus externus. As the disease progresses, otorrhea and conductive hearing loss may develop (4). OE is categorized under four groups as; localized or pervasive, acute or chronic (5). Acute form of OE is primarily bacteria derived (90% of all cases). It can also develop due to fungi, viral and allergic dermatological disorders. In OE formation, some of inducing factors are water exposure on external ear, high temperature or high humidity in the environment, lowness or absence of cerumen, continuous stimulation with foreign objects in external auditory canal, hearing aids plugged into external auditory canal, ear plugs, psoriasis, dermatitis and radiation therapy (6).

Vitamin D plays a vital role in human body. In recent studies, the relationship between vitamin D deficiency and otologic

disorders has been documented. Vitamin D deficiency has been associated with otitis media with effusion (7), retraction pocket formation and cholesteatoma (8), tympanostomy tubes (9), allergic dermatitis (10) and chronic otitis media (11). In many of the human tissues and human cells, vitamin D as exists 1,25-dehydroxcolecalciferol [1,25-$(OH)_2D_3$] or cholecalciferol and its active form is recognized as vitamin D_3 (12). It manifests anti-inflammatory and immunomodulator function and displays vitamin D and vitamin D binding protein (VDBP) (13). Vitamin D creates its effect through vitamin D receptor (14). Vitamin D decreases the expression of matrix metalloproteinase (MMPs) (15) considered to be secreted by the skin colonizer bacteria in fibroblast (HFL-1) cells; therefore, vitamin D causes inhibition in MMP activity (16). Studies have shown that inflammatory skin disorders of vitamin D are partially mediated by human neutrophile elastase (HNE) (17) and MMPs (18).

In this study, we aim to demonstrate the importance of 25-OH vitamin D levels among patients with recurrent acute OE. By means of this research, it will be feasible to prevent potential complications due to recurrent acute OE and diminish labor force losses as well as treatment costs. Best of our literature review has validated that there is not yet any research exhibiting the relationship between recurrent acute OE and 25-OH vitamin D levels.

Methods

This research was conducted among a total of 147 patients having applied to Private Corlu Reyap Hospital and Private Kesan Hospital Otorhinolaryngology Outpatient Clinic between June 2019 and December 2019. Fifty-nine patients within the age range of 18-45 years (mean age 32.3 years) with recurrent acute OE were selected. Recurrent acute OE diagnosis of our patients was based on patient history and physical examination that involved autoscopy. Patients whose one of the ears displayed recurrent OE at least three or more times in the last two years were included in the scope of this research for which two groups were formed as those having received recurrent OE diagnosis and those not having recurrent OE diagnosis. Fifty-nine patients were diagnosed with recurrent acute OE and 88 control group patients were examined to estimate serum blood levels of 25-OH vitamin D. Blood serum levels of the patients were tested in otorhinolaryngology outpatient clinic and OE diagnoses were recorded for all patient groups.

The control group consisted of patients having applied with complaints irrelevant to OE, whose serum 25-OH vitamin D levels were measured, and the control group patients were selected from those who did not filed any hospital application due to OE two years before the visit.

Inclusion criteria: Those having received recurrent acute OE diagnosis based on patient history and examination.

Exclusion criteria: Having a systemic disease, having a chronic disease except recurrent acute OE disease, receiving vitamin D therapy within the last 30 days, neuromuscular and immunological disorders, craniofacial and defined genetic abnormalities. Patients below 18 and above 45 years were also excluded. Research protocol was approved by the Ethics Committee (protocol no: 2020.08.01.08, date: 04.02.2020) of Namik Kemal University Medical Faculty and written informed consents of the patients were also collected.

In every group, the mean age of the patients was as follows; control group (1): 31.58±5.688 years; group (2): 32.95±7.160 years.

Blood Serum Collection and Biochemical Analyses

Prior to the test measurement, 8-12 hours of hunger is required. Analyses of serum blood levels were performed and serum levels of 25-hydroxyvitamin D_3 [$25(OH)D_3$] were included in the study. Blood samples were taken from venous blood. To quantify the 25-OH vitamin D chemiluminescence microparticle immuno examination (CMIA) measurement system, an Alinity i (Abbott) device and 25-OH vitamin D reagent kit 08P45 were used. Blood samples, anti-vitamin D coated paramagnetic microparticular and diluents were mixed and incubated. Existing 25-OH vitamin D in the sample was removed from the VDBP and bound to microparticules coated with anti-vitamin D. To form a reaction mixture, vitamin D acridinium marked conjugate was added. After one washing session, pre-trigger and trigger solutions were mixed in the solution. Lastly, the obtained chemiluminescence reaction was measured in relative light unit (RLU) form. There was a relationship between the 25-OH vitamin D amount in the sample and RLU detected by the optical components of the system. In the serum, the targeted vitamin D range was a minimum of 30-40 ng/mL.

Statistical Analysis

Statistical analysis was performed via SPSS 22 software program. Intergroup comparisons were conducted by employing independent t-test. Accuracy of the test was computed as 95%. $P<0.05$ was accepted as the statistically significant level.

Results

This research provides 25-OH vitamin D levels of a total of 147 patients. 25-OH vitamin D results of group (1) varied between 15 and 54 ng/mL and 25-OH vitamin D results in group (2) moved in between 3 and 42 ng/mL. In the control group (1), the mean ratio was 32.13, the mean standard error was 0.826. In group (2), the mean ratio was 20.54 and the mean standard error was 0.896. In this study, the mean age of the control group (1) patients was 31.58±5.688 years. The mean age of patients with recurrent acute OE in group (2) was 32.95±7.160 years. Compared to the control group, serum 25-OH vitamin D levels in recurrent OE group were measured to be in a statistically

low level (p=0.000). Research groups were designed as below (Table 1, Figure 1).

Table 1. The serum [25(OH)D_3] levels of patients. Comparison of serum [25(OH)D_3] levels

	Control group (1) (n=88)	Group (2) (n=59)	p*
25-OH vitamin D (mean±standard error mean)	32.13±0.826	20.54±0.896	0.000

Control group (1): Patients with no recurrent acute OE, group (2): Patients with recurrent acute OE.
Control group (1) (n=88) and group (2) (n=59), *p<0.0001.
OE: Otitis externa

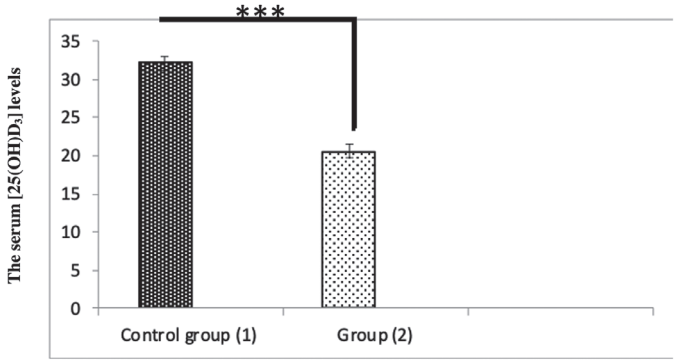

Figure 1. The serum [25(OH)D_3] levels of patients. Comparison of serum [25(OH)D_3] levels control group (1) (n=88) and group (2) (n=59) ***p<0.0001

Discussion

A review of relevant literature shows that there is not yet any research having examined 25-OH vitamin D levels among patients with recurrent acute OE. In our research, we detected that blood serum 25-OH vitamin D levels of patients with recurrent acute OE were in statistically significantly low level.

25-OH vitamin D is the best indicator of vitamin D level in our body. Today, 25-OH vitamin D level below 20 ng/mL in human blood is accepted as deficiency, 20-30 ng/mL level is accepted as inadequacy and ≥30 ng/mL level as adequate. Thresholds and significance of these values are based on a myriad of studies and a range of reference guidelines (19). Among our patients who had recurrent acute OE, vitamin D results were measured as 20.54 ng/mL. In another research, it was reported that 50 percent of the children with otitis media with effusion displayed vitamin D levels below 50 nmol/L (9).

Recurrent acute OE diagnosis of our patients was made based on patient history and a physical examination that entailed autoscopy. Patients whose one of the ears displayed recurrent OE at least three or more times in the last two years were included in the scope of this research. Chronic OE is a clinical manifestation lasting longer than four weeks or displaying four or more attacks in one year term (20). As for our patients, clinical manifestation of OE continued shorter than four weeks. In our research, clinical indications among the patients were characterized by pain, irritation and erythematous external canal. Thirteen of all cases (22%) manifested seropurulent discharge and conductive hearing loss. Among 45 (76.2%) recurrent acute OE cases, we detected very intense ear pain. Among five patients (8%), there was pain in jaw joint part in tandem with difficulty in chewing. In our cases, 40 patients (67.7%) maintained cerumen insufficiency. A conducted research proved the protective role of cerumen in recurrent OE (21). Despite genetic disposition, age-related factors that call for individual metabolism are equally effective in cerumen manifestation. In our cases, there was not any family history and 15 cases (25.4%) shared their swimming-related history in our research.

The mean age of our patients was computed as 32.95±7.160 years. Although OE can be monitored in every age group, it is mostly detected above the age of two years, mainly observed between the ages of 5 and 14 years (22). In acute OE progress, the history of using a hearing aid is vital since its usage increases a predisposition for OE formation. None of our patients had a background in using a hearing aid. OE is more common in regions with a hot and humid climate, while our research was taken place in cool regions. Despite the presence of regional variations, a vast majority of OE cases emerge during summer season and between June and August. Our cases were evaluated between the months June and December.

In recurrent chronic otorhinolaryngology disorders, bacterial biofilms were found to be related to acute otitis media, chronic tonsillitis and chronic rhinosinusitis (23-25). Human cathelicidin linked with vitamin D is a peptide termed as LL-37 and displays a bactericide activity. *In vitro* studies showed that breakdown product of human cathelicidin's free C-terminal LL-37 could stop the formation of *Pseudomonas aeruginosa* biofilms (26). Our previous research also displayed the effect of n-acetylcysteine and acetylsalicylic acid on bacterial biofilm (27).

In an international research, it was reported that among adults in New Zealand, serum vitamin D concentrations were typically at maximum level in March and minimum level in August (28). In our research, blood sampling was performed during June and December when vitamin D levels were expected to be in the midrange.

Conducted studies have revealed that vitamin D plays a critical mediator role in the production of antimicrobial peptides (cathelicidins and defensins) (29). It was also reported that vitamin D significantly elevates the production of cathelicidin, which has a direct antimicrobial function in keratinocytes (30). In another research, findings have proved that high MMP levels in ear canal and interrelation with HNE levels could play a role in the chronic OE pathogenesis (31). We hold the belief

that vitamin D deficiency played a role through the means of cathelicidin, MMP and HNE in recurrent acute OE.

For treatment, we applied extensive local hygiene, topical antibiotics, systemic antimicrobial therapy and anti-inflammatory agent to our patients. Besides, in the treatment of OEs chronic form, debridement, acidification and topical corticosteroid applications are well reported in the literature (32).

Chronic OE has been associated with low life quality (33). Its negative influence on the life quality was also declared by our patients. It is suggested to conduct a differential diagnosis between OE and middle ear disorder, malignant OE and cholesteatoma (34). Results of our research suggests that 25-OH vitamin D deficiency is an age and gender independent factor that can promote the growth of recurrent acute OE.

It should be noted that there are certain factors limiting this research. First, we conducted this study with a limited number of patients. Second limitation is not having measured MMPs and VEGF levels.

Conclusion

In this research, we attempted to manifest vitamin D levels among patients with recurrent acute OE and we demonstrated clinical significance of vitamin D. In recurrent acute OE, the cause of resistance against medical treatment could be related to low vitamin D levels. Future prospective studies on vitamin D could better illustrate the importance of these vitamins in the resistance against medical treatment in case of otorhinolaryngologic disorders and emergence of recurrent attacks. Having some awareness on the relationship of vitamin D with recurrent acute OE would play a salient role in preventing complications of disorders, lowering labor force losses and treatment costs. For the patients with recurrent acute OE and vitamin D deficiency, vitamin D supplementation could ameliorate characteristic clinical indications of the disease. In the future, researches with larger quantities of patients could provide further contributions to the topic.

Ethics

Ethics Committee Approval: Research protocol was approved by the Ethics Committee (protocol no: 2020.08.01.08, date: 04.02.2020) of Namık Kemal University Medical Faculty.

Informed Consent: Written informed consent of the patients was collected.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.B., Design: K.K., Data Collection or Processing: F.B., K.K., Analysis or Interpretation: F.B., Literature Search: F.B., Writing: A.T.

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Examining the effect of *in vitro* toothbrushing and the effect of different whitening toothpaste usages on the color change of a nanofilled composite

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Keywords: Composites, discoloration, whitening, toothpaste

ABSTRACT

Aims: Whitening has recently been one of the most demanded treatments in modern dentistry practice. The authors tested three hypotheses related to color changes on universal nanofilled composite resin samples promoted by *in vitro* tooth brushing with the usage of different whitening toothpastes.

Methods: For this *in vitro* study, a total of 8 different whitening toothpaste test groups and a control group, where each test group included 10 randomly selected composite resin samples, were formed. After all composite samples were light cured and polished, they were stored in distilled water, kept immersed in red wine and subjected to brushing, consecutively. Colors of the composite samples were measured by using a colorimeter device after each process.

Results: For all test groups, we observed that the discolorations on the samples due to immersion in red wine were reduced significantly after brushing with or without a toothpaste. Color improvement promoted by one whitening toothpaste brand containing hydrogen peroxide as bleaching agent was significantly higher than in the control group. Statistically similar color improvement values were observed among whitening toothpaste test groups, when compared to each other.

Conclusions: From the results of our study, it can be concluded that: Firstly, only one of the whitening toothpastes showed statistically significant color improvement over the control group; secondly, the color improvements promoted by whitening toothpastes, used in this study, were statistically similar.

Introduction

Dental whitening has recently been one of the most demanded treatments in modern dentistry practice. Individuals desire a whiter tooth color to have an attractive smile (1). The most important disadvantage of composite resins, commonly used in the teeth restorations due to increasing interest in dental aesthetics, is the colorations caused by internal and external factors (2,3). The internal factors that cause coloration are chemical dissolution of the resin matrix, the structure of the composite resins (4) and the fillers due to incomplete polymerization (5). Extrinsic factors, on the other hand, create color change on the outer surface (6). In the literature, it has been shown that chromogenic beverages such as red wine and coffee can lead to surface colorations on composite restorations (4,7-9).

These surface colorations in composite restorations can be partially or completely eliminated by brushing with toothpaste (10). Among the methods that people use to have better aesthetic appearances, using whitening toothpastes is very common.

In order to provide the teeth to be perceived whiter, optical agents such as blue pigment (blue covarine) are added into the ingredients of some toothpastes. This type of toothpastes allows the teeth to appear whiter right after brushing, by enabling the optical agents, which create a yellow-to-blue color change in the teeth, to be stored on the tooth surface. This has been proven in many clinical and *in vitro* studies (11,12).

The bleaching process takes place mainly by the conversion of peroxides into free radicals. During the bleaching process, long-chained organic molecules that cause coloration are oxidized by peroxide agents with free oxygen and separated into smaller molecules with lighter colors. These compounds are rendered colorless by cleavage of double bonds in the chain, separation of compounds, or oxidation of other chemical structures (13). Hydrogen peroxide is added to some whitening toothpaste ingredients in low concentrations to benefit from this feature of peroxides.

The charcoal, the first use of which for oral hygiene dates back to Hippocrates of Ancient Greek (14), is included in the formulas of some whitening toothpastes to improve bleaching efficiency. However, in the literature, it is stated that there is not sufficient scientific evidence for the cosmetic and health benefits of commercially available charcoal-based toothpastes (15).

The enzyme, included in whitening toothpastes, provides a whitening effect as a result of removing the stained biological film layer by breaking down the organic molecules of the pellicle (16).

Hypotheses Tested

In this *in vitro* study, we tested three hypotheses: First, stating that the color improvement promoted by brushing (with or without toothpaste) would be statistically significant; second, stating that the color improvement promoted by whitening toothpaste would be significantly higher than the one without toothpaste (control group); last, stating that the color change promoted by different whitening toothpaste brands would not be significant when compared to each other.

Methods

In this *in vitro* study, we used a light-curing universal nanofilled composite resin (Filtek Ultimate Universal Restorative A1 Enamel - 3M ESPE, St. Paul, USA) as aesthetic dental restorative material.

We used the following whitening toothpastes; R.O.C.S. Sensation Whitening (EuroCosMed, Russia), Splat Blackwood (Splat-Cosmetica, Moscow, Russia), Curaprox Black is White (Curaden Pharma GmbH, Switzerland), Colgate Optic White (Colgate, New York, USA), Signal White Now CC (Unilever, France), Parodondax Whitening (GlaxoSmithKline, Middlesex, UK), Sensodyne True White (GlaxoSmithKline, EU) and Beverly Hills Formula Perfect White Black (Purity Laboratories, Ireland). The bleaching ingredients contained in whitening toothpastes used in our study are shown in Table 1.

Preparation of Samples and Test Groups

90 disc-shaped composite samples (n=90) were prepared in the metal mold with a diameter of 6 mm and a thickness of 2 mm (17). After covering transparent mylar strip on samples, they were light cured under a 1 mm glass lamina for 20 seconds by using the Woodpecker LED-B light device (Guilin Woodpecker, Guangxi, China). Then, for the finishing and polishing of each composite sample; dry, medium, fine and superfine OptiDisk (Part No. 4200, Kerr, Switzerland) polishing discs were used for 10 seconds each, consecutively. We polished the same side of the samples and put a mark on the non-polished sides for standardization. All samples were stored in distilled water for 4 weeks at room temperature (18). A total of 9 test groups, each including 10 randomly selected (n=10) composite samples, were formed to test 8 different whitening toothpastes and one control group (artificial saliva). All samples were kept in closed containers at room temperature during every step of the study.

Measuring the Color Change

Surface color was measured three times on the center of the polished side of each sample by using a colorimeter device (Minolta CR-321, Osaka, Japan) and the mean values were recorded in every step of the study. Before each measurement cycle, the colorimeter device was calibrated for L, a, b values (L=93.05, a=-4.84, b=6.95). Calibrations were performed in the same medium using the white calibration plate. The standard

Table 1. Bleaching ingredients contained in whitening toothpastes used in the study

Whitening toothpastes	Charcoal	Enzyme	Optical agent	Hydrogen peroxide	Pentasodium triphosphate	Silica	Hydrated silica
R.O.C.S. Sensation Whitening	-	+	-	-	-	+	-
Splat Blackwood	+	-	-	-	-	-	+
Curaprox Black is White	+	-	+	-	-	-	+
Colgate Optic White	-	-	-	+	-	+	-
Signal White Now CC	-	-	+	-	-	-	+
Parodondax Whitening	-	-	-	-	+	-	+
Sensodyne True White	-	-	-	-	+	-	+
Beverly Hills Formula Perfect White Black	+	-	-	-	-	-	+

white background was used during the measurements of the composite samples and the calibration of the instrument was checked before each measurement.

After all samples in all test groups had been kept immersed in artificial saliva for 24 hours at room temperature to help the formation of a pellicle, initial color values of the samples were measured.

Following to this process, the samples were kept immersed in red wine (Kalecik Karası 2014, contains 12% alcohol) for 24 hours; washed with tap water for 10 seconds; and dried with paper, and the color measurements mentioned above were done for the second time.

At the last step, composite samples belonging to different test groups were brushed with different whitening toothpastes dedicated to that group. On the other hand, the samples in the control group were brushed only with artificial saliva. Toothpastes were mixed with artificial saliva at a rate of ½ (by weight) before brushing. We used Oral-B Genius 8000 (Braun, Germany) electric toothbrush with Oral-B CrossAction (Braun, Germany) brush head. Composite resins were subjected to brushing manually for 1 min with a standardized pressure (pressure sensor gives a visual notification through the LED SmartRing, which illuminates red when too much pressure is applied) and 10,500 oscillations/rotations per minute. The samples were washed with tap water for 10 seconds and dried with paper, and then, the final color measurements were done.

Statistical Analysis

Statistical analyses were performed with SPSS 22.0 (Statistical Package for Social Sciences, IBM Inc., USA) software. To evaluate the color change in test groups after each process (immersed in red wine, brushing), dependent samples t-test (paired-t) was employed. The differences between test groups were analyzed by using ANOVA and Tukey post-hoc tests. The results were evaluated at ($p<0.05$) significance level.

Results

It was observed that the two consecutive processes, immersion in red wine for 24 hours and brushing with whitening toothpaste, affected the color of all samples, where ΔE1 shows the color change after immersion in red wine and ΔE2 shows the color change after brushing, both measured against the initial color of the samples. For all test groups, we observed that the discolorations on the samples which occurred after immersion in red wine were significantly reduced after brushing (with or without toothpaste), but not to the clinically acceptable level (ΔE2 ≥3.3 for all test groups) (Table 2).

Regarding the effects of different whitening toothpaste brands on color change, it was observed that only the color improvement promoted by Colgate Optic White group was significantly higher than in the control group. Color improvement promoted by other whitening test groups was nonsignificant when compared to the control group (Table 3).

It was observed that the color improvement promoted by different whitening toothpaste brands was statistically similar, when compared to each other (Table 3).

Among the whitening toothpastes, the one containing hydrogen peroxide (Colgate Optic White) provided the best color improvement, whereas the one containing pentasodium triphosphate (Parodondax Whitening) provided the poorest color improvement (Table 1, 3).

Discussion

Whitening toothpastes have taken place among the dental whitening products that individuals can easily reach and apply as a result of their increased aesthetic expectations (19). However, the physical and chemical effects of whitening toothpastes, containing different active ingredients, on the composites commonly used in the restoration of teeth are not well known.

In this study, we investigated the change in color of the samples of universal nanofilled composite resin material, which were immersed in red wine, after being brushed with eight different brands of whitening toothpastes.

Table 2. ΔE1 and ΔE2 average and standard deviation

Test groups	ΔE1 (color change after immersion in red wine, compared to initial color)	ΔE2 (color change after brushing, compared to initial color)	p
R.O.C.S. Sensation Whitening	9.65±1.604	5.86±1.55	0.000*
Splat Blackwood	9.61±2.257	5.78±1.367	0.000*
Curaprox Black is White	10.27±2.325	6.55±2.193	0.004*
Colgate Optic White	11.33±1.967	6.87±1.923	0.000*
Signal White Now CC	9.35±0.884	6.02±1.961	0.000*
Parodondax Whitening	9.59±3.293	5.78±1.062	0.001*
Sensodyne True White	11.10±1.444	7.71±2.509	0.000*
Beverly Hills Formula Perfect White Black	9.76±1.085	6.90±1.702	0.001*
Control Group (artificial saliva)	9.87±3.141	7.75±3.586	0.003*

*There is statistically significant difference ($p<0.05$)

Table 3. ΔE3 average and standard deviation

Test groups	ΔE3 (color change after brushing, compared to immersion in red wine)	p
R.O.C.S. Sensation Whitening	4.63±1.218 a	0.008*
Splat Blackwood	5.64±1.973 a	
Curaprox Black is White	6.32±2.395 a	
Colgate Optic White	7.10±2.855 ab	
Signal White Now CC	5.49±1.169 a	
Parodondax Whitening	4.47±2.329 a	
Sensodyne True White	4.49±1.188 a	
Beverly Hills Formula Perfect White Black	5.13±1.421 a	
Control Group (artificial saliva)	4.00±1.341 ac	

*The same letter represents statistical similarity. Different letters indicate statistically significant difference (p<0.05)

The changes in the color of composite resins used in restorations, particularly in the anterior region, due to internal and external factors cause patient dissatisfaction and this is considered to be the aesthetic deficiency of materials (20,21).

External factors causing the coloration on resin materials are various colored solutions such as red wine, coffee, cola and tea (5). In some studies, it has been stated that tea and coffee are major causes of coloration, while some other studies have reported that red wine increases coloration the most (5,6,8). In our study, red wine was used for coloring the composite samples.

In our study, although two toothpastes (Curaprox Black is White and Signal White Now) containing optical agent as a bleaching agent partially removed the wine stains on the samples, the color improvement they provided was not at clinically acceptable level (ΔE ≥3.3).

In a study held by Philpotts et al. (18) (2017), investigating the effect of silica-based whitening toothpaste, containing blue covariant, on the color of the aesthetic restorations on extracted human teeth, the red wine stains on the samples were partially removed by a silica-based toothpaste; however, complete removal of the stains could not be achieved. In our study, two silica-based toothpastes (R.O.C.S. Sensation Whitening - ΔE2=5.86±1.55; Colgate Optic White - ΔE2=6.87±1.92) partially removed the red wine stains on the samples, but the color improvement they promoted was not at clinically acceptable level (ΔE ≥3.3), similar to those observed by Philpotts et al. (18) (2017).

In the literature, it is stated that there is not sufficient scientific evidence for the cosmetic and health benefits of commercially available charcoal-based toothpastes (15). In our study, the charcoal-based toothpastes that we investigated showed statistically nonsignificant color improvement compared to other whitening toothpastes.

Although it is stated in the literature (22) that the enzyme-containing toothpastes significantly reduce external staining compared to others, the enzyme-containing toothpaste used in our study (R.O.C.S. Sensation Whitening) did not show a significant superiority in terms of the removal of the stains compared to those without enzymes.

Atalayın et al. (23) (2018) applied a one-year brushing simulation with three different whitening toothpastes (Pro-Expert Strong Teeth-İpana, 3D White Luxe Perfection-İpana containing hydrated silica as abrasive ingredient; White Ruscello-GC containing calcium carbonate as abrasive ingredient) in their *in vitro* study, examining the after-brushing color changes of the four different composite resin samples (Componeer-Coltene, Brillant Ever Glow-Coltene, Essensia-GC, Harmonize-Kerr) stained with coffee. They observed that stains in all groups were removed at the clinically acceptable level (ΔE ≤2). In our study, however, the stains on the samples could not be eliminated at the clinically acceptable level after brushing with the toothpastes. This is considered to be due to the fact that the brushing simulation time (which is 15 days) applied in our study was much shorter.

Omata et al. (6) (2006), in their study on the coloration of microhybrid resin composite (Clearfil AP-X) samples, observed that the red wine stains were reduced when the stained composite samples were brushed for 10 seconds, with an electric toothbrush soaked in water without any toothpaste. Similarly, in our study, when the composite samples in the control group were brushed for 1 minute, with electric toothbrush soaked in artificial saliva, the red wine stains decreased.

In our study, it was observed that Colgate Optic White toothpaste, which contains hydrogen peroxide as bleaching agent, provided the best color improvement (ΔE3=7.10±2.85), which was significantly higher than in the control group (artificial saliva).

The current study had some limitations. We should consider that *in vitro* studies are difficult to fully reflect the oral environment. This situation may be related to the accuracy of the results about color changes on universal nanofilled composite resin samples, promoted by whitening toothpastes.

Conclusion

According to the results obtained in the present study, considering the *in vitro* study restrictions and regarding the hypotheses tested respectively, it can be concluded that:

- For all test groups, the discoloration on the samples that occurred due to immersion into red wine were reduced significantly after brushing (with or without toothpaste), but not to the clinically acceptable level (ΔE ≥3.3).

- Colgate Optic White toothpaste, containing hydrogen peroxide as bleaching agent, promoted the maximum color

improvement on the samples, which was significantly higher than in the control group (artificial saliva).

- The color improvements promoted by whitening toothpaste brands, used in this study, were statistically similar, when compared to each other.

The results of this study showed that first and third hypotheses tested were true while the second one was partially true.

Ethics

Ethics Committee Approval: Our study was prepared in 2019, therefore, it was not presented to any ethics committee.

Informed Consent: It is not required for our study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.D., E.A.O., Design: F.D., E.A.O., S.K., F.T.T., Data Collection or Processing: E.B., N.A., F.D., Analysis or Interpretation: F.D., E.A.O., S.K., Literature Search: F.D., E.B., N.A., Writing: F.D., E.A.O.

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Socio-demographic and clinical profile of mixed opioid and amphetamine type stimulant dependent subjects in Malaysia: A preliminary report

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ABSTRACT

Aims: In recent years, the increasing trend of amphetamine type stimulant (ATS) use among opioid dependent subjects was strikingly afflicted worldwide, including Malaysia. To study socio-demographic and clinical characteristics of mixed opioid and ATS dependent subjects undergoing methadone maintenance therapy (MMT) program in order to provide supportive information and enhance the management of mixed drug abuse related to health and social effects.

Methods: A descriptive study was carried out in Methadone Clinic in Kuala Terengganu among mixed opioid and ATS dependent patients (n=36) who fulfilled the inclusion and exclusion criteria, and all of them were interviewed.

Results: The mean standard deviation (SD) age of patients was 40.98 years (SD: 4.64), and the most common age of initiation was 15-20 years (42%). Most of the educational background was up to high school level (44%). Morphine and methamphetamine were the most commonly abused substances. The majority of 83.1% used injections as their method of opioid ingestion, while 65.3% preferred chase to ingest ATS. About 30.6% of drug-dependent subjects in this study were on MMT for a 4-year duration and 63.5% of them were on methadone dosage between 45 and 70 mg/day. Most of them demonstrated no history of arrest and conviction for drug use (63.9%) or other criminal acts nonrelated to drug abuse (94.5%).

Conclusions: This study provides update and supportive information of socio-demographic and clinical characteristics of mixed opioid and ATS dependent subjects undergoing the MMT program that may contribute towards comprehensive management to overcome the significant surge of ATS use among opioid dependents.

Introduction

Drug addiction imposes a major threat to public health and social issue worldwide involving opioids addiction, methamphetamine, amphetamine type stimulant (ATS), cocaine, cannabis and other psychoactive substances (1). Malaysia is facing a serious public health problem associated with drug addiction. Through a historic window, this problem was initiated in Malaysia by hippy culture during the 1970s during the period of Vietnam War, which introduced cannabis and heroin to local residents (2). A cumulative total of 512,767 drug users were identified as reported by National Anti-Drugs Agency (NADA) between 1988 and 2017, representing 1.6% of the total Malaysian population (NADA, 2017) (3). The actual figure of the total drug users may exceed more than half a million as the national database has only reported individuals who have been arrested and convicted for illicit drug use and sent to mandatory

institutional rehabilitation (4). The annual number of reported drug addicts by the NADA in 2010 to 2017 is approximately 15,000 to 30,000 per annum with a peak of 30,844 in 2016 and the least cases were detected in 2012 with 15,101 cases.

Heroin maintains the first rank of the most frequently used drug from 2010 to 2013 with a peak of 75.07% of total abused drugs in 2013. The percentage of heroin addiction per year shows a steady decline from 2014 to 2017 in which it reduced by half in 2017, which accounts for 39.1% (3).

The scenario is evidenced by the growing problem of usage of ATSs including crystal methamphetamine and various other methamphetamine and/or amphetamine-containing substances/ pills in Malaysia and the surrounding countries (5). The rise of methamphetamine use in South East Asia has attracted significant attention as it emerges as the world's fastest-growing methamphetamine market and the report that this drug is the primary drug of concern in Southeast Asia (SEA) countries (6). According to Chawarski et al. (7) (2012), ATS use in Malaysia was insignificant before 1987. Over the next several years, the total ATS use increased gradually before spiking up after 1997. By 2018, the total ATS use was 75% of the participants. Vicknasingam et al. (8) (2010) found that approximately 60% of opioid injection drug users reported lifetime use of ATS and 29% also reported lifetime injection of ATS in many regions in Malaysia. Between 2010 and 2017, it was observed that ATSs addiction ranged around 30-40% of the total drug abuse per year. The percentage started to rise steadily from 2014 and eventually resulted in more than half of the total drug used in 2017, which exceeded the heroin usage by 20.88% (3). The rising of ATSs usage is also evidenced by the increasing number of admissions in recent years for amphetamines use. In 2015, the total number for admission related to drug treatment was 6,032, of which opiates accounted for 71% (4,287), while the amphetamines use-related admission was 1,571, accounting for 26% of the total in 2015, and having an estimated increase of 47% compared to 2014 (839 admissions).

Of this total, methamphetamine (crystalline form) represented 77% (1,213 admissions) of the amphetamines-related treatment admissions in 2015 (9-11). The male population continues to represent the majority (96.4%) of cumulative drug addict cases in Malaysia, with a ratio of 1 female for every 26 males. About 41.7% of reported cases are amongst young people between the ages of 13 and 29 years. In term of ethnicity, 80.6% of Malays, 7.5% of Chinese, and 6.8% of Indians were drug addict with other ethnics contributing 5.1% (3).

Opioids including synthetic or naturally occurring alkaloid (benzylisoquinoline alkaloids) are derived from the opium poppy plant (12). Of all, morphine and heroin are the most known and used opioids, while others include methadone, buprenorphine, codeine, tramadol, oxycodone, and hydrocodone (6). They are prescribed clinically for its analgesic properties and in treating opioid dependence. Excessive opioid use, in the absence of proper medical supervision, can lead to fatal respiratory depression (13). Opioids are also available in the form of liquid, solid, and powder (14). Apart from its powerful analgesic effects, opioid intake may also induce relaxation, 'high' feeling, as well as other side effects such as physical dependency, tolerance, respiratory depression, sedation, constipation, nausea, and death.

ATS are manufactured composites that consist of two main sub-type substances: amphetamine and ecstasy. Amphetamine group substances include amphetamine, methamphetamine and their derivatives, such as methcathinone, fenethylline, and methyl-phenidate (9). Methamphetamine (street name is frequently known as "crystal", "glass", "speed", "ice") is being manufactured simply in illicit laboratories from readily accessible, cheap elements. Ephedrine or pseudoephedrine is the most commonly used precursors for methamphetamine synthesis (14). Amphetamine group substances are being prescribed for several clinical conditions under strict rules and regulation (15). Ecstasy group substances are synthesized from amphetamine derivatives, including methylenedioxy-N-methylamphetamine (MDMA) and MDMA-like drugs. They are classified as 'entactogens' with no therapeutic use that has been recognized so far.

Unlike opioid, which is commonly administered by injection, ATS is available in various forms like tablets, crystal, and liquid which are smoked, snorted, injected, or used per rectal (16). ATS administration, particularly the amphetamine-group substances, induce euphoric in users, heighten their confidence level, and increase their alertness, arousal, libido, energy level, and physical strength. Besides, ATS also raises blood pressure, heart and respiratory rates (14). Meanwhile, a wrongful usage of methamphetamine and ATS may lead to a severe complication, including neurological damage to the brain, acute renal failure, and toxic effect to cardiovascular system (17).

Comprehensive management and intervention strategy were launched to overcome the alarming increase of ATS use among opioid dependents. The objective of this study is to provide the socio-demographic and clinical characteristics of mixed opioid and ATS dependent subjects undergoing the methadone maintenance therapy (MMT) program in order to provide supportive information and help in enhancing the management with recent knowledge of drug abuse pattern in relation to health and social effects.

Methods

A descriptive, cross-sectional study was carried out in Methadone Clinic in Kuala Terengganu. The study protocol was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia [National Medical Research Register-18-1989-41507 (IIR)]. The sample was recruited

among patients undergoing the MMT program in this clinic, who fulfilled the inclusion criteria as follows: (1) Diagnosis for COATS based on The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; (2) male, aged from 18 to 50 years; (3) no previous history or current mental disorder including major depression, psychoses, or bipolar disorder; (4) seronegative for human immunodeficiency virus (HIV); and (5) Malay (third generation). Only 36 out of a total 70 male patients attending the MMT program in this clinic, who fulfilled the criteria, were enrolled in this study. No female patient registered in this MMT clinic. All subjects gave voluntarily signed informed consent after the explanation of the study procedures and objectives. A pre-designed questionnaire form which was based on the Addiction Severity Index criteria was used for data collection and subject's evaluation. The questionnaire contained the subject's personal details, including age, race, religion, relevant family history, and physical assessment [body mass index (BMI) and blood pressure measurement]. Besides, socio-economic status, such as marital status, occupation, educational level, were also included. A detailed history of substances abused was also taken including the age of drug initiation, duration, mode of ingestion, duration on MMT program, frequency of methadone use, methadone dosage, and involvement with criminal arrest or conviction.

Statistical Analysis

Descriptive statistics were applied for demographic data of subject analysis using the GraphPad Prism (GraphPad Prism version 6 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

Socio-demographics Characteristics

All subjects recruited in this study were Malay male, and the minimum and maximum ages for the subjects were 31 and 49 years, respectively. Approximately 42% and 14% of them were employed and self-employed, respectively, while 44% were unemployed. About 37.5% of the drug-dependent subjects were married, whereas more than 52.5% were single, and the remaining 10.0% were divorced. As for the educational background, at least 44% of the drug-dependent subjects had studied up to high school. All the subjects had a normal range of BMI and blood pressure measurement. Details of the socio-demographic characteristics were shown in Table 1.

The minimum of opioid and ATS initiation age among the subjects in this study was 15 years, while the maximum was 30 years with the mean and standard deviation of 23.23 and 4.301, respectively, and 58.6% of drug-dependent subjects were on both opioid and ATS addiction for more than 10-year duration. The most commonly used drug for opioids was morphine while that for ATS was methamphetamine. The majority of 83.1% used injections as their method of opioid ingestion while 65.3% preferred chase to ingest ATS. About 84.2% (n=30) used opioid 1 to 5 times on a daily basis while most of the drug-dependent subjects used ATS 1 to 5 times per week, which accounted for 70.7% (n=25). Most of them demonstrated no history of arrest and conviction for drug use (63.9%) and other crimes which were nonrelated to drugs (94.5%) (Table 2). About 30.6% (n=11) of drug-dependent subjects in this study were on MMT for a 4-year duration while the longest duration on MMT among them was 11 years, accounting for 5.6% (n=2) of all subjects. Majority of the drug-dependent subjects were on methadone dosage between 45 and 70 mg/day, representing approximately 63.5%

Table 1. Socio-demographic characteristics of mixed opioid and amphetamine-type stimulant dependent subjects undergoing methadone maintenance therapy program in Bukit Tunggal Health Clinic

Age group (years)		
31-40	15	41.7
40-50	21	58.3
BMI		
Underweight	1	2.8
Normal range	30	83.3
Overweight	5	13.9
Blood pressure (mm/Hg)		
Systolic blood pressure		
100-110	1	2.8
110-120	8	22.2
120-130	27	75
Diastolic blood pressure		
60-70	3	8.3
71-80	21	58.3
81-90	12	33.4
Gender		
Males	36	100
Females	0	0
Education level		
Primary	4	11
High school	32	89
Employment status		
Unemployed	16	44
Employed	5	14
Self-employed	15	42
Religion		
Muslim	36	100
Non-Muslim	0	0
Marital status		
Married	14	37.5
Single	19	52.5
Divorced	3	10

BMI: Body mass index

(n=23). The highest methadone dosage among them was 120 mg/day, which accounted for 2.8% (n=1), while 28% (n=10) were on methadone dosage of 40 mg/day and less with the lowest methadone dosage of 2.5 mg/day, representing 2.8% (Table 3).

Discussion

The present study reported a similar demographic pattern with the previous study regarding predominantly male gender among drug-dependent subjects (18). Mohamed et al. (19) (2008) reported the significant association of male gender and drug dependence in which male participants showed a higher tendency for substance and drug dependence. Tuchman (20) (2010) suggested that women faced more obstacles regarding access to substance abuse compared to men. Above all, multiple factors affecting the difference in gender-related drug use disorder include epidemiology, social background, biological responses, underlying psychiatric illness, progressions to drug dependence, difficulty to access treatments, follow-up, and completion. The average age of our subjects was 40.94 (4.64) years, while the drug addiction initiation age was ranging between 15 and 30 years, which is in line with the increasing trend worldwide that substance abuse begins at a young age (21).

Previous studies linked the opioid-dependent patients on methadone maintenance with higher preference and consumptions of sugary foods that lead to weight gain and higher BMI (22,23). However, the mean BMI for the drug-dependent subjects in this study was 21.15 (3.08). The possible explanation is because of the ATS use among drug-dependent subjects. Effects of amphetamines on hypothalamic receptors would lead to a norepinephrine release together with slight dopamine and serotonin release that resulted in central nervous system stimulation and increased activity, decreased appetite - hence, resulting to a weight reduction (24). In addition, previously in the 1940s and 1950s, amphetamine and amphetamine derivatives had become primary drug used in treating obesity in the United States and was approved by US Food and Drug Administration (25).

MMT program is effective and gives numerous benefits for opioid dependence subjects, which includes health benefits as well as markedly improved family stability, social functioning, and well-being (26). Most of our drug-dependent subjects were either employed or self-employed, which represented by 36% and 14%, respectively. Employment involved working as a security guard, a waiter at restaurant, welder, and many more. Approximately 37.5% of them were married and had children while 52.5% remained single.

Table 2. Clinical characteristics of the mixed opioid and amphetamine-type stimulant drug-dependent subjects undergoing methadone maintenance therapy program in Bukit Tunggal Health Clinic

Drug addiction initiation age (years)		
15-20	15	42
21-25	13	36
26-30	8	22
Duration on mixed drug addiction		
<10 years	21	41.4
>10 years	15	58.6
Main drug		
Opioid		
Morphine	29	80.6
Heroin	7	19.4
ATS		
Methamphetamine	36	100
Others	0	0
Methods of ingestion		
Opioid		
Injection	30	83.1
Chase	5	14.3
Both	1	2.6
ATS		
Injection	9	24.9
Chase	23	65.3
Both	2	9.8
Patterns of drug use		
Frequency of daily opioid use		
1-5 times	30	84.2
>5 times	6	15.8
Frequency of weekly ATS use		
1-5 times	25	70.7
>5 times	11	29.3
History of drug use arrest and conviction		
Yes	13	36.1
No	23	63.9
History other crime arrest and conviction		
Yes	2	9.8
No	34	90.2

ATS: Amphetamine-type stimulant, MMT: Methadone maintenance therapy

Table 3. Clinical characteristics of methadone usage among mixed opioid and amphetamine-type stimulant drug-dependent subjects undergoing methadone maintenance therapy program in Bukit Tunggal Health Clinic

Duration on MMT (years)		
1-5	24	66.7
5-10	10	27.8
>10	2	5.5
Methadone dosage (mg)		
1-40	10	27.8
41-80	19	52.8
81-120	7	19.4

MMT: Methadone maintenance therapy

The current study is confined to Malay subjects which represent the largest ethnicity in Malaysia population. NADA (2017) reported that the majority of drug users involved Malay ethnicity compared to others. Hence, we have selected Malay subjects from three generations of Malay ethnicity (his father, mother, grandfather, and grandmother must be of Malay origin). This is important in preventing ethnicity bias as Malaysia is a multi-racial country.

Besides, despite the increasing trend of drug use among women in recent years, as reported by NADA, (2017), the male population still accounts for the majority of the drug users in Malaysia. Mohamed et al. (19) (2008) reported the significant association of male gender and drug dependence in which male participants showed a higher tendency for substance and drug dependency. Tuchman (20) (2010) suggested that women faced more obstacles regarding access to substance abuse compared to men. Above all, multiple factors affecting the difference in gender-related drug use disorder include epidemiology, social background, biological responses, underlying psychiatric illness, progressions to drug dependence, difficulties in accessing treatment, follow up, and completion.

According to drug report by NADA (2017) and Ibrahim et al. (27) (2012), teenagers represented 2.67% of all those arrested and charged as drug dependents with the majority of them aged between 14 and 15 years, which accounted for 57.5% of all teenagers involved. This report also revealed that crystalline methamphetamine was the drug of choice which was commonly used among both teenagers and youth group. A possible explanation for this might be due to the easy access and the low price of the drug that made it affordable for the students to try this potent drug and subsequently become addicted (28). Besides, this drug can be taken through various routes including smoking, injection, swallowing or snorting. All of these may be the key factors that excite teenagers and youth to get and try them.

Another possible factor contributing to this scenario is due to the locality of Malaysia, which is situated in the SEA and shares a border with Thailand. UNDOC (6) (2019) has reported that the SEA region is recognized as the fastest-growing methamphetamine market worldwide. Apart from being produced in Malaysia on a small scale, ATS and other synthetic drugs are brought in Malaysia through trafficking routes across Thailand (29,30).

Majority of drug-dependent subjects preferred injection as their route of choice in using opioid [59.3% (n=22)]. This finding is consistent with that of Desrosiers et al. (29) (2016) where most of the subjects take opioid through injection. The reason for such preference might be due to the fast effect of the drug even with a small amount. As for ATS, our study reported the chase method (“chase the dragon”) as the most preferred route, which accounts for 73.6% (n=26). Through the usage of this method, the methamphetamine is vaporized by crushing the tablets and then heated on tin foil. The formed fumes are smoked in the same way to the ‘chasing the dragon’ used in opioid smoking (31). MMT program has been introduced as substitution therapy to opioid addiction in Malaysia since 2005, and currently, this program becomes widely available in almost all health care centres throughout Malaysia (32). In this study, a total of 30.6% (n=11) of drug dependents were on for a 4-year duration while the longest duration on MMT among them was 11 years [5.6% (n=2)]. This result is in line with previous reports stating that MMT program is effective at improving the quality of life of drug-dependent subjects including significantly reducing relapse of opioid use, mortality rates, blood-borne illness such as hepatitis B, hepatitis C, and HIV/AIDS; in addition to improving family stability and increase employment potential (33,34). Being a single-dose therapy on a daily basis at low cost with tolerable side effects are also the contributing factors rendering this MMT program successful and effective for the drug-dependent subjects.

Approximately 63.5% (n=23) of our subjects are on average of 45-70 mg/day of methadone. This finding was supported by a previous review by Faggiano et al. (35) (2003) in which it was suggested that maintaining the patient on higher methadone dosages (60-100 mg/day) was more effective to ensure patient’s compliant on MMT program and it helped to reduce the opioids use relapse during the program compared to lower dosages. However, the optimal dosage still subjected to the clinical ability and patient’s condition. This finding was further supported by a study of Mohamad et al. (36) (2010), which also recommended that a higher dosage of methadone might reduce the risk of illicit opioid abused.

The present study has several limitations. The relatively small sample size for this study is due to our objectives that aimed only drug dependent subjects who were on both opioid and ATS addiction at the same time, which made it harder to recruit eligible volunteers. Another limitation was the place of recruitment, where we recruited the drug dependent subjects receiving MMT in methadone clinic hosted by Health Clinics in Kuala Terengganu only, instead of involving other possible potential facilities such as drug rehabilitation centre and prison. Besides, our study is also confined to Malay male adult population, which only represents the sub-group population from a general population from specific location in Malaysia. As we excluded other races such as Chinese and Indian population, hence our result only represents the effect of drug dependence to peripheral dopamine systems among Malay male population. It makes these findings less generalisable to represent Malaysian male population as a whole.

Conclusion

This study provides update and supportive information of socio-demographic and clinical characteristics of mixed opioid

and ATS dependent subjects undergoing the MMT program that may contribute towards comprehensive management to overcome the significant surge of ATS use among opioid dependents.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia [National Medical Research Register-18-1989-41507 (IIR)].

Informed Consent: All subjects gave voluntarily signed informed consent after the explanation of the study procedures and objectives.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: I.A., Concept: R.A.B., I.A., Design: N.K.M.J., A.A.N., R.A.B., I.A., Data Collection or Processing: N.K.M.J., A.A.N., R.A.B., Analysis or Interpretation: N.K.M.J., A.A.N., R.A.B., I.A., S.I., Literature Search: N.K.M.J., Writing: N.K.M.J., I.A.

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Evaluation of risk factors and causative pathogens in bloodstream infections in cancer patients

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ABSTRACT

Aims: Bloodstream infection in cancer patients may cause delays in chemotherapy, prolonged hospital stay, difficulties in diagnosis and treatment, inappropriate treatment, high mortality rates and increased health care costs. The aim of this study was to evaluate the risk factors and causative pathogens in bloodstream infection in cancer patients.

Methods: The study was planned as a retrospective case-control study. The study included patients who were followed up and treated with the diagnosis of hematologic malignancy or solid tumor and who were found to have bloodstream infection during the period from 01/01/2014 to 12/31/2018 and patients with similar characteristics as the control group.

Results: A total of 312 (276 hematologic malignancy, 36 solid tumor) bloodstream infections were included. The presence of central venous catheter (CVC), steroid use, presence of urinary catheter, neutrophil count, neutropenia duration and total hospitalization day were determined as independent risk factors of bloodstream infection in patients with hematologic malignancy. The presence of CVC, total parenteral nutrition, renal failure and neutrophil count were determined as independent risk factors of bloodstream infection in patients with solid tumor. In patients with hematologic malignancy, 113 *Escherichia coli* and 59 *Klebsiella pneumoniae* were detected. In patients with solid tumor, 7 *E. coli* and 5 *K. pneumoniae* were detected.

Conclusions: Risk factors are higher in the development of bloodstream infection in patients with hematologic malignancy and patients should be closely monitored. Multidrug-resistant bacteria might also be isolated from the blood cultures of patients. Therefore, it is recommended to start with broad-spectrum antibiotics in bloodstream infections.

Introduction

Despite improvements in antimicrobial treatment and supportive care services, bloodstream infections are an important cause of morbidity and mortality (1). Bloodstream infections in malignant patients are among the life-threatening complications leading to delays in chemotherapy, prolonged hospital stay, difficulties in diagnosis and treatment, inappropriate treatment, high mortality and increased health care costs (2).

The frequency of bloodstream infections is between 11% and 38% and the mortality rate reaches 40% (3). The presence of multidrug-resistant bacteria increases the morbidity and mortality rates in these patients (1). In recent years, Gram-

negative bacteria have been detected as causative agents, but this varies according to the geographical region (2).

Candidemia means the presence of *Candida* species in the blood. When *Candida* is detected in blood cultures, the source of the infection should be investigated. Candidemia is an indicator of disseminated candidiasis in many patients (4).

The aim of this study was to determine the risk factors, the distribution and frequency of isolated agents, and to determine the resistance status in bloodstream infections in cancer patients.

Methods

The study was planned as a retrospective case-control study. Approval was obtained from the University of Health Sciences Turkey Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Ethics Committee (approval date: 02/20/2019 and decision no: 2019-03/209).

The study included patients who were followed up and treated with the diagnosis of hematologic malignancies and solid tumors and who were found to have bloodstream infections during the period from 01/01/2014 to 12/31/2018, and patients with similar characteristics but no growth in culture materials were included in the control group.

The control group was selected from the patients who presented to the hospital in the same period, had no clinical signs and symptoms suggestive of bloodstream infection and had negative blood culture. The patients in the study and control groups were matched in terms of hospitalization period and inpatient unit type. The files of the patients were analyzed retrospectively and the data obtained were transferred to the forms for evaluation.

The analysis of the risk factors for the development of bloodstream infections was performed from the data obtained until the development of bloodstream infection. For the control group, risk analysis was planned from the data of hospitalization periods.

When fever was ≥38.3 °C in patients with malignancies, at least 2 vials of blood cultures were drawn from each patient. Blood cultures were obtained from different peripheral veins of patients with central venous catheter (CVC), including one from the catheter.

The automated BacT/ALERT 3D (bioMerieux, Marcy-l'Etoile, France) system, which detects the growth of blood cultures by signal, was used. Antibiotic susceptibility tests of factors were performed according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). According to the Center for Disease Control and Prevention (CDC) definitions, the patients were divided into two groups as primary bloodstream infections and catheter-related bloodstream infections.

The blood samples were inoculated into automated BacT/ALERT 3D (bioMerieux, Marcy-l'Etoile, France) system for blood cultures. C. albicans were separated by using the germ tube test for typing. The non-albicans species were typed with VITEK® 2 Compact System (bioMerieux, France). Amphotericin B, fluconazole, flucytosine, voriconazole, caspofungin, itraconazole sensitivity tests were performed with VITEK® 2 Compact System (bioMerieux, France). EUCAST was used in the antifungal test.

Results of the patients were recorded electronically from the health records. The demographic data of the patients and their other information at the time of cultures were recorded in a pre-prepared form. Patients with microbial growth in their blood and/or catheter cultures were included in the study.

Eighteen-year-old and older patients with malignancies, who had clinical, laboratory and microbiological bloodstream infection history, were included in the study. Patients who were under the age of 18 years, pregnant patients and those without malignancies were excluded from the study.

Statistical Analysis

SPSS (IBM SPSS Statistics 24) program was used in the statistical analyses. The data were entered into statistical software program and analyzed by using the same computer software program. In order to interpret the findings, frequency tables and descriptive statistics were used.

The categorical descriptive data were presented as frequency distribution and percentages (%) and the measurable descriptive data were presented as mean±standard deviation and median (the largest, the smallest values).

The "χ^2-cross tables" were used to evaluate the relationship between two qualitative variables. The "Mann-Whitney U" test (Z-table value) statistics were used to compare the two non-normally distributed independent groups. The "Binary Logistic Regression" analysis was used to determine independent risk factors. $P<0.05$ was considered statistically significant.

Results

The data of 276 patients with hematologic malignancy and bloodstream infections and data of 36 patients with solid tumor and bloodstream infections were evaluated.

A total of 312 (276 hematologic malignancy, 36 solid tumor) bloodstream infections were detected in these patients. Ninety seven (44.3%) of the patients with hematologic malignancy were female and 122 (55.7%) were male. The mean age of the patients was 44.5±16.2 years. One hundred-nine (49.7%) of the study (case) group patients had acute myeloid leukemia, 48 (21.8%) had non-Hodgkin lymphoma and 44 (20.1%) had acute lymphoblastic leukemia (Table 1). Sixteen (44.4%) of the patients with solid tumor were female and 20 (55.6%) were male. The mean age of the patients was 56.0±14.4 years. Thirty six (100%) patients had solid tumors (Table 2).

Table 1. Comparison of demographic characteristics and risk factors of the patients with hematologic malignancy and the control group

	Patients with hematologic malignancy (n=276, %)	Control (n=153, %)	p value
Gender			
Female	97 (44.3%)	55 (35.9%)	χ^2=2.595 p=0.107
Male	122 (55.7%)	98 (64.1%)	
Disease			
AML	109 (49.7%)	70 (45.8%)	χ^2=7.538 p=0.480
ALL	44 (20.1%)	29 (19.0%)	
CML	3 (1.4%)	-	
CLL	1 (0.5%)	-	
HCL	1 (0.5%)	-	
MM	8 (3.7%)	4 (2.6%)	
MDS	4 (1.8%)	4 (2.6%)	
NHL	48 (21.8%)	45 (29.4%)	
HL	1 (0.5%)	1 (0.6%)	
Hospitalization in the last three months	155 (56.2%)	85 (58.6%)	χ^2=1.840 p=0.399
Intensive care unit stay	20 (7.2%)	4 (2.6%)	χ^2=3.170 p=0.075
Presence of central venous catheter	167 (60.5%)	42 (27.5%)	χ^2=43.052 **p<0.001**
Total parenteral nutrition	37 (13.5%)	3 (2.0%)	χ^2=14.002 **p<0.001**
Renal failure	29 (10.5%)	2 (1.3%)	χ^2=11.093 **p<0.001**
Diabetes mellitus	29 (10.5%)	10 (6.5%)	χ^2=1.429 p=0.232
HSCT (allogeneic, autologous)	22 (8.0%)	14 (9.2%)	χ^2=0.058 p=0.810
Allogeneic HSCT	15 (5.4%)	11 (7.2%)	χ^2=0.269 p=0.604
Autologous HSCT	10 (3.6%)	3 (2.0%)	p=0.258
Disease status			
Newly diagnosed	98 (35.5%)	50 (32.7%)	χ^2=7.817 p=0.167
Remission	74 (26.8%)	58 (37.9%)	
Refractory	30 (10.9%)	15 (9.8%)	
Relapse	58 (21.0%)	265 (13.1%)	
Stable	14 (5.1%)	20 (5.9%)	
Not evaluated group	2 (0.7%)	9 (0.6%)	
Name of the given chemotherapy regimen			
Induction	96 (35.8%)	47 (37.0%)	χ^2=7.574 p=0.056
Consolidation	83 (31.0%)	53 (41.7%)	
Salvage regimen	83 (31.0%)	24 (18.9%)	
Palliative chemotherapy or other chemotherapies	6 (2.2%)	3 (2.4%)	
Steroid use	77 (27.9%)	20 (13.1%)	χ^2=15.141 **p=0.001**
Quinolone prophylaxis	31 (11.2%)	14 (9.2%)	χ^2=0.260 p=0.610

Antibiotic use in the last three months	139 (50.4%)	49 (32.0%)	χ^2=3.443 **p<0.001**
Another bloodstream infection in the last three months	71 (25.7%)	8 (5.2%)	χ^2=27.523 **p<0.001**
Presence of another infection	85 (30.8%)	28 (18.3%)	χ^2=7.923 **p=0.005**
Presence of urinary catheter	48 (17.4%)	5 (3.3%)	χ^2=16.852 **p<0.001**
Presence of mucositis	201 (72.8%)	84 (54.9%)	χ^2=14.182 **p<0.001**
Additional disease	76 (27.5%)	41 (26.8%)	χ^2=0.027 p=0.869
Discharge status			
Discharge	211 (76.4%)	147 (96.1%)	χ^2=27.461 **p<0.001**
Mortality	65 (23.6%)	6 (3.9%)	

The "χ^2-cross tables" were used to investigate the relationship between two qualitative variables.
AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia, CML: Chronic myeloid leukemia, CLL: Chronic lymphocytic leukaemia, HCL: Hairy cell leukemia, MM: Multiple myeloma, MDS: Myelodysplastic syndrome, NHL: Non-Hodgkin lymphoma, HL: Hodgkin lymphoma, HSCT: Hematopoietic stem cell transplantation

The hospitalization status in the last three months, intensive care unit stay, hematopoietic stem cell transplantation, disease status, given chemotherapy regimens, quinolone use and additional disease were not significantly different between the patients with hematologic malignancy and the control group in patients with hematological malignancy (Table 1). The hospitalization status in the last three months, given chemotherapy regimens, steroid use, antibiotic use in the last three months and additional disease were not significantly different between the patients with solid tumor and the control group in patients with solid tumor (Table 2).

There was a significant difference in the presence of CVC between the patients with hematologic malignancy and the control group in patients with hematologic malignancy (p<0.001). The patients [167 (60.5%)] had CVC, 51 (18.5%) were ports and 116 (42.8%) were permanent central catheters. A significant difference wasalso found between the patients with solid tumor and the control group in terms of the presence of CVC in patients with solid tumor (p<0.001). Twenty-seven (75%) patients had CVC; 16 (44.4%) were port and 11 (30.6%) were permanent central catheter.

The total parenteral nutrition (TPN) history, renal failure, steroid use, antibiotic use in the last three months, another bloodstream infection in the last three months, presence of another infection, presence of urinary catheter, mucositis and mortality statuses were significantly different between the patients with hematologic malignancy and the control group in bloodstream infections (p<0.001, p=0.001, p=0.001, p<0.001, p<0.001, p=0.005, p<0.001, p<0.001 and p<0.001, respectively) (Table 1). The intensive care unit stay, TPN history, renal failure, disease status, another bloodstream infection in the last three months, presence of another infection, presence of urinary catheter, mucositis and mortality statuses were significantly different between the patients with solid tumor and the control group in bloodstream infections (p=0.005, p=0.001, p<0.001, p=0.013, p=0.025, p<0.001, p<0.001, p=0.006 and p=0.005, respectively) (Table 2).

There was no significant difference in terms of diabetes mellitus between the patients with hematologic malignancy and the control group (p=0.232). There was no significant difference in terms of diabetes mellitus between the patients with solid tumor and the control group (p=0.343).

The mean neutrophil count was 30/mm^3 in the patients with hematologic malignancy and the mean neutrophil count was 600/mm^3 in the control group. There was a statistically significant difference (p<0.001). There was a statistically significant difference in terms of neutropenia duration and total hospitalization days between the patients with hematologic malignancy and the control group (p<0.001 and p<0.001, respectively) (Table 3). The mean neutrophil count was 4.395/mm^3 in the patients with solid tumor and 3.460/mm^3 in the control group (p=0.470). There was a statistically significant difference in terms of neutropenia duration and total hospitalization day between the patients with solid tumor and the control group (p=0.010 and p<0.001, respectively) (Table 3).

The mean duration of neutropenia before infection was 9.5±9.6 (7.0) days and the mean of total hospitalization days before infection was 18.4 days in the patients with hematologic malignancy (Table 3). The mean duration of neutropenia before infection was 1.2±2.0 (1.0) days and the mean of total hospitalization days before infection was 8.7 days in the patients with solid tumor (Table 3).

The presence of CVC, steroid use, antibiotic use in last three months, presence of urinary catheter, neutrophil count, neutropenia duration and total hospitalization days

Table 2. Comparison of demographic characteristics and risk factors of the patients with solid tumors and the control group

	Patients with solid tumor (n=36, %)	Control (n=37, %)	p value
Gender			
Female	16 (44.4%)	16 (43.2%)	χ^2=0.000
Male	20 (55.6%)	21 (56.8%)	p=1.000
Disease			
Solid tumor	36 (100%)	37 (100%)	χ^2=0.004 p=0.998
Hospitalization in the last three months	17 (47.2%)	21 (556.8%)	χ^2=0.337 p=0.561
Intensive care unit stay	7 (19.4%)	-	**p=0.005**
Presence of central venous catheter	27 (75.0%)	1 (29.7%)	χ^2=13.224 **p<0.001**
Total parenteral nutrition	15 (41.7%)	2 (5.4%)	χ^2=11.477 **p=0.001**
Renal failure	17 (47.2%)	1 (2.7%)	χ^2=17.144 **p<0.001**
Diabetes mellitus	9 (25.0%)	5 (13.5%)	χ^2=0.901 p=0.343
Disease status			
Remission	1 (2.8%)	-	χ^2=10.766 **p=0.013**
Refractory	-	1 (2.7%)	
Stable	-	8 (21.6%)	
Metastatic	35 (97.2%)	28 (75.7%)	
Name of the given chemotherapy regimen			
Consolidation	-	1 (4.2%)	χ^2=6.396 p=0.094
Salvage regimen	-	1 (4.2%)	
Palliative chemotherapy	26 (96.3%)	17 (70.8%)	
Other chemotherapies	1 (3.7%)	5 (20.8%)	
Steroid use	5 (13.9%)	1 (2.7%)	p=0.093
Antibiotic use in last three months	12 (33.3%)	5 (13.5%)	χ^2=2.980 p=0.084
Another bloodstream infection in the last three months	5 (13.9%)	-	**p=0.025**
Presence of another infection	16 (44.4%)	2 (5.4%)	χ^2=12.941 **p<0.001**
Presence of urinary catheter	16 (44.4%)	2 (5.4%)	χ^2=12.941 **p<0.001**
Presence of mucositis	1 (33.3%)	2 (5.4%)	χ^2=7.46 **p=0.006**
Additional disease	15 (41.7%)	12 (32.4%)	χ^2=0.330 p=0.566
Discharge status Discharge	29 (80.6%)	37 (100%)	**p=0.005**
Mortality	7 (19.4%)	-	

The "χ^2-cross tables" were used to investigate the relationship between two qualitative variables

were determined as independent risk factors of bloodstream infection in the patients with hematologic malignancy (Table 4). The presence of CVC, history of TPN renal failure and neutrophil count were determined as independent risk factors of bloodstream infection in the patients with solid organ tumor (Table 4).

In the patients with hematologic malignancy, 113 (51.7%) *Escherichia coli*, 59 (27.1%) *Klebsiella pneumoniae* and 19 (8.6%) *Pseudomonas aeruginosa* were found to be positive for Gram-negative bacteria. Extended-spectrum beta-lactamase (ESBL) positivity was found to be 110 (50.5%), carbapenemase

Table 3. Comparison of patients with hematologic malignancy and solid tumor and control group

	X̄±SD	Median (IQR)	X̄±SD	Median (IQR)	p value
Hematologic malignancy (n=276) and control group (n=153)					
Age	44.5±16.2	46.0 (27.0)	43.9±16.2	48.0 (29.5)	Z=-0.853 p=0.394
Neutrophil count	366.1±1164.7	30.0 (97.5)	2018.8±4075.4	600.0 (2470.0)	Z=-9.116 **p<0.001**
Neutropenia duration	9.5±9.6	7.0 (7.0)	6.0±8.7	3.0 (10.0)	Z=-5.471 **p<0.001**
Total hospitalization, days	36.5±16.9	31.0 (19.0)	6.2±11.6	27.0 (19.0)	Z=-12.350 **p<0.001**
Solid tumor (n=36) and control (n=37) groups					
Age	56.0±14.4	57.5 (16.2)	54.0±14.8	57.0 (15.5)	Z=-0.618 p=0.536
Neutrophil count	5397.5±5122.7	4395.0 (8887.5)	3780.0±2289.8	3460.0 (3315.0)	Z=-0.723 p=0.470
Neutropenia duration	1.2±2.0	1.0 (2.7)	0.3±1.2	0.0 (0.0)	Z=-2.587 **p=0.010**
Total hospitalization, days	20.6±12.3	18.0 (12.7)	4.3±4.5	3.0 (2.5)	Z=-6.665 **p<0.001**

"Mann-Whitney U" test (Z-table value) statistics were used to compare the two non-normally distributed independent groups.
X̄: Mean, SD: Standard deviation, IQR: Interquartile range

Table 4. Determination of factors affecting disease risk status in the hematology group and oncology group

	B	Standard error	Wald	df	Sig.	OR	95% CI	
							Lower	Upper
Hematology group								
Presence of central venous catheter	1.0	0.3	9.5	1	**0.002**	2.7	1.4	5.0
Steroid use	1.9	0.5	13.5	1	**<0.001**	6.6	2.4	18.2
Antibiotic use in last three months	0.7	0.3	5.3	1	**0.021**	2.1	1.1	3.9
Presence of urinary catheter	2.1	0.7	8.4	1	**0.004**	8.6	2.0	36.8
Neutrophil count	-0.001	0.000	14.1	1	**<0.001**	0.9	0.9	0.9
Neutropenia duration	-0.140	0.0	26.6	1	**<0.001**	0.9	0.8	0.9
Total hospitalization, days	0.2	0.0	61.6	1	**<0.001**	1.2	1.1	1.2
CCR=87.4%; $\chi^2_{(8)}$=5.923, p=0.656								
Oncology group								
Presence of central venous catheter	2.0	0.9	4.8	1	**0.029**	7.1	1.2	41.4
TPN	2.5	1.2	4.4	1	**0.036**	11.7	1.2	115.3
Renal failure	4.3	1.4	9.7	1	**0.002**	76.9	5.0	174.2
Presence of urinary catheter	2.7	1.5	3.4	1	0.063	15.6	0.8	283.8
Neutrophil count	0.05	0.02	7.1	1	**0.008**	1.05	1.1	1.9
CCR=86.3%; $\chi^2_{(8)}$=4.032, p=0.854								

B: Regression coefficient, Wald: Significance test of coefficient, df: Degrees of freedom, Sig.: Significance, OR: Odds ratio, CI: Confidence interval of OR, CCR: Correct classification rate, TPN: Total parenteral nutrition

positivity was found to be 39 (17.9%) and multidrug resistance was found to be 122 (56.0%). In the patients with solid tumor, 7 (41.2%) *E. coli*, 5 (29.4%) *K. pneumoniae* and 3 (17.6%) *P. aeruginosa* were found to be positive for Gram-negative bacteria. ESBL positivity was found to be 8 (47.1%), carbapenemase positivity was found to be 1 (5.9%) and multidrug resistance was found to be 5 (29.4%) (Table 5).

In the patients with hematologic malignancy, 20 (38.6%) *Staphylococcus epidermidis* was the most common causative agent in Gram-positive bacteria, followed by 13 (25.0%)

Staphylococcus hominis and 6 (11.5%) *Staphylococcus haemolyticus*. Methicillin resistance 44 (84.6%), penicillin resistance 46 (88.5%) and vancomycin resistance 1 (1.9%) were found. In the patients with solid tumor, 4 (25.0%) S. epidermidis were the most common causative agent in Gram-positive bacteria, followed by 3 (18.7%) *Staphylococcus aureus* and 3 (18.7%) *Enterococcus faecium*. Methicillin resistance 10 (62.5%), penicillin resistance 13 (81.2%) and vancomycin resistance 1 (6.3%) were found (Table 5).

In the patients with hematologic malignancy, two *Candida krusei* and *Candida tropicalis* were isolated, whereas *Candida albicans* was isolated in only one patient. All of the fungal agents were susceptible to voriconazole, one was moderately susceptible to amphotericin, and two were moderately susceptible to flucytosine. All of the fungal agents were not resistant to caspofungin and micafungin and two of them were resistant to fluconazole. One *C. albicans* and one *C. tropicalis* were isolated in patients with solid tumor. It was only moderately sensitive to *C. tropicalis* amphotericin and there was no resistance to all other antifungal drugs in both fungal agents.

Discussion

In patients with malignancy, the immunosuppressive effects of chemotherapy as well as immune defects due to underlying hematological disorders increase the risk of infection in patients (5). In addition, the application of chemotherapy does not only reduce the number of neutrophils, but also cause chemotactic and phagocytic disorders (6).

The incidence of bloodstream infections in patients with malignancy is 11.7% and this rate is reported to be between 11.8% and 33.3% in different geographical regions (7).

In one study, the incidence of bloodstream infection was found to be eight times higher in the patients with hematologic malignancy than in the patients with solid tumor due to the underlying disease (2). In this study, 276 bloodstream infection were detected in the patients with hematologic malignancy and 36 bloodstream infection in the patients with solid tumor.

The secondary bloodstream infection and catheter-related bloodstream infection are more common in the patients with solid tumor, whereas primary and mucosity-related bloodstream infections are more common in the patients with hematologic malignancy (2). In this study, primary bloodstream infection was found to be 161 (58.3%), catheter-related bloodstream infection was found to be 115 (41.7%) in the patients with hematologic malignancy, whereas primary bloodstream infection was found to be 18 (50%), catheter-related bloodstream infection was found to be 18 (50%) in the patients with solid tumor. Primary bloodstream infection rate was found to be higher in patients with hematologic malignancy.

Table 5. Gram-negative bacteria and Gram-positive bacteria

Variable (patients with hematologic malignancy)	n	%
Gram-negative bacteria		
Escherichia coli	113	51.7
Klebsiella pneumoniae	59	27.1
Pseudomonas aeruginosa	19	8.6
Enterobacter cloacae	6	2.8
Acinetobacter baumannii	7	3.2
Aeromonas hydrophila	3	1.4
Pseudomonas spp.	5	2.3
Sphingomonas paucimobilis	1	0.5
Klebsiella oxytoca	1	0.5
Stenotrophomonas maltophilia	3	1.4
Acinetobacter lwoffii	1	0.5
Extended-spectrum beta-lactamase	110	50.5
Carbapenemase	39	17.9
Multi-drug resistance	122	56.0
Variable (patients with solid tumor)		
Gram-negative bacteria		
Escherichia coli	7	41.2
Klebsiella pneumoniae	5	29.4
Pseudomonas aeruginosa	3	17.6
Klebsiella oxytoca	1	5.9
Stenotrophomonas maltophilia	1	5.9
Extended-spectrum beta-lactamase	8	47.1
Carbapenemase	1	5.9
Multi-drug resistance	5	29.4
Variable (patients with hematologic malignancy)		
Gram-positive bacteria		
Staphylococcus haemolyticus	6	11.5
Corynebacterium spp.	2	3.8
Staphylococcus epidermidis	20	38.6
Enterococcus faecium	3	5.8
Staphylococcus mitis	2	3.8
Staphylococcus hominis	13	25.0
Staphylococcus aureus	3	5.8
Staphylococcus warneri	1	1.9
Kocuria kristinae	1	1.9
Streptococcus spp.	1	1.9
Methicillin resistance	44	84.6
Penicillin resistance	46	88.5
Vancomycin resistance	1	1.9
Variable (patients with solid tumor)		
Gram-positive bacteria		
Staphylococcus epidermidis	4	25.0

Enterococcus faecium	3	18.7
Staphylococcus hominis	2	12.5
Staphylococcus aureus	3	18.7
Enterococcus faecalis	1	6.3
Streptococcus spp.	2	12.5
Leuconostoc spp.	1	6.3
Methicillin resistance	10	62.5
Penicillin resistance	13	81.2
Vancomycin resistance	1	6.3

The risk factors for bloodstream infection caused by Gram-negative bacteria include bone marrow transplantation, liver failure, lower respiratory tract diseases, renal failure, immunsupresif treatments, diabetes mellitus and invazive procedures of uriner tract (8-11). The risk factors for bloodstream infection might include weight loss, inability to feed, wounds on the mucosa and skin, operations of urinary tract and gastrointestinal tract, obstructions, immunosupresif treatments in patients with solid tumor (12). Operations, old age, additional diseases, advanced stage of cancer could be risk factors for bloodstream infection in some hospitals (13).

In patients with hematologic malignancy and patients with solid tumor, the presence of CVC, TPN renal failure and another bloodstream infection in the last three months were found to be risk factors for bloodstream infection in this study (p=0.013). The presence of CVC, steroid use, antibiotic use in last three months, presence of urinary catheter, neutrophil count, neutropenia duration and total hospitalization day were determined as independent risk factors of bloodstream infection in the patients with hematologic malignancy. The presence of CVC, TPN, renal failure and neutrophil count were determined as independent risk factors of bloodstream infection in the patients with solid organ tumor.

Although Gram-negative bacilli were common causative agents in the patients with malignancy in previous studies in the 1960's and 1970's, recent studies have reported that Gram-positive bacteria might be the most common causative agents (14,15). In this study, Gram-negative bacilli were found to be the most common factors in the patients with hematologic malignancy and/or solid tumor.

E. coli is the most frequently isolated bacteria. In contrast, *P. aeruginosa* were reported to be the most commonly isolated agent from bloodstream infection in the patients with malignancy in different countries (16,17). In this study, *E. coli* was the most frequently isolated bacteria in both patient groups, followed by *K. pneumoniae* and *P. aeruginosa.*

In a study, the rate of ESBL producing *Enterobacteriaceae* strains was reported to be 34.1% in the patients with hematologic malignancy and 65.9% in the patients with solid tumor (16). In this study, ESBL positivity was found to be 50.5% in the patients with hematologic malignancy and 47.1% in the patients with solid tumor.

The increase in ESBL producing strains has caused more use of carbapenems and this has ended up with increased resistance (18). In a study conducted by Trecarichi et al. (15), it was reported that carbapenem resistance could reach up to 38% in the patients with hematologic malignancy. In this study, carbapenem resistance was found to be 17.9% in the patients with hematologic malignancy and 5.9% in the patients with solid tumor. The rate of multidrug-resistant gram-negative bacteria was higher in patients with hematologic malignancy.

In the United States between 2005 and 2017, 55% reduction in MRSA was observed following the introduction of multifaceted infection control measures in *S. aureus* infections (19). Worldwide, the prevalence of methicillin-resistant *S. aureus* in bloodstream infection is <1% in Scandinavia, 40% in Japan, Israel and Europe (20). In this study, the rate of Gram-positive bacteria was lower in both patient groups. The number of methicillin-resistant agents was 44 (84.6%) in the patients with hematologic malignancy and 10 (62.5%) in the patients with solid tumor.

Candidiasis is an important nosocomial infection which is seen both in adults and in children, especially in hospitalized patients (21,22). And common source of infection is the endogenous flora of the patient (23). The risk factors for candidemia may include age, trauma, or burns, as well as CVC, TPN, broad-spectrum antibiotics use, high APACHE scores, acute renal failure, hemodialysis, history of surgery, especially abdominal surgery, gastrointestinal system perforations, and anastomotic leaks (21).

In this study, the above-mentioned risk factors were identified as risk factors for candidemia in both patient groups. *Candida* growth was found to be very low in both groups. There were five growths in the patients with hematologic malignancy and two in the patients with solid tumor.

The current study had some limitations. As a result of its retrospective design, the duration of CVC, duration of steroid therapy and duration of urinary catheter could not be obtained from the health records.

Conclusion

As a result, risk factors are higher in the development of bloodstream infection in the patients with hematologic malignancies. Therefore, the patients with hematologic malignancies should be closely monitored for developing blood stream infections. Reducing these risk factors may contribute to reduce the incidence of bloodstream infection. Multidrug-resistant bacteria might also be isolated from the blood cultures of the patients with cancer. Therefore, whenever it is necessary,

should be kept in mind it is recommended to start with broad-spectrum antibiotics in bloodstream infections in patients with cancer.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from Clinical Research Ethics Committee of University of Health Sciences Turkey Ankara Dr. Abdurrahman Yurtaslan Training and Research Hospital (approval date: 02/20/2019 and decision no: 2019-02/200).

Informed Consent: Retrospective study.

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Authorship Contributions

Concept: D.M., A.M., Design: D.M., M.K.Ç., E.D., Data Collection or Processing: D.M., A.M., N.K., M.K.Ç., E.D., S.Ç., F.A., M.E., Analysis or Interpretation: D.M., N.K., S.Ç., F.A., Literature Search: D.M., Writing: D.M.

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Therapeutic efficiency analyses of mandibular advancement devices using polysomnography, smartphone sleep applications, and simple pulse oximetry

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Keywords: Snoring, obstructive sleep apnea, mandibular advancement device, pulse oximeter device, smartphone sleep applications

ABSTRACT

Aims: Oral appliances are non-invasive solutions for obstructive sleep apnea (OSA). The aim of the present study was to determine the therapeutic efficiency of mandibular advancement devices (MAD), using polysomnography (PSG), smartphone sleep applications (SSA), and simple pulse oximetry (SPO) for OSA patients.

Methods: Totally 18 mild-moderate OSA patients (8 females and 10 males, aged between 34 and 83 years) with subjective snoring complaints were selected. The diagnostic PSG tests were accepted as initial PSG. Each patient was subjected to SPO and SSA at 3 different nights at home. The mean diagnostic values of oxygen desaturation index (ODI), snoring intensity score (SIS) and snoring percentage (SP) were obtained. Non-titratable-customized MADs (NTC-MAD) with 60-75% of maximal mandibular protrusion were fabricated. SPO and SSA measurements were repeated at the 1st, 4th, 12th, and 24th weeks of the treatment process. At the 24th week of the treatment, the PSG tests were repeated and all PSG, SPO, and SSA values were compared with initial diagnostic values.

Results: There was no significant difference in body mass index and neck circumference values during the treatment ($p>0.05$). The success rate of NTC-MAD treatment was about 68.7% according to the success criterion of MAD treatment as the decrease of apnea-hypopnea index below 5 per hour. ODI, SIS, and SP values showed a significant decrease during the treatment ($p<0.01$). A maximum decrease in SPO and SSA data were obtained at the 12th week of NTC-MAD use ($p<0.05$).

Conclusions: NTC-MADs significantly decreased snoring intensity and frequency and may be accepted as effective devices in the treatment of OSA.

Introduction

Obstructive sleep apnea (OSA) is one of the common syndromes of sleep-related breathing disorders according to the "International Classification of Sleep Disorders-3" (1,2). OSA, which causes snoring and sleep fragmentations, resulting in excessive day time sleepiness, originates from apneas and hypopneas (2). OSA severity is defined as mild for apnea-hypopnea index (AHI) ≥5 and <15, moderate for AHI ≥15 and ≤30, and severe for AHI >30 events/h (2).

The principal treatment methodology for OSA patients is positive airway pressure (PAP) (2,3). In patients with mild to moderate OSA, oral appliances such as mandibular advancement devices (MADs) and tongue advancement devices are alternately indicated especially for patients intolerant to continuous PAP therapy (3).

The American Academy of Dental Sleep Medicine (3) recommended titratable-customized MADs (TC-MAD) for patient comfort and the ability to permit modifications in the amount of mandibular protrusion for treatment efficacy. However, several studies (4-7) demonstrated similar therapeutic efficiency of non-titratable-customized MADs (NTC-MADs) in the treatment of simple snoring (SS) and OSA.

The treatment efficacy of oral appliances is generally identified by polysomnography (PSG). Important physiological

sleep markers such as AHI, oxygen desaturation index (ODI), and respiratory disturbance index are obtained with PSG (2,3,8). However, the rareness of qualified PSG centers and late appointment times are still the main factors restricting its usage. Pepin et al. (9) proposed measurement and comparison of snoring sound magnitudes as an alternative method for the follow-up of SS and OSA patients and claimed that at least 15% of snorers had an AHI >15 events/h. It was shown that the acoustic characteristics of snoring differed between SS and OSA patients (10).

The latest remarkable developments in the telecommunication industry and smartphone technologies have enabled to monitor and compare magnitudes of snoring at home by using a wide range of smartphone sleep applications (SSA). Manufacturers of SSA claim that these applications enable users to record snoring sounds, snoring frequencies, and snoring intensities.

The effect of oral appliances on SS and OSA has been studied extensively by using PSG, type-3 home sleep study device, and subjective questionnaires. However, there are no studies in the literature evaluating the therapeutic effects of MADs in OSA patients using both SSA and simple pulse oximeter (SPO). The aim of the present study was to determine the efficacy of NTC-MAD therapy on snoring intensity, snoring frequency, and oxygen desaturation periods in patients with snoring problems using SSA and SPO methods.

Methods

The present study was approved by the Clinical Trials Ethics Committee of Gulhane Military Medical Academy (Ankara, Turkey) (2015-KAEK-84/ 24th December 2015) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. The present study was registered at ClinicalTrials.gov (NCT04427111). The participants were selected from OSA patients presenting with subjective snoring (n=37) to the Department of Prosthodontics between December 2015 and January 2017. The exclusion criteria for the study were; having severe OSA, having previous surgery for OSA, and carrying a high risk of cardiovascular, respiratory, neurological or psychiatric disorders (n=12). Patients with an inadequate dental anchor for MAD treatment, temporomandibular joint dysfunction, and Angle Class 3 maxillomandibular relation (n=7) were also excluded. OSA patients who were not eligible for MAD treatment were informed and directed to other treatment options. Totally 18 mild-moderate OSA patients with subjective snoring complaints were included in the study. Of 18 patients, eight patients (44.4%) were female and 10 patients were (56.6%) male. The average age of the patients was 49.33 years, ranging between 34 and 83 years.

PSG, ODI, and SSA values were used to determine and compare the therapeutic efficacy of NTC-MAD treatment in the present study. The diagnostic PSG values of the subjects (before NTC-MAD use) were accepted as initial PSG (PSG-i) values. Final PSG (PSG-f) measurements were performed after 24 weeks of NTC-MAD treatment with NTC-MADs *in situ*. Body mass index (BMI) and neck circumference values were also recorded before NTC-MAD treatment and at the 24th week of the treatment of NTC-MAD. The success criterion of NTC-MAD treatment was defined as the decrease of AHI below 5 per hour. To detect a reduction in AHI, a minimum sample size of 13 participants was considered to obtain 80% power at a 95% confidence to demonstrate a significant difference in AHI of three months after the NTC-MAD therapy, as previously reported (4). A 10% dropout rate was assumed. It was indicated that 15 pairs of subjects demonstrated the therapeutic effect of NTC-MAD in the present study.

To determine initial ODI (ODI-i) values, all subjects were first informed about the proper usage of SPO (Beurer PO 80, BEURER GmbH, Ulm, Germany) in detail. Subjects were instructed to press the start button and attach the device's probe on the extremity of their index finger before falling asleep. To determine initial SSA values, all subjects were first informed about the proper usage of SnoreLab SSA software (Reviva Softworks Ltd., London, UK). Then, the subjects were instructed to download SSA on their smartphones. All subjects were recommended to place their smartphones next to their bed with the microphone facing towards the subjects, to keep the charger connected, and to sleep alone at the quietest room of the home during SSA recordings. Also, the subjects were instructed to use SSA and SPO in-home environment during sleep for three nights due to minimizing the environmental impact on recordings. The average SSA and SPO values obtained in three nights were calculated and initial mean values of ODI, snoring intensity score (SIS), and snoring percentage (SP) were obtained.

After obtaining ODI-i and SSA values, NTC-MADs were fabricated. Irreversible hydrocolloid impressions (Italgin Chromatic Alginate, BMS, Capponoli, Italy) were made from both dental arches. The impressions were poured with type 3 dental stone (Denstone 3, Ata Yapı Ürünleri San. ve Tic. Ltd., Turkey). Autopolymerizing acrylic resin splints (Self Curing for Ortho Chrystal, BMS, Capponoli, Italy) in 2-2.5 mm thickness were fabricated on the models of upper and lower jaws and were tried intraorally. Interferences on maximal retrusive and protrusive positions were eliminated. Maximal mandibular protrusion value (MMPV) and retrusive positions were marked on splints (Figure 1A, 1B). To determine efficient mandibular protrusion value (EMPV), MMPV was measured for each patient and the 60-75% (4,11) of MMPV was calculated and marked (Figure 1C). An interincisal space of 6 mm was set for each patient (4). The most comfortable protruded mandibular position situated between 60 and 75% of MMPV was determined according to

patient response and registered with wax rims. (Figure 1D) The registered models were mounted on a semi-adjustable articulator (AAA43002 CT, Amann Girrbach Artex, Koblach, Austria). The upper and lower acrylic resin splints were secured to each other in the posterior region with auto polymerizing acrylic resin and NTC-MADs were obtained. Subsequently, the NTC-MADs were trimmed, polished, and placed on dental arches (Figure 1E, 1F). The patients were instructed to wear their NTC-MAD every night. After the initial of NTC-MAD therapy, each patient performed the previously described ODI and SSA procedures at the 1st, 4th, 12th, and 24th weeks of the treatment protocol.

Consequently, totally 17 measurement values for each subject were recorded; PSG-i values, PSG-f values, ODI-i values, ODI 1st week values (ODI-1w), ODI 4th week values (ODI-4w), ODI 12th week values (ODI-12w), ODI 24th week values (ODI-24w), initial SIS (SIS-i) values, SIS 1st week values (SIS-1w), SIS 4th week values (SIS-4w), SIS 12th week values (SIS-12w), SIS 24th week values (SIS-24w), initial SP values (SP-i), SP 1st week values (SP-1w), SP 4th week values (SP-4w), SP 12th week values (SP-12w) and SP 24th week values (SP-24w) were obtained.

Statistical Analysis

All statistical analyses were performed with the SPSS 22.0 software package (SPSS Inc., Chicago, Ill). Continuous variables were expressed as the means with standard deviations. The Kolmogorov-Smirnov test was used to confirm that the data were within the ranges of normal distribution. The t-test (two-tailed for paired samples) was used to compare the differences between initial and final values. To evaluate the effect of MAD on ODI, SIS, and SP, one-way repeated measures analysis of variance test (ANOVA) was used. The Bonferroni-Holm correction was used to correct the multiple comparisons. The type 1 error probability associated with this test was set as (α=0.05).

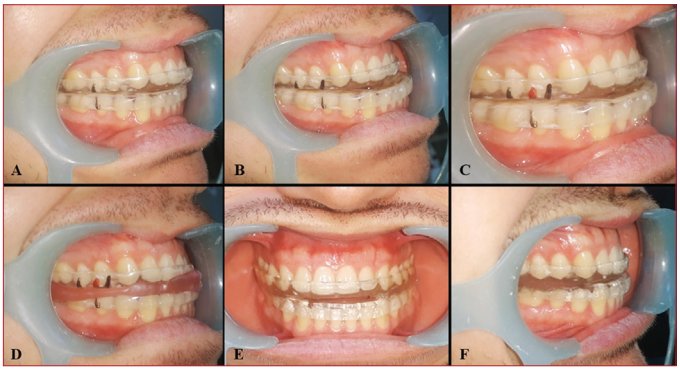

Figure 1. Setting the mandibular protrusion rate at 60-75% of the maximal mandibular protrusion. A) Retrusive position, B) maximal mandibular protrusion value (MMPV), C) efficient mandibular protrusion value (EMPV) (60-75% of MMPV). D) Fixing EMPV with wax rims. E and F) Intraoral views of non-titratable customized mandibular advancement device

Results

All patients completed the study protocol. No significant difference was found between the mean BMI and neck circumference values comparing the 1st (BMI=29.62±5.56 kg/m^2 and neck circumference=38.22±4.12 cm) and 24th weeks (BMI=28.84±5.10 kg/m^2 and neck circumference=38.22±4.12 cm) of the treatment [t(17)=1.748, p=0.98 and t(17)=1.758, p=0.97 respectively].

PSG-i and 24th week (PSG-f) mean PSG values were shown in Table 1. In the comparison of PSG-i and PSG-f values, it was determined that all AHI, ODI, and SI values were significantly decreased. The average decrease of AHI, ODI, and SI values were 5.67±3.66/h (p<0.001), 4.25±3.49/h (p<0.001) and 51.48±51.25/h (p=0.005), respectively. According to PSG results, the success of NTC-MAD treatment was 68.7% for OSA patients.

The mean ODI, SIS, and SP values were depicted in Table 2. Intragroup comparison of ODI, SIS, and SP at 5 different time intervals (ANOVA) showed a significant difference (p<0.001). A significant difference was found between ODI-i and ODI-1w, ODI-4w, ODI-12w, and ODI-24w (p<0.05). The highest decrease in ODI values was reached at the 12th week of the treatment. No significant difference was found between ODI-12w and ODI-24w (p=0.181) (Figure 2A). As with ODI values, a significant difference was also found between SIS-i and SIS-1w, SIS-4w, SIS-12w, and SIS-24w (p<0.001) except SIS-12w and SIS-24w (p=0.113) (Figure 2B).

Intragroup comparison of SP values showed that there was a significant difference at 5 different time intervals (p<0.001). The Bonferroni-Holm correction displaced no significant difference between SP-i and SP-1w (p=0.56), SP-4w and SP-12w (p=1) and SP-12w and SP-24w (p=0.510) (Figure 2C).

Table 1. Initial polysomnography (PSG) and 24th week PSG-final data (mean value±standard deviation)

	PSG-i	PSG-f	t value	p value
AHI (events/h)	10.59±5.23	4.91±2.6	6.574	**<0.001***
ODI (events/h)	7.61±4.88	3.35±2.39	5.169	**<0.001***
SI (events/h)	173.17±131.93	122,26±31,18	3.48	**0.005***

*Indicates significant changes for intragroup comparison (t-test).
AHI: Apnea-hypopnea index, ODI: Oxygen desaturation index, SI: Snoring index, PSG-i: Initial polysomnography, PSG-f: Final PSG

Discussion

The objective of the present study was to determine the efficacy of NTC-MAD therapy on snoring intensity, frequency, and oxygen desaturation periods in mild-moderate OSA patients with snoring problems. For the differential diagnosis of patients referring to snoring complaints, AHI was accepted to be the main physiological sleep parameter in the present study. Besides, the AHI values were also used for the evaluation of the NTC-MAD's treatment success in OSA patients (2,3).

MAD treatment success rate determined by AHI varies between 19% (12) and 71% (4). Age, gender, BMI, the severity level of OSA, and MAD type may influence the success of MAD treatment (3,13,14). It is emphasized that EMPV is the most determinative parameter affecting customized MAD treatment success (3). Aarab et al. (4) reported that 50% to 75% of MMPV had higher treatment efficiencies. Marklund et al. (11) reported that using more than 75% of MMPV increased the complication probability.

Table 2. Pairwise comparison of oxygen desaturation index, snoring intensity score and snoring percentage at different time intervals

	Time interval	Mean±SD	f value	p value
ODI	ODI-i	7.82±5.39	25.17	**p<0.001***
	ODI-1w	6.62±4.48		
	ODI-4w	4.37±2.99		
	ODI-12w	3.5±2.67		
	ODI-24w	3.14±2.32		
SIS	SIS-i	72.96±31.14	39.45	**p<0.001***
	SIS-1w	61.09±26.97		
	SIS-4w	48.85±24.35		
	SIS-12w	40.20±22.33		
	SIS-24w	36.03±22.14		
SP	SP-i	32.61±17.29	21.46	**p<0.001***
	SP-1w	29.40±14.60		
	SP-4w	21.29±11.95		
	SP-12w	19.81±10.64		
	SP-24w	17.35±8.97		

*Indicates significant changes for intragroup comparison (ANOVA).
SD: Standard deviation, ODI: Oxygen desaturation index, SIS: Snoring intensity score, SP: Snoring percentage

In the present study, the NTC-MADs were fabricated by using 60-75% of MMPV to provide optimal treatment efficacy and to minimize the side effects. All patients use the appliances appropriately without side effects that restrict the use of appliances. The treatment success of the present study was similar to those in which EMPV was expressed as being the 60-75% of MMPV, either with TC-MADs or NTC-MADs (4,6,15-17). In these studies, the success of the MAD treatment is determined by full night PSG. However, the insufficient number of qualified sleep laboratories and the expensiveness of the PSG procedure obliged the clinicians to use home testing devices for the diagnosis and follow-up of sleep disorders. One of these home testing devices is SPO (18). SPOs are capable of calculating the ODI and monitoring automatically the desaturation fluctuations during sleep. The major concern about ODI is that hypopneas and short apneas do not always cause a 3-4% decrease in oxygen saturation. In such cases, the ODI parameter is far from being determinative (18).

Due to the coherence of AHI and ODI values in morbid obesity, ODI was especially used for the follow-up procedures of OSA in morbidly obese patients with AHI >10 (19). Recently, Ernst et al. (20) reported that the correlation between AHI and ODI was larger in patients with normal weight and proportionally decreased by the increase in BMI. This result highlighted the important role of ODI during the follow-up of OSA patients.

In the present study, a significant decrease in ODI (4.68 events/h) was found using NTC-MADs. Incoherence with our study, Ghazal et al. (21) reported a decrease of 4.50 events/h in ODI with TC-MADs. Similarly, Barnes et al. (22) found a decrease of 4.30 events/h in ODI with TC-MADs. Zhou and Liu

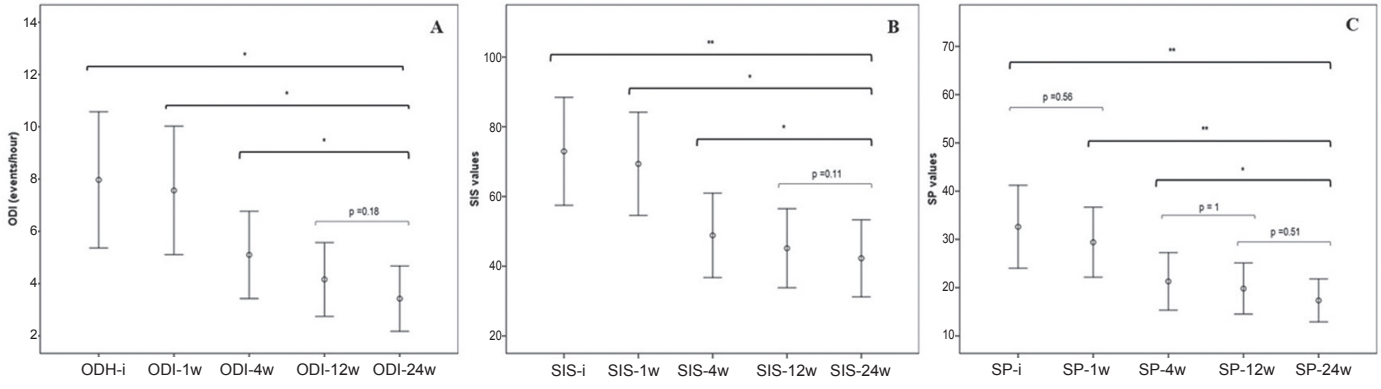

Figure 2. Multiple comparisons of simple pulse oximeter and smartphone sleep application values (Bonferroni-Holm correction) (*p<0.05, **p<0.01, 1. standard deviation). A) Oxygen desaturation index, B) snoring intensity score, C) snoring percentage

ODI: Oxygen desaturation index, SIS: Snoring intensity score, SP: Snoring percentage

(6) reported that compared to TC-MADs, NTC-MADs achieved an equivalent reduction in ODI in adult patients with OSA and reported a decrease of 25.00 events per hour in ODI with the use of an NTC-MADs. The authors physiologically determined individual EMPVs using the PSG test and titration for each patient. That proper method for determining EMPV explains the huge difference in ODI values compared to the present study.

In all of the above-mentioned studies, the mean differences in ODI were only determined at the end of the 12th or 24th weeks. There is not any study that reported the mean ODI difference in periodical time intervals within the treatment procedure. According to the results of the present study, it can be deduced that the maximum reduction in ODI can be achieved in 12 weeks.

MADs are not only indicated for the treatment of OSA patients but also for SS patients. The effects of MADs on snoring were mostly assessed by subjective tests such as questionnaires and visional analog scale (3,5). However, some researchers suggested using sensors and microphones in a soundproofing environment for recording and evaluating snoring sounds objectively (10,23). Snoring sound frequency could be evaluated as an important parameter for differential diagnosis between SS and OSA because the snoring sounds of OSA patients recorded in sleep laboratories have a frequency higher than 800 Hz (10) and SS patients usually have frequencies between 110 and 190 Hz (below 500 Hz) (23).

Remarkable technological advancement of communication provides recording snoring sounds at the patient's home by using smartphones. And also, SSAs monitor the snoring sounds as numeric data. Although there are many SSAs, few studies report about the reliability of SSAs. An excellent positive correlation was reported between SSAs and sleep laboratory sound recordings of 93% to 96% in a soundproof environment (24,25).

According to studies evaluating snoring sounds, the MAD treatment decreased SIS (26,27). Walker-Engström et al. (26) determined that NTC-MADs with 75% of MMPV caused a higher decrease in SIS. Besides, Bloch et al. (27) stated that NTC-MADs provided a greater reduction in subjective snoring intensity than TC-MADs. In the present study, SIS values started to decrease just from the 1st week of NTC-MAD treatment. O'Sullivan et al. (28) reported that MADs reduced SIS even at the first night. Smith and Battagel (29) reported that the use of MAD for a month caused a 64% reduction in SIS. However, the SIS reduction rate (43%) in this study was not as high as theirs. That difference may be explained with the determination difference of EMPV existing between two studies. While Smith and Battagel (29) used one-night titration to determine EMPV, a standard EMPV (60-75% of MMPV) was used in the present study. Considering the obtained SSA data, it was determined that the maximum reduction in SIS with the use of NTC-MADs could be obtained at the 12th week.

It was determined in the present study that SP significantly decreased (39%) at the 4th week and that level remained constant till the end of the study. Umemoto et al. (7) and Lee et al. (30) reported a 40% and 34% decrease in SP values, respectively, with the use of NTC-MADs. However, Umemoto et al. (7) found a decrease of 9% in SP values with TC-MADs while that of NTC-MADs was 40%. The authors concluded that due to their more stable and compact structure that keeps the mandible at a constant protrusive position, NTC-MADs were much more efficient in SP than TC-MADs.

Numerous sleep medicine authors recommended the use of TC-MADs due to some advantages like providing the titration of MADs to optimize its efficiency, keeping the mandible more flexible, and causing fewer side effects (2,3). However, NTC-MADs were used in the present study as in some previous studies (3,5,26,27) reporting that the treatment efficiencies of NTC-MADs and TC-MADs were similar. Further, some studies declared that NTC-MADs were more efficient in snoring due to structural characteristics (6,7). Besides, NTC-MADs are much more economic devices compared to TC-MADs, thus, low-costing NTC-MADs were used for SS and OSA patients with snoring complaints. The other limitations are that no information exists about how to calibrate SnoreLab to calculate SIS and SP and that SSAs may be affected by ambient sounds (25). A wide range of smartphones with various technological properties may lead to differences in the determination of MAD treatment success. Also, the age and susceptibility of the patients to the technology may affect the results of the sleep studies.

SSAs are still very new and there is not enough reliable information about them. However, technological developments can be considered as a messenger soon that SSAs, which have advanced algorithms that are less affected by smartphone hardware and ambient sounds, will take their place in the markets.

Conclusion

Within the limitations of this *in vivo* study, the following conclusions were drawn:

1. The use of NTC-MADs for 24 weeks was found to be an efficient method for decreasing AHI, ODI, SI, SIS, and SP.

2. Significant reduction in ODI and SIS was observed at the end of the 1st week and continued to decrease regularly until the 12th week.

3. A significant decrease in SP was determined at the 4th week.

It was concluded that NTC-MADs significantly decreased snoring intensity and frequency and they are effective devices in the treatment of SS and OSA.

Ethics

Ethics Committee Approval: The trial was approved by Clinical Trials Ethics Committee of Gülhane Military Medical Academy in Ankara, Turkey (protocol number: 2015-KAEK-84, date: 24th December 2015).

Informed Consent: All patients gave their written informed consent to participate in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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The awareness of chronic obstructive pulmonary disease among smokers admitting to pulmonary medicine outpatient clinic: Single center experience

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ABSTRACT

Aims: Smoking is the strongest risk factor for chronic obstructive pulmonary disease (COPD), but the awareness of COPD among smokers has been found at low levels worldwide. The aim of current study was to assess the COPD awareness level among smokers and to determine the factors associated with awareness.

Methods: The current study involved the smokers admitted to the smoking cessation outpatient clinic, pulmonary medicine outpatient clinic, and pulmonary medicine clinic of an Training and Research Hospital. A self-administered COPD awareness survey was applied and the question of "Have you ever heard the term of COPD?" was asked to all participants. According to the survey scores, participants were categorized into groups as "Poor", "Good" and "Very Good" Awareness.

Results: All included 531 participants were asked the question of "Have you ever heard the term of COPD?", and 490 (92.3%) of them answered that question as "Yes". Statistically significant differences were found between the participants saying "Yes" and those saying "No" in the terms of the age range, smoking status, and education status with the numbers and percentages of 117 (23.87%), 246 (50.2%), and 170 (34.69%) ($p=0.039$, $p<0.001$, and $p=0.004$), respectively. The numbers and percentages of "Poor Awareness", "Good Awareness" and "Very Good Awareness" groups were 211 (39.75%), 299 (56.3%), and 21 (3.95%), respectively.

Conclusions: The awareness level of COPD among smokers admitted to pulmonary medicine department was detected as "Good", and this "Good" level was predicted to be associated with the publicly sponsored broad anti-smoking campaigns including smoking cessation polyclinics offering free service.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities. COPD is in close relationship with significant exposure to noxious particles, especially tobacco (1). With a prevalence of 5 to 14% in general population, COPD is currently the 4th leading cause of death in the world but is projected to be the 3rd leading cause of death by 2020 (2-7). The risk of developing COPD among smokers is more than 40% (8). In Turkey, the prevalence of tobacco smoking and COPD disease is 31.6% and 3.6%, respectively (9).

COPD onset time for smokers has not been clear yet. COPD patients even if with an end-stage, may usually perceive their symptoms like dyspnea and weakness as an expected age-relevant condition rather than COPD (10). Additionally, it has been declared that, under or misdiagnosed percentages of COPD patients were very high (8,11-13). The initial step of prevention and treatment for COPD is the awareness of this COPD disaster. Currently, no validated method and survey were defined to evaluate the awareness level of public and especially smokers on COPD (14-21).

In GARD study conducted to evaluate COPD awareness in general population of Turkey, it was observed that

approximately half of the participants did not know that COPD was a lung disease and was associated with smoking (22). In a survey conducted in current smokers, results also showed that awareness was lower than expected in Turkey (23). However, especially in recent years, it is prospected that educational activities such as increasing social trainings, warnings on cigarette packs, public spots, smoke free zones and smoking cessation polyclinics may have changed this situation positively. With this point of view, in this current study, we aimed to reveal the awareness and knowledge of COPD in smokers. In this way, we aim to make awareness assessments especially for active smokers, who are the target audience, and to help to plan the appropriate measures and education programs with the results to be drawn from this study.

Methods

This study is a cross-sectional epidemiologic study performed in the Pulmonary Medicine Department of University of Health of Sciences Turkey, Gülhane Training and Research Hospital (Ankara, Turkey) between the dates of May 01, 2018 and December 31, 2019. Current smokers admitted to the Smoking Cessation Outpatient Clinic and Pulmonary Medicine Outpatient Clinic were invited to participate in this current survey. The volunteer participants were applied to self-administered questionnaire that consisted of three different sections with the titles of A, B and C.

Section A: The personal data including age, gender, body mass index, education status, occupation, clinical findings such as symptoms (cough, dyspnea, etc.) and symptom score of dyspnea in accordance with the Modified Medical Research Council scale of the participants were gathered.

Overall tobacco exposure burden of the participants was assessed by package/year (p/y) calculation, and the nicotine addiction level was evaluated with the Fagerström Test for Nicotine Dependence Test Score (FTND). Participants were defined as “mild”, “moderate” and “severe” smokers according to the tobacco exposure years of less than 10 p/y, 10-30 p/y and more than 30 p/y, respectively. Participants were also grouped as mild, moderate and severe nicotine addicts according to their FTND scores (FTND ≤3, 4-7 and ≥8, respectively).

Section B: The participants were asked whether they had ever heard about COPD and they had any information about COPD. Their information resources were also asked.

Section C: The participants were asked a 20 questioned-COPD Awareness Level Survey consisting of four different question concepts as the ‘perception’ episode including the questions numbers of 1, 2, 4, 5 and 6, the ‘knowledge’ episode including the questions numbers of 3, 7, 16, 17 and 18, the ‘acceptance’ episode including the questions numbers of 8, 10, 11, 12 and 19 and the ‘expectation’ episode including the questions numbers of 9, 13, 14, 15 and 20 (Table 1). The distribution of episodes’’ questions was harmonized in order to provide answering without any bias.

The questions of survey were answered by participants as ‘absolutely disagreed’, ‘disagreed’, ‘indecisive’, ‘agreed’ and ‘absolutely agreed’. Each question was scored with 1 to 5 points according to these answers (except for 13th, 14th and 17th questions which were valued as absolutely disagreed as 5 points, disagreed as 4 points, indecisive as 3 points, agreed as 2 points and absolutely agreed as 1 point in order to reduce the predictability of the survey by the participant). This reversed valued questions helped to avoid the bias answering. The answers were reorganized into three groups as follows; the ‘absolutely disagreed’ and ‘disagreed’ were combined as ‘disagreed participants’, the ‘absolutely agreed’ and ‘agreed’ were combined as ‘agreed participants’ and ‘indecisive’ was presented as ‘undecided participants. Then, Total Awareness Score was calculated by summing all the answer points for each participant. Considering the Total Awareness Scores, the participants were levelled into three groups named as “POOR”, “GOOD” and “VERY GOOD” awareness with the total points of 0-69, 70-85 and 86-100, respectively.

Ethics committee approval was received for this study from the Ethics Committee of Gülhane Training and Research Hospital with the number of 18/111 at April 24, 2018. Written informed consent was obtained from patients who participated in this study.

Statistical Analysis

Relationships of investigated parameters were evaluated statistically. SPSS for Mac 20.0 package program (SPSS Inc, Chicago, IL) was used for statistical evaluation. Data were summarized as the mean and standard deviation (SD) for the continuous variables, as absolute value and percentages for the categorical variables. The normality of the continuous variables was analyzed with the Kolmogorov-Smirnov test and Shapiro-Wilks test. Chi-square test for the categorical variables and Student’s t-test or Mann-Whitney U statistical tests were used according to the suitability to the normal distribution for the continuous variables. In the assessment of correlations, Spearman’s correlation analysis was used for data with non-parametric distribution. P value less than 0.05 was considered as statistically significant with a 95% confidence interval.

Results

A total of 531 [343 men (64.5%), 188 women (35.5%)] participants were enrolled to the study and all were applied the COPD Awareness Survey shown in Table 1. The participants were included from smoking cessation outpatient clinic, pulmonary medicine outpatient clinic and pulmonary medicine clinic with the numbers and percentages of 398 (75%), 119

Table 1. The chronic obstructive pulmonary disease awareness survey

	Disagreed		Indecisive	Agreed	
	Completely Disagreed	Disagreed	Indecisive	Agreed	Completely Agreed
1. COPD is a very frequent disease.					
2. COPD has a severe mortality rate.					
3. COPD progresses with an exacerbation.					
4. COPD is a chronic disease.					
5. COPD is a preventable disease.					
6. COPD is a treatable disease.					
7. This disease develops with the obstruction of the airways.					
8. Exposure of non-smokers to cigarette smoke in a smoking environment may cause COPD.					
9. Cessation of smoking may mostly prevent COPD.					
10. Exposure to outdoor air pollution may cause COPD (especially fumes from vehicles exhaust in traffic).					
11. Exposure to organic and inorganic occupational dusts and chemicals may cause COPD.					
12. Smoke from substances such as wood, dung, bushes, coal burned for heating and cooking at home may cause COPD.					
13. The deficiency of vitamin A, C and E may play a role in the development of COPD.					
14. Alcohol use may play a role in the development of COPD.					
15. Although rare, some people may genetically develop COPD.					
16. Although patients are in the risk group, they may not have complaints.					
17. The most important complaints of the patients are cough and sputum production.					
18. Complaints of the COPD patients are more intense during the morning.					
19. The most common disease differentiated with COPD is asthma.					
20. Influenza and pneumonia vaccines are needed to prevent from COPD.					

COPD: Chronic obstructive pulmonary disease

(22.9%) and 14 (2.6%), respectively. The social-demographics and smoking characteristics of participants were detected in Section A and summarized in Table 2.

In Section B, all 531 participants were asked the question "Have you ever heard the term of COPD?". Four hundred ninety (92.3%) of the participants answered this question as "YES". Considering the age range, the participants in the age range of 40-49 years had a statistically significant higher rate with the number and percentage of 117 (23.87%) (p=0.039). According to smoking status, the moderate group (10-30 p/y) had significantly higher values and percentage of 246 and 50.2% (p<0.001). When taking education status into consideration, in all education levels, the percentage of answering as "YES" was higher compared to the participants answering as "NO" and the statistically prominent number and percentage of 170 and 34.69% was determined at primary/secondary school graduate (p=0.004) respectively. The general characteristics of those who heard and did not hear the term of COPD were summarized in Table 3.

After the question "Have you heard of COPD?", the second question "Do you know what COPD is?" was asked to all participants. Four hundred twenty-four of 490 participants who said they heard about COPD stated that they had information about COPD. That is, a total of 107 participants stated that they did not know what COPD was.

The predicted symptoms of COPD and primary learning resource of COPD were also evaluated in Section B (Table 4). Dyspnea was described as the first most predicted symptom of COPD by 452 of the participants. In the disease-symptom pairing related to COPD, the symptoms were listed by frequency of response as dyspnea (87.1%), sputum (79.6%), cough (67.8%), wheezing (64.7%), tiredness (54.5%), chest pain (45.7%) and weight loss (30.4%).

Table 2. Demographics and smoking characteristics of all participants

		Man n=343 (64.5%)	Woman n=188 (35.5%)	All patients n=531 (100%)
Age (mean years±SD)		42.8±16.8	44.3±12.3	43.4±15.4
BMI (mean±SD)		26.4±5.3	25.8±4.5	26.2±5.1
Smoking pack-year (mean±SD)		25.9±19.4	23.9±16.4	25.3±18.4
Education status* (n, %)	**Uneducated**	2 (25%)	6 (75%)	8 (1.5%)
	Pri./Sec. school	126 (64.6%)	69 (35.4%)	195 (37.2%)
	High school	99 (58.6%)	70 (41.4%)	169 (32.3%)
	University	110 (72.4%)	42 (27.6%)	152 (29%)
Occupation status (n, %)	**Unemployed**	10 (50%)	10 (50%)	20 (4%)
	Officer	43 (67.2%)	21 (32.8%)	64 (13%)
	Worker	173 (84.8%)	31 (15.2%)	204 (41.2%)
	Student	22 (81.5%)	5 (18.5%)	27 (5.5%)
	Soldier	17 (100%)	-	17 (3.4%)
	Retired	51 (71.8%)	20 (28.2%)	71 (14.3%)
	Housewife	-	92 (100%)	92 (18.6%)
Smoking status (n, %)	**Mild**	84 (71.8%)	33 (28.2%)	117 (22.04%)
	Moderate	144 (56.2%)	112 (43.8%)	256 (48.2%)
	Severe	115 (72.8%)	43 (27.2%)	158 (29.76%)
FTND level (n, %)**	**Mild**	34 (56.7%)	26 (43.3%)	60 (11.5%)
	Moderate	200 (70.2%)	85 (29.8%)	285 (54.6%)
	Severe	100 (56.5%)	77 (43.5%)	177 (33.9%)

(*Seven patients' education status and **nine patients' FTND level were not available).
FTND: Fagerström Test for Nicotine Dependence, SD: Standard deviation, Pri./Sec. School: Primary/secondary school, BMI: Body mass index

The participants were also asked for the resource of their information about COPD. Media (television/newspaper) was the first most popular information resource (46.7%). The highest percentage of doctors as an information resource was present at the participants aged over 60 years with the percentage of 25.9% (Table 4). The distribution of participants' information resources by demographic and cigarette addiction status were summarized in Table 4.

In Section C of the study, a COPD awareness questionnaire with 20 question was applied to the participants (Table 1). The distribution of the answers given to the questions was evaluated. The first three questions confirmed with the highest percentage by men and women were the 9th, 7th and 19th questions with the percentages of 86.3%, 82.5%, 79.6% and 86.2%, 80.9% and 78.2%, respectively.

Confirmation rates of the participants to the question episodes of 'Perception', 'Knowledge', 'Acceptance' and 'Expectation' were also evaluated. The mean numbers and percentages of perception, knowledge, acceptance and expectation questions answered as agreed by all participants were respectively 361.6 (68.1%), 281.6 (53.02%), 381.8 (71.88%) and 250.2 (47.14%). Considering the percentages of survey explained above, the acceptance was detected as a prominent concept at current smokers on COPD awareness and perception, knowledge and expectation followed acceptance.

According to the answers given to the questions of survey, the total COPD Awareness Survey Score of the participants was calculated. Considering the scores of the survey, the mean score was 77.5±7.5 in all participants, 76.7±6.9 in females and 77.9±7.8 in males. Correlations were investigated to determine whether there was a relationship between the COPD awareness score and demographic, social and clinical data. Possible correlations between awareness scores with age, BMI, cigarette package-year (p/y), education level, duration of smoking, and FTND score of participants were assessed. We observed a positive significant correlation between COPD awareness score with cigarette package/year (p=0.023, r=0.099).

According to the mean±SD results of the normally distributed results, the participants' scores were divided into 3 main groups; "Poor Awareness", "Good Awareness" and "Very Good Awareness". Those whose total scores were within the 'mean score±SD' range were defined as "Good Awareness", those with higher scores than the 'mean score+SD' upper limit as "Very Good Awareness" and the those with a lower score than the 'mean score–SD' as "Poor Awareness". Hereby, the groups "Poor Awareness", "Good Awareness" and "Very Good Awareness"

Table 3. The social and clinical features of the participants those who have heard of chronic obstructive pulmonary disease and those who have not heard

Parameters		Have you ever heard of COPD? n (%)		p
		No	Yes	
Gender	Female	10 (24.39)	178 (36.32)	0.125
	Male	31 (75.61)	312 (63.67)	
Age range	<30	15 (36.58)	108 (22.04)	**0.039**
	30-39	7 (17.07)	93 (18.99)	
	40-49	2 (4.87)	117 (23.87)	
	50-59	10 (24.39)	98 (20.00)	
	>60	7 (17.07)	74 (15.10)	
FTND level*	Mild	5 (12.19)	55 (11.22)	0.398
	Moderate	26 (63.41)	259 (52.85)	
	Severe	10 (24.39)	167 (34.08)	
Smoking status	Mild	20 (48.78)	97 (19.79)	**<0.001**
	Moderate	10 (24.39)	246 (50.20)	
	Severe	11 (26.83)	147 (30.00)	
Education status**	Uneducated	1 (2.44)	7 (1.43)	**0.004**
	Pri-Sec.	25 (60.97)	170 (34.69)	
	High school	11 (26.83)	158 (32.24)	
	University	4 (9.76)	148 (30.20)	

(*Nine patients' FTND level were not available and **seven patients' education status and).
COPD: Chronic obstructive pulmonary disease, FTND: Fagerström Test for Nicotine Dependence, Pri./Sec.: Primary/Secondary

Table 4. The information resources on chronic obstructive pulmonary disease

		n	Informed on COPD 424 (79.85%)					Uninformed on COPD 107 (20.15%)
			Doctor n (%)	Nurse n (%)	Tv/Media n (%)	Internet n (%)	Others n (%)	
All participants		531	101 (23.8%)	49 (11.5%)	198 (46.7%)	27 (6.4%)	49 (11.55)	107 (20.15%)
Gender	Female	188	40 (21.8%)	16 (8.5%)	71 (37.8%)	11 (5.9%)	15 (7.9%)	35 (18.6%)
	Male	343	61 (17.8%)	33 (9.6%)	127 (37%)	16 (4.7%)	34 (9.9%)	72 (21%)
Age range	<30	123	20 (16.3%)	10 (8.1%)	50 (40.6%)	6 (4.9%)	10 (8.1%)	27 (22%)
	31-39	100	18 (18%)	10 (10%)	36 (36%)	4 (4%)	15 (15%)	17 (17%)
	40-49	119	23 (19.3%)	11 (9.2%)	46 (38.7%)	7 (5.9%)	4 (3.4%)	28 (23.5%)
	50-59	108	19 (17.6%)	13 (12.1%)	40 (37%)	5 (6.2%)	12 (11.1%)	19 (17.6%)
	>60	81	21 (25.9%)	5 (6.2%)	26 (32.1%)	5 (6.2%)	8 (9.9%)	16 (19.7%)
FTND*	Mild	60	12 (20%)	9 (15%)	18 (30%)	3 (5%)	6 (10%)	12 (20%)
	Moderate	285	56 (19.7%)	24 (8.4%)	116 (40.7%)	16 (5.6%)	22 (7.7%)	51 (17.9%)
	Severe	177	31 (17.5%)	15 (8.5%)	61 (34.5%)	6 (3.4%)	20 (11.3%)	44 (24.8%)

(*Nine patients' FTND level were not available).
COPD: Chronic obstructive pulmonary disease, FTND: Fagerström Test for Nicotine Dependence

defined the participants with total survey score of "0-69", "70-85" and "86-100", respectively. The numbers and percentages of "Poor Awareness", "Good Awareness" and "Very Good Awareness" leveled groups were 211 (39.75%), 299 (56.3%), and 21 (3.95%), respectively. At the end of the awareness assessment, the proportion of "Very Good Awareness" was found to be 3.97% (n=21) in the entire population. "Very Good Awareness" rates according to the education levels were 0%, 15%, 30% and 55%, respectively. Although there was no statistical significance, it was found that awareness of COPD increased as the level of education increased (Table 5).

Table 5. Chronic obstructive pulmonary disease awareness scores and correlations

		COPD awareness score (n, %)			p
		Poor Awareness	Good Awareness	Very Good Awareness	
FTND level*	Mild	29 (48.3%)	27 (45%)	4 (6.7%)	0.176
	Moderate	114 (40%)	158 (55.4%)	13 (4.6%)	
	Severe	65 (36.7%)	108 (61%)	4 (2.3%)	
Smoking status	Mild	47 (40.2%)	65 (55.6%)	5 (4.3%)	0.830
	Moderate	107 (41.8%)	140 (54.7%)	9 (3.5%)	
	Severe	57 (36.1%)	94 (59.5%)	7 (4.4%)	
Education status**	Uneducated	2 (25%)	6 (75%)	-	0.176
	Pri.-Sec.	80 (41%)	112 (57.4%)	3 (1.5%)	
	High school	67 (39.6%)	96 (56.8%)	6 (3.6%)	
	University	60 (39.5%)	81 (53.3%)	11 (7.2%)	
Gender	Man	140 (40.8%)	189 (55.1%)	14 (4.1%)	0.750
	Woman	71 (37.8%)	110 (58.5%)	7 (3.7%)	
Age range	<30	50 (40.7%)	67 (54.5%)	6 (4.9%)	0.445
	30-39	48 (48%)	49 (49(%)	3 (3%)	
	40-49	50 (42%)	66 (55.5%)	3 (2.5%)	
	50-59	34 (31.5%)	69 (63.9%)	5 (4.6%)	
	>60	29 (35.8%)	48 (59.3%)	4 (4.9%)	

(*Nine patients' FTND levels and **seven patients' education status were not available).
COPD: Chronic obstructive pulmonary disease, FTND: Fagerström Test for Nicotine Dependence

Discussion

COPD is a chronic and progressive airway disease. It still ranks the 4th among all causes of death in the world, and its frequency is expected to increase further in the coming years. Given that smoking is the most important predictive risk factor for COPD, it would not be wrong to say that it is a public health problem that can be prevented and controlled mostly. Despite all these definitive medical facts, the public awareness is still low, unfortunately. Individuals and patients cannot fully identify the disease and the name of COPD; moreover, patients express their illnesses with wrong diagnoses such as asthma, bronchitis etc. On the other hand, there are problems in conceptual perception regarding COPD treatment. Most patients perceive the inhaler bronchodilator treatments that are prescribed to them only as "air' and believe that they are not given any medications. This unconsciousness also adversely affects patients' compliance to treatment. Given all these facts, it is obvious that raising public awareness about COPD is a public health responsibility.

Considering the COPD Awareness Survey Scores of participants with the mean points of 77.5±7.5 points, it was found to be corresponded to "Good Awareness Level".

In Turkey, such as many countries in the world, the perception and knowledge of COPD is thought to have increased in recent years thanks to smoking restrictions, smoke-free airspace applications, very serious control mechanisms related to the sale and consumption of tobacco products, informative messages added on cigarette packs, public spots, smoking cessation polyclinics, social programs and trainings. In our clinical practice, the feedbacks received from individuals and patients have revealed the dominant idea that social knowledge about COPD has increased, but of course, more concrete data are needed to turn this idea into an objective scientific thesis. At this point, based on the references taken from previous studies, it is commonly accepted that face-to-face interviews and objective surveys give very useful results. In screening studies for COPD awareness, positive changes in awareness rates have been observed in the last 20 years. However, these positive developments differ considerably among countries.

In a Spain-based study, which is one of the comparative old examples of such studies, awareness rates for 2012 and 2002 were compared, and it was found that COPD awareness increased from 8.6% to 17.0% in the general population (24,21). In a telephone survey study conducted in Spain, only 8.6% of 6758 people knew COPD (24). Smokers were reached in a hybrid survey study conducted in Canada in 2010. While 72% of active smokers knew about cancer and 56% knew about sleep apnea, only 36% said they had heard of COPD before (25). However, when compared to the Canada's COPD awareness rate in 2005, the increase from 17% to 36% can be considered as a partial positive development (26,27).

In a French study conducted in 2011, only 8% individuals knew the term of COPD and 66% associated the term COPD

with respiratory disease in the population aged 40 to 75 years (17). We see that higher awareness rates are starting to be identified as we come to more recent times. For example, in a Danish study, published in 2018, including 1002 participants, the percentages of knowing the symptoms of COPD were 86.4%, 89.2%, 81.5% and 85.1% at smokers, ex-smokers, non-smokers and all participants, respectively (28). In GARD study carried out in Turkey in 2013, COPD awareness of the general population was found to be 49.6% (22). Unfortunately, these positive developments have not been detected in studies in Korea. In Korean studies, awareness of COPD among smokers was between 0.4% and 26.5% (15,29). In this study, we aimed to investigate this awareness in smokers, which was the main target group for the development and prevention of COPD. The data we obtained through the face-to-face interviews and questionnaire study gave us very positive results on the awareness of COPD in active smokers, the highest risk population for COPD.

The mean age of the applicants to quit smoking was 43.4±15.4 years, which represented an age group suitable for the early diagnosis of COPD (1,4). Considering the gender distribution, men made up the majority. This situation has been interpreted in relation to the higher incidence of smoking in men in our country (9). At the evaluation of educational status, the positive relationship between the desire of quitting smoke and the education level of smokers was stood out. Our examination of nicotine dependency rates of those who accepted to quit smoking and participate in our study showed us that individuals with low and high mean FTND scores were in the minority and most of the participants had moderate FTND scores. In other words, it can be said that individuals with moderate FTND scores are more enthusiastic about quitting smoking.

In this current study, the high percentage of “YES” answers (92.3%) given by the smokers to the question of “Have you ever heard of COPD?” is a significant indicator of high awareness to COPD as the beneficial result of anti-smoking strategies like an establishing smoking cessation outpatient clinics. This rate is significantly higher than the rates reported in some studies involving the entire population or smokers only. Different mechanisms may have played role here. The first and the most hopeful is the significantly increased awareness of COPD in the community, especially among smokers. This increase in hearing COPD can be considered as an indicator of that anti-smoking strategies are working, although reducing smoking rates is the main goal of these strategies. The second possible mechanism is that the population of this study consists of participants who applied to the smoking cessation unit of a tertiary hospital voluntarily, reside in the capital and have a high awareness of receiving health care. This participant profile may not fully reflect the countryside. In other words, when it spreads to the general public, this awareness rate will probably reduce. In a survey conducted in 2016 in Turkey involving active smokers, it was reported that only 34% of the population recognized the term “COPD” as a “pulmonary disease” (23). In addition, since it covers only enthusiasts for smoking cessation, but not all smokers, it can be thought that the participants are a high perception population about the smoking-related health problems.

While there is no significant difference in terms of gender distribution and FTND levels between those who have heard of COPD and those who have not heard before, there was a significant difference between age groups, smoking intensity and education levels (Table 3). Similarly, in the GARD study conducted in Turkey, there was no significant difference between the genders and the awareness rates decreasing in older age (22). In this current study, it was found that those with a low intensity of smoking were unaware of the disease more often. While 66.93% of those who had heard of the disease before were at least high school graduates, only 36.59% of those who had never heard before were at least high school graduates. The education levels of the participants seem to be very decisive in this regard.

Among the resources of information about COPD, TV/media was the top rated for all participants. While the TV/media sources became more prominent in the younger age group, physicians came to dominant for with advancing age as the information resources. This is a result that supports the view that personal information acquisition sources are closely related to social life habits. This result should be taken into account in the future educational activities for the younger age group who are close to the media. It can be interpreted that family physicians should show more sensitivity to COPD in middle and older age groups. Similarly, in the results of Spain and Korea, TV was shown as the most common source of information (15,21).

When we analyzed the answers given to our COPD awareness survey questions, we found that the highest awareness was in the ‘acceptance’ questions and the lowest awareness was in the ‘expectation’ questions. This concept is very specific to the current study and no evident mentioning on this concept was present in the concerning studies (14-29). While awareness of smokers about risk factors in the development of COPD was higher, levels of knowledge about prevention of COPD and follow-up of the disease were found lower. This shows that smokers have mostly heard of COPD, but they need to know better about the disease.

We observed a significant positive correlation between COPD awareness score and cigarette p/y levels. We interpret this relationship that awareness of COPD and therefore concerns about the disease have increased in intensive smokers over time, perhaps due to the early onset of symptoms, perhaps from the warnings of people in their close circles, or as a result of seeing the stimulating relatives on cigarette packs more

frequently. In general, we also observed a close relationship between COPD awareness and education level. The frequency of 'very good awareness' was encountered most frequently among participants who were university graduates. According to the total score of COPD awareness survey, 17 of the 20 participants evaluated in the 'very good awareness' group were at least high school graduates. Increasing the level of education should be considered as one of the points of diffraction in social struggle with COPD. The fact that our study was conducted in volunteers who applied to smoking cessation units can be considered as a subjective limitation.

Conclusion

The awareness level of COPD among smokers admitted to pulmonary medicine department was detected as "Good", and this "Good" level is predicted to be associated with the publicly sponsored broad anti-smoking campaigns including smoking cessation outpatient clinics offering free service.

The high-level acceptance of the participants on COPD Awareness encouraged us to abolish COPD in Turkey and all around the world. Since high percentages of positive answers given to admission questions compared to other sections were prominent in the survey, it is advisable to increase the awareness level of smokers should be set as a priority target in policies to combat COPD. Thus, smoking rates and COPD frequency could be reduced together. More general data can be obtained with this type of study to be carried out by spreading to the general public. The smoker part of the public must be taken into consideration to struggle with the smoking pandemic. Especially, campaigns aiming to correct the acceptance of smokers would increase the success of smoking cessation strategies. We believe that current study and the subsequent ones to be carried out in this concept from now on will guide the fight against smoking and COPD.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gülhane Training and Research Hospital with the number of 18/111 at April 24, 2018.

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.A., O.S., Concept: Y.A., O.S., Design: Y.A., N.Ö., Data Collection or Processing: A.Ç., Y.A., Analysis or Interpretation: D.D., C.T., Writing: Y.A., N.Ö.

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Dens invaginatus: A report of two cases

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Keywords: Dens in dente, classification, tooth abnormalities, incisor, dental papilla

ABSTRACT

Dens invaginatus is an uncommon developmental anomaly of teeth arising due to the invagination of the dental papilla. The affected teeth show an infolding of enamel and dentine even sometimes extending into the root causing perforation. The malformation exhibits a wide variety of presentation. On the other hand, it can affect any teeth including primary, permanent and supernumerary teeth. The frequently affected teeth are maxillary lateral incisors. It usually results in pulpal necrosis when the invagination is extending into the pulp chamber. In most cases, it is diagnosed as an incidental finding in a routine dental examination as an alteration of the crown structure or as a radiographic finding. An early prophylactic approach is the most effective means of treatment. However, endodontic management of the affected tooth is often required.

Introduction

"Dens invaginatus" is a form of tooth dysmorphogenesis formed mainly due to the infolding of enamel and dentin or inflection of the lingual pit of an incisor before calcification. This form of dental anomaly which is also termed as dens in dente or dilated composite odontome was first reported in human tooth by Ploquet in 1794 (1). Different causes have been attributed to this anomaly; Kronfeld in 1934 put forth the theory of focal growth retardation, Fischer and Sprawson attributed infection as a cause in 1936 and 1937, respectively. In 1937, Rushton attributed it to a rapid proliferation of a part of the inner enamel epithelium into the developing dental papilla, and Euler in 1939 and Atkinson in 1943 stated an increased localized external pressure as a cause. According to Bruszt in 1950, it may be a result of two tooth germs fusing together. Gustafson and Sundberg stated traumatic injury as a probable etiology. In 1957, according to Oehlers, the distortion of enamel organ might result in malformation. The absence of signaling molecules for morphogenesis and genetic factors was also reported as a causative factor (2,3). Though it can affect primary, permanent and supernumerary teeth, the most frequently affected teeth are the lateral incisors in the maxillary arch. This may be due to the effect of external force exerted on the lateral incisor tooth bud by the adjacent teeth during the development (4).

The prevalence rate of dens invaginatus is about 0.04% to 10%. Male predilection with a ratio of 3:1 is noted (5). There are numerous classifications that were put forward to describe the different forms of dental invaginations, but Oehler's classification, which is based on radiological criteria, is widely used. Oehler has classified the anomaly into three variants depending on the extent of invagination (6).

Type 1: Minor form of coronal enamel lining that does not extend beneath the cemento-enamel junction (CEJ).

Type 2: Form of enamel lining extending into the root but limited to a blind sac, that may or may not be communicating with the dental pulp.

Type 3 A: Invagination having no communication with the pulp but extends into the root forming a lateral communication with the periodontal ligament space by a pseudoforamen.

Type 3 B: Invagination completely lined by enamel and sometimes cementum, extending into the root, perforating at the apex by a pseudoforamen.

Case Presentations

Case 1

A 20-year-old female was admitted to our department with a complaint of pain in her right upper anterior tooth for a month. The pain was pricking type, intermittent in nature, aggravated during mastication and relieves on its own and non-radiating in nature. On intraoral examination, the right maxillary lateral incisor appeared to have an altered morphology (Figure 1A). There was no carious lesion present on the tooth surface, but the tooth showed tenderness on vertical percussion. Based on the findings of the clinical examination, it was pre-diagnosed as apical periodontitis. Informed consent was obtained before taking clinical photographs of the patient.

Intraoral periapical radiographic (IOPAR) examination of the right maxillary lateral incisor revealed altered morphology with a coronal enamel invagination that was parallel to the pulp canal and perforated the root laterally (Figure 1B). The periapical area showed an ill-defined radiolucency similar to rarefying osteitis. Based on the radiographic appearance, a confirmed diagnosis of dens invaginatus (type 3 A) was established. Endodontic therapy of the tooth was planned but the patient did not turn up for further treatment.

Case 2

A 19-year-old male was admitted to our department with a complaint of abnormal shape of the left upper anterior tooth since its eruption, with no history of pain and symptoms. On intraoral examination, crowding of the maxillary central incisors was noted and the left maxillary lateral incisor displayed a different morphology with a pit in the incisal edge of the tooth. There was no evident carious lesion on the tooth surface (Figure 2A). Informed consent was obtained before taking clinical photographs of the patient.

IOPAR of the left maxillary lateral incisors revealed a coronal enamel invagination that extended to the CEJ (Figure 2B). Based on the radiographic feature, a definite diagnosis of dens invaginatus (type 1) was established. Prophylactic sealing of the invagination of the tooth was planned.

Discussion

Dens invaginatus is diagnosed as an incidental finding in a routine dental examination as a difference in the crown structure or as a radiographic finding. The altered crown morphology or a

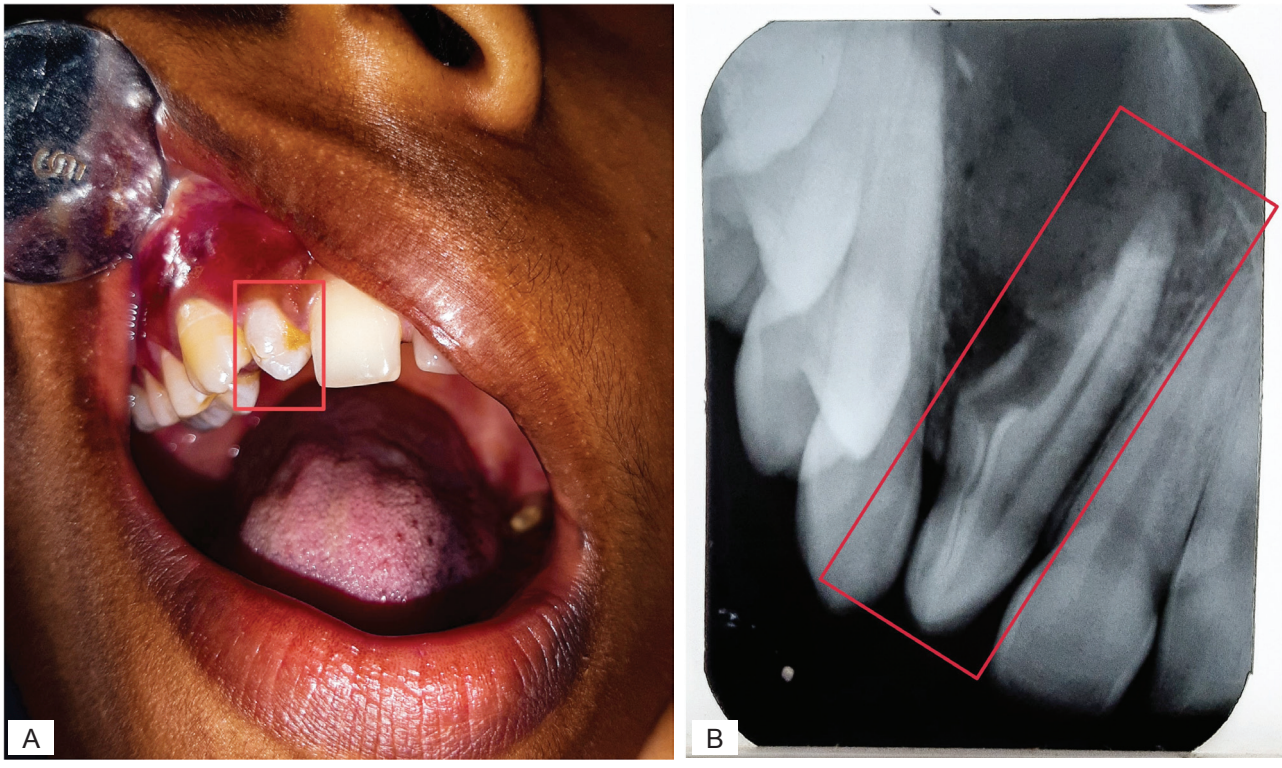

Figure 1. A) Right maxillary lateral incisor with an altered morphology of the clinical crown. B) Intraoral periapical radiographic of right maxillary lateral incisor showing a deep invagination with lateral perforation on the distal aspect of the root and a small periapical radiolucency

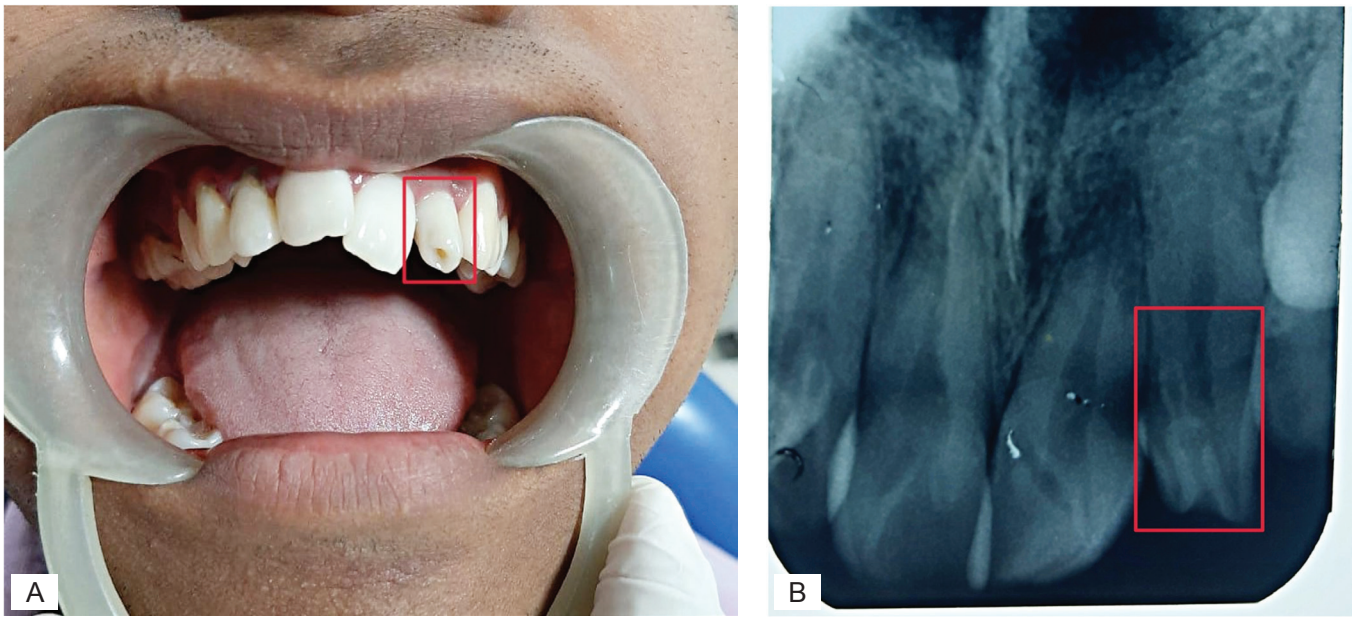

Figure 2. A) Altered morphology of the clinical crown of left maxillary lateral incisor with a deep pitting on the incisal aspect. B) Intraoral periapical radiographic showing coronal enamel invagination in the maxillary left lateral incisor extending downwards till the cemento-enamel junction

deepened foramen coecum may help in the clinical identification, but sometimes the affected teeth may not show any clinical alteration of the crown. In most cases the affected crown may be in conical or barrel-shaped. The altered or conical shape of the incisors may lead to significant esthetic discomfort (7). Early diagnosis is important to determine the necessary treatment because coronal invaginations may extend into the pulp or may allow the entry of irritants into the pulp or periapical area. In most cases, the pulp is separated from the invagination by a thin layer of enamel and dentine, so the invasion of the bacteria or irritants through the invagination may reach the pulp easily and may predispose to pulpal and periapical pathology (8).

Here in the first case, there was altered crown morphology with perforation of root structure, whereas the second case had coronal malformation without any symptoms. Undiagnosed and untreated coronal invaginations may lead to periapical pathology as noticed in our case. The management of dens invaginatus involves prophylactic closure or sealing of the invagination, non-surgical endodontic therapy, endodontic surgery (9), extraction with intentional replantation (10), based on the type of invagination.

Conclusion

A thorough knowledge of the clinical views and radiographic presentation of dens invaginatus may help for the identification of the case. There are various classifications available in the literature, describing the different forms of invaginations. They may help us to understand the various presentations of dens invaginatus, enabling early diagnosis and providing appropriate treatment measures.

Ethics

Informed Consent: Informed consent was obtained before taking clinical photographs of the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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A rare cause of acute malnutrition: Non-germinoma

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ABSTRACT

Energy needs of children are high, hence, malnutrition adversely affects body functions of children. Supportive therapy must be starter early in order to prevent weight loss, morbidity and mortality. Malignancies are frequent among the etiology of malnutrition. Considering the coexistence of malnutrition and childhood cancers, early diagnosis is essential. In this study, we aimed to present an adolescent patient with acute malnutrition diagnosed as non-germinoma.

Introduction

Malnutrition is excessive deficiency of energy, protein or other nutrients. Energy needs of children are higher than adults and energy reserves are also limited. Disequilibrium of nutrients cause adverse effects on tissue, body function and clinical outcome (1). Malnutrition causes mortality and morbidity in children because of the increased risk of infection, disrupted immune system, delayed in wound healing, and dysfunctioning in gastrointestinal barrier (1). According to the functional categorization of weight loss, the reasons were divided into three categories as excess calorie need (diabetes mellitus, chronic chest and heart disease, hyperthyroidism, inflammatory diseases), insufficient calorie intake (malignancies, psychiatric disorders), and absorption disorders (diabetes mellitus, celiac disease, lactose intolerance, pancreatic insufficiency, gastroesophageal reflux) (2). We here report an adolescent patient hospitalized for acute malnutrition and diagnosed with non-germinoma.

Case Presentation

A previously healthy 17-year-old boy was admitted to the Department of Pediatric Gastroenterology with a 4-month history of nausea which was more severe in the morning and was not associated with eating, decreasing loss of appetite and significant weight loss.He reported weight loss from 58 to 41.5 kg in in the last four months. His complaints had begun with flu-like symptoms, fever, stomachache, nausea, vomiting, and myalgia. He reported to have lost around seven kilogram (10%) during the first week of the symptom initiation. His physician prescribed intramuscular antibiotics for five days. He reported symptom relief following this treatment. However, a complete recovery was not present and the patient continued to lose weight to 41.5 kg, reaching 28% during these four months. On admission, he was conscious, and his temperature was 36.7 °C; heart and respiratory rates and systolic and diastolic blood pressure values were normal. He was 41.5 kg (p<3), 170 cm (p10-25) and his body surface area was 1.32 m^2. According

to physical examination, he was pallor, cachectic. Other examinations were normal. In addition, he was consulted to the Department of Child Mental Health and Disease, anxiety and depression were detected secondarily. On the other hand, he was examined for substance abuse because his father convicted of substance trafficking and he was neglected by the family. For the purpose of excluding substance abuse, he was observed during hospitalization. In the process of diagnosis, ondansetron (0.15 mg/kg/dose) was given to cease nausea. Total parenteral nutrition therapy was given for the purpose of preventing weight loss. Oral nutrition was continued to ensure that the total daily energy intake was 3000 kcal/day and enteral feeding solution was also started. Endoscopy was performed for diagnosis. Proton pump inhibitor was given to him because gastritis was shown via gastric biopsy. However, the cause of this sudden severe weight loss could not be explained. Thyroid-stimulating hormone (TSH) and free T4 were 2.49 mIU/mL and 0.59 ng/dL, respectively. Secondary hypothyroidism was diagnosed because TSH did not respond to free T4 level. Then, follicle stimulating hormone, luteinizing hormone, prolactin, cortisol (morning serum), and insulin-like growth factor-1 were 0.2 mIU/mL, <0.2 mIU/mL, 223 ng/mL, 1.48 mcg/dL, and 32.28 ng/mL, respectively. These results and ACTH stimulation test showed that his diagnosis was panhypopituitarism. Hence, the mass between suprasellar cistern, optic chiasm and prepontine cistern was detected via pituitary magnetic resonance imaging (MRI). A stereotactic biopsy was taken from the supercellular cistern and nongerminoma was diagnosed. Serum alpha-fetoprotein (AFP) and beta-hCG were 2.85 ng/mL and 1.55 IU/L, respectively. Because of normal level of AFP and beta-hCG and no two or more foci on cranial MRI, no positive sign on spinal MRI and negative cerebrospinal fluid-cytology, it was considered as non-metastatic standard risk non-germinoma. Hence, three cures of PEI (cisplatin, etoposide, ifosfamide) were given. A cure of PEI was given after reevaluation. Finally, treatment was achieved successfully via 54 gy (TrueBeam) radiotherapy. His weight was recorded 60 kg after the treatment. He is still being followed up in child gastroenterology, endocrinology and oncology monthly. He was informed about his disease and procedure, and the informed consent was received from the patient.

Discussion

The patient had nausea and vomiting. Nausea is a subjective term which can be defined as a feeling in the predictive symptom of vomiting. Vomiting occurs by repetitive forceful contraction of abdominal muscles which cause higher pressure in abdominal part and gastric content discharge into the mouth. Many reasons which may be physiologic responses to underlying disease related to gastrointestinal tract, central nervous system or other system may cause nausea and vomiting (3). Causes of nausea and vomiting include medication, infections, gastroesophageal reflux, bile reflux gastritis, eosinophilic gastroenteritis, helicobacter disease, pancreatitis, anatomic abnormalities, central nervous systems diseases (migraine, increased intracranial pressure, congenital malformation, seizure disorders), psychiatric disease (psychogenic vomiting, anxiety disorders, depression, anorexia nervosa, bulimia nervosa), and endocrinologic and metabolic causes (4).

Malnutrition in any disease, including cancer, is the result of combination of decreased intake, increased loss (including malabsorption) and risen needs (5). A significant factor that contributes to malnutrition in pediatric oncology patients is inflammation because cytokines released by the tumor may alter protein, lipid and carbohydrate metabolism and cause weight loss (6). Changes in metabolism of carbohydrate, fat and protein have been shown in oncologic patients (5). Moreover, increased lipid breakdown between these changes leads to the depletion of lipid stores and changes in carbohydrate metabolism, and all these changes cause energy loss. In addition, there is an increased protein cycle and loss of normal compensation mechanisms seen in hunger. The final result is malnutrition, which is especially associated with loss of lean body mass (5).

It is suggested that nutritional state is vulnerable to infections by causing hormonal changes and poor cytokine response in childhood cancer patients. Secondly, it is argued that nutritional status may be an effective factor on mortality in malignancy patients (7). While initiating therapy, malnutrition may decrease the effectiveness of anticancer therapy by decreasing the absorption of chemotherapeutic drugs and poorly tolerated dose density (7).

Both the malnutrition at the diagnosis of malignancy and the malnutrition in the third month after diagnosis were associated with a decrease in the survival rates. Unlike malnutrition detected in diagnosis, insufficient feeding in the third month may be prevented by strict monitoring of the nutritional status and, if necessary, rapid intervention (7). Our patient had acute malnutrition (41.5 kg, p<3) at the time of diagnosis and reached the ideal body weight (60 kg, 10-25 p) at the end of treatment with strict follow-up of the nutritional status of him during the disease process.

Conclusion

Malnutrition causes severe morbidity and mortality in children because of increased risk of infection, disruption in immune system, delay in wound healing, and dysfunction in gastrointestinal barrier. One of the reasons of energy deficiency is malnutrition because of that energy intake is insufficient to meet energy need. In childhood cancer patients, a significant factor on insufficient feeding is inflammation, and malnutrition is frequent at diagnosis time in this population. Consideration of coexistence of malnutrition and childhood cancers is important for early diagnosis and reduction of morbidity and mortality.

Ethics

Informed Consent: He was informed about his disease and procedure, and the informed consent was received from the patient.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.H., M.A., Concept: D.H., M.A., Design: N.B., Data Collection or Processing: D.H., M.A., Analysis or Interpretation: D.H., N.B., Literature Search: D.H., M.A., Writing: D.H., M.A., N.B.

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Synchronous multicentric astrocytoma with different histological types in both supratentorial and infratentorial regions

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Keywords: Multicentric glioma, pilocytic astrocytoma, anaplastic astrocytoma

ABSTRACT

Synchronous multicentric gliomas are lesions with different histological features and they occur in the same time at different parts of the brain without anatomical continuity. We aimed to describe a multicentric astrocytoma with different histopathological types located in supratentorial and infratentorial regions of the brain. Our patient was operated first for the posterior fossa tumor, then he underwent the removal of frontal tumor. The posterior fossa tumor was pilocytic astrocytoma and the frontal tumor was anaplastic astrocytoma. Synchronous multicentric astrocytoma with different histology is very rare and should be treated in different sessions for better clinical outcome.

Introduction

Gliomas are the most common malignant intracranial tumors that may be observed in any part of central nervous system including cranial and spinal regions, as well as in the spinal cord (1-3). They may be solitary or multicentric in the cranium. Multicentric gliomas are very rare and unique lesions for neurosurgeons with an incidence around 2-10% (1,4). Gliomas are defined as multicentric when no macroscopic or microscopic continuity is found between the lesions (1,4,5).

Multicentric gliomas are more frequent in middle-aged men and they show rapid progression (5,6). The prognosis is poor and the overall survival of these patients is usually short. These tumors are mostly seen in the supratentorial region (1,6). It is very rare to observe in supra- and infratentorial locations simultaneously (5,7).

We presented a unique case of multicentric glioma, which was located in both supra- and infratentorial regions with different histological types. We reported clinical, radiological and surgical features of this rare case.

Case Presentation

A 26-year-old male patient was admitted to our outpatient clinic for headache. Neurological examination was normal. The cranial magnetic resonance imaging (MRI) revealed a lesion with cystic and solid components of 38x52x43 mm in the infratentorial region. It was hypointense in T1W series, heterogeneous hyperintense in T2W series with heterogeneous contrast enhancement of solid component, presumed as pilocytic astrocytoma (Figures 1A, 1B). In addition, there was another lesion in the right frontal lobe, in the subcortical white

matter, which was 42x47x41 mm in size, hypointense in T1W and hyperintense in T2W series (Figures 1C, 1D).

The patient was hospitalized, and 2-staged surgery was planned, first for posterior fossa tumor and the second for frontal lobe tumor. In the first stage, suboccipital craniectomy was performed and cystic white-gray tumor was totally removed. Histological diagnosis was pilocytic astrocytoma grade 1. Histologically, the tumor was composed of spindle cells (Figure 2A) showing compact development pattern in most areas on the fibrillary background and in some areas, abundant Rosenthal fibers (Figure 2B) were observed. p53 expression was negative, GFAP was strongly positive. Partially focal infiltrative pattern was observed in most areas. Ki-67 proliferation index was around 1%.

In the second stage, 1-month after the first stage, right frontal tumor was removed using frontal craniotomy. Histopathological diagnosis was Anaplastic Astrocytoma grade 3. Histologically, p53 expression was widely positive, and neoplastic cells were negative for ATRX expression (Figure 2C). Ki-67 proliferation index was 10% (Figure 2D).

The patient was discharged without neurological deficit. Chemotherapy (temozolomide 100 mg) and radiotherapy (60 Cy/30 Fr for frontal lesion) were administered after surgical treatment. The patient's follow-up at the end of the second year showed no signs of recurrence or residual in cranial MRI (Figures 1E,H). The patient's neurological condition was still stable.

Discussion

Multicentric gliomas were first reported by Bradley in 1880 (7). The frequency and etiology of multicentric gliomas are not well known (1,6-8). It is important to differentiate multifocal and multicentric gliomas. Multifocal tumors are known to be caused by the invasion of commissural or tract fibers, but multicentric gliomas occur in a distant region and there is no connection path between these tumors (1,3,6-8).

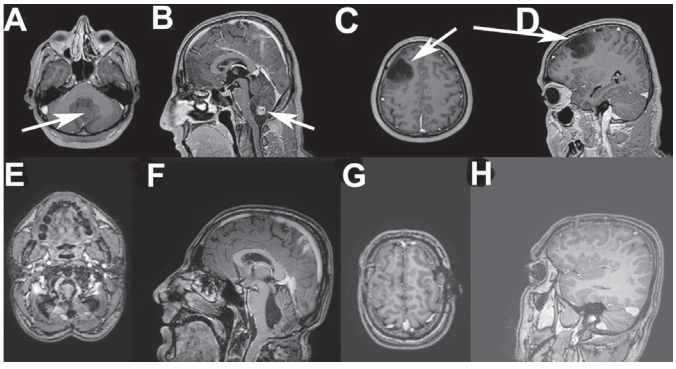

Figure 1. Preoperative axial (A) and sagittal (B) T1W magnetic resonance imaging scans show contrast-enhancing mass lesion (marked with white arrows) in the posterior fossa, axial (C) and sagittal (D) T1W images show right frontal hypointense tumor (marked with white arrows) in the supratentorial region. Postoperative axial T1W (E) and sagittal T1W (F) scans confirmed total resection of posterior fossa tumor (pilocytic astrocytoma), axial (G) and sagittal (H) supratentorial slices confirmed total removal of right frontal tumor (anaplastic astrocytoma)

The pathogenesis of multiple gliomas is still unclear. Willis (9) stated that this was a two-stage process. First, almost all of the brain tissue undergoes a neoplastic transformation and becomes susceptible to neoplastic development. In the next stage, abnormal cell proliferation causes a number of warnings (9). Another hypothesis was interpreted by the long-distance invasion and migration capacity of glioma cells (10).

Cerebral metastasis, brain abscesses, and lymphomas should be considered in the differential diagnosis of multicentric gliomas (1,11). The exact diagnosis is made after surgical removal of tumors via classical craniotomy with microsurgical approach. However, endoscopic endonasal approach may be used for gliomas located in the skull base. Meanwhile, the reconstruction of skull base defect is always a challenge for endoscopic approach (12). In our patient, the diagnosis of supratentorial tumor was anaplastic (grade 3) astrocytoma and the infratentorial tumor was a pilocytic (grade 1) astrocytoma. The time period between the 2 surgeries was one month.

Inoue et al. (5) reported a case of multicentric glioma similar to our case, but not presented at the same time. In their case, infratentorial tumor was detected 7 months after the surgery for supratentorial glioma. In addition, the diagnosis of supratentorial tumor was diffuse (grade 2) astrocytoma and infratentorial tumor was glioblastoma (grade 4). In our case, the diagnosis of supratentorial was anaplastic astrocytoma (grade 3) and infratentorial was pilocytic astrocytoma (grade 1). As mentioned above, multicentric glioma cases are mostly seen

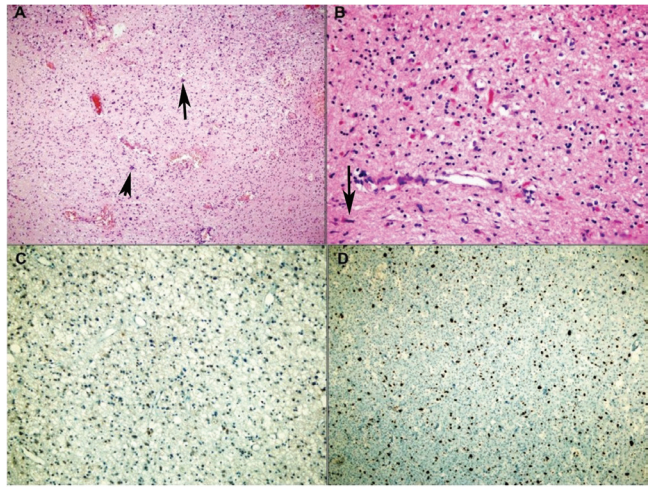

Figure 2. A) Histological examination shows that the tumor was composed of spindle cells (marked with black arrows). B) There is compact development pattern in most areas on the fibrillary background and in some areas abundant Rosenthal fibers (marked with black arrow). C) Immunohistochemical examination shows that p53 expression was widely positive, negative for ATRX neoplastic cells. D) Ki-67 proliferation index was 10%

in the supratentorial region (1,4,6,7). Nakhl et al. (6) presented a case of multicentric glioma but all tumors were located in supratentorial region. In our case, the tumors were in different regions and there was no connection between the tumors.

Predisposing factors for multicentric gliomas are neurofibromatosis type 1, multiple sclerosis, and radiation exposure (1,2,7). Especially, radiation is an important factor on the development of brain gliomas (2,13). Multicentricity is usually associated with poor prognosis (1,10). Our case was a young male patient. He had no radiation history, no other systemic disease such as neurofibromatosis and genetic disorders. He underwent successful removal of both tumors. There was no recurrence in the second year follow-up with adjuvant chemotherapy and radiotherapy in the postoperative period.

Conclusion

Synchronous multicentric astrocytomas in two different regions of the brain with different histological diagnosis are very rare. These tumors should be removed in different sessions with a reasonable time period. In addition to microscopic spread, genetic factors may also have an effect on pathophysiology of multicentric astrocytomas. Proper surgical intervention and postoperative adjuvant treatments have significantly contributed to the patient's survival.

Ethics

Informed Consent: The patient gave written informed consent to publish the case report without revealing his identity.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K., Design: A.D., Data Collection or Processing: M.S., Analysis or Interpretation: M.S., Literature Search: A.D., Writing: A.D., S.K.

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

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Unusual endophthalmitis with macular infarction

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Keywords: Endophthalmitis, infarction, *Klebsiella*, macula

ABSTRACT

Macular infarction due to intraocular aminoglycoside injection is well known. Recently, few atypical organisms have been reported to cause macular infarction after endophthalmitis. Our case is a 69-year-old female patient who presented with redness and discharge in the right eye. She had had intraocular ranibizumab injection for diabetic macular edema two days ago. The vision was counting fingers at one meter without a clear view of the fundus. The patient underwent vitreous tap, and intraocular injection of vancomycin and ceftazidime was performed. The vitreous culture was positive for *Klebsiella pneumoniae*. Two days later, fundus visibility was better. However, the vision was counting fingers at 50 centimeters, and macula was pale. Therefore, we planned pars plana vitrectomy. *Klebsiella pneumonaie* is generally considered to be a cause of endogenous endophthalmitis, especially in patients with liver abscess or fulminant pneumonia. We herein wanted to report the first case of *Klebsiella pneumonaie* endophthalmitis with no endogenous focus resulting in macular infarction.

Introduction

Intravitreal injections (IVI) have become the mainstay therapy for various retinal diseases following the demonstration of beneficial roles by multicenter clinical trials. On the other hand, endophthalmitis is one of the most feared complications. The incidence of endophthalmitis following IVI varies from 0 to 0.092% (1,2). It is crucial to identify the causative organism due to therapeutic challenges in unknown etiologies. A detailed history is essential to narrow down the list of organisms. Macular infarction following intravitreal amikacin injections is well known (3). *Enterococcus faecalis* and *Staphylococcus epidermidis* are previously reported as the causative agents of macular infarction in endophthalmitis as well (4). *Klebsiella pneumoniae* is a causative organism of endogenous *endophthalmitis*, especially in diabetic patients with accompanying hepatic or renal abscess (5,6). In this report, we present the first case with macular infarction secondary to exogenous *Klebsiella pneumoniae* endophthalmitis with no underlying systemic pathology.

Case Presentation

A 69-year-old white female patient presented with pain, redness, blurred vision, and discharge in the right eye two days after intravitreal ranibizumab injection. She was diabetic, and she had a diagnosis of proliferative diabetic retinopathy with diabetic macular edema before the IVI. At the initial examination, her best-corrected visual acuity was 20/1250 in the right eye and 20/25 in the left eye. On slit-lamp examination, hazy cornea and mixed cells with fibrin in the anterior chamber were observed. Vitreous was hazy with a severe cellular response. The afferent pupillary defect was visible in the involved right eye. Ocular motility was normal though the pain was worse with eye movements. Poor red reflex was observed, but B-scan ultrasonography revealed

an attached retina. A diagnosis of acute endophthalmitis was made with clinical findings of light sensitivity, ocular pain-decreased vision, and severe cellular response in vitreous. The patient underwent vitreous and anterior chamber tap followed by the injection of vancomycin (1 mg/0.1 cc) and ceftazidime (2.25 mg/0.1 cc). The patient was admitted and started on topical fortified vancomycin (50 mg/mL), ceftazidime (100 mg/mL), and prednisolone acetate every hour as well as peroral moxifloxacin (400 mg) once daily. On the following day, there was no clinical improvement, and vision further decreased to 20/25000. We proceeded with vitrectomy. During the surgery, a pale macula with multiple retinal hemorrhages was observed (Figure 1). Following surgery, fluorescein angiography (FA) revealed macular ischemia, and optical coherence tomography showed subretinal fluid (Figure 2).

Culture of vitreous fluid was obtained, and following proper inoculation and two days of incubation, the growth of Gram-negative bacilli was observed. Conventional biochemical analysis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker, MA, USA) revealed the identification as *Klebsiella pneumoniae*. Antimicrobial susceptibility tests were applied in accordance with the European Committee on Antimicrobial Susceptibility Testing criteria (Figure 1D) (7). The species showed susceptibility to cefepime, cefotaxime, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, and netilmicin; intermediate susceptibility to piperacillin - tazobactam; resistance to amoxicillin - clavulanate and ampicillin - sulbactam. *Klebsiella pneumoniae* endophthalmitis is a commonly endogenous and delayed diagnosis of endogenous endophthalmitis. The endogenous microorganism can lead to not only visual loss but also an increased risk of mortality. Infectious disease and internal medicine consultations were requested. Blood culture, blood analysis, and related imaging modalities (thoraco-abdominal computed tomography and abdominal ultrasonography) for any abscess revealed no endogenous focus of infection.

At her 1-month follow-up visit, the vision was still poor. However, subretinal fluid resolved without any intervention (Figure 2). At her final visit (four months after diagnosis), the vision was unchanged with the resolution of anterior chamber reaction, and a few preretinal macular hemorrhages were observed. Figure 2G and Figure 2H show retinal and vascular change by time.

Discussion

Intravitreal aminoglycoside toxicity is generally acknowledged as a predisposing factor for macular infarction. Therefore, we substituted ceftazidime for aminoglycosides due to potential hazards in the treatment of bacterial endophthalmitis (7,8). Ceftazidime is a third-generation cephalosporin with particularly good coverage of Gram-negative bacteria with low toxicity in comparison with aminoglycosides. Intravitreal vancomycin is considered to be safe if injected at doses of 1 gram and intervals of 48 hours (9). On the other hand, macular infarction, possible retinal toxicity of aminoglycosides, may also be caused by infective agents like *Enterococcus faecalis* and *Staphylococcus epidermidis* (3,4).

Klebsiella pneumoniae is commonly the causative agent of endogenous endophthalmitis. Endogenous endophthalmitis, comprising 2% to 15% of all endophthalmitis, is a rare but devastating ocular infection when an organism crosses

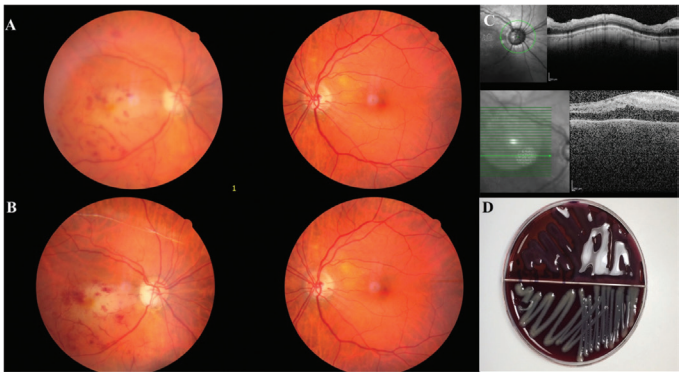

Figure 1. Before pars plana vitrectomy retinal images were not clear. These images are taken following vitrectomy. A) Same day, following pars plana vitrectomy. The pale macula and retinal hemorrhages are seen. B) One week after following pars plana vitrectomy. Macular ischemia is advanced. C) The retinal nerve fiber layer has not been affected yet. Subretinal fluid and retinal edema are seen due to vascular ischemia and inflammation. Macular thickness is 448 µm D) *Klebsiella pneumonaie* subculture after 24th incubation

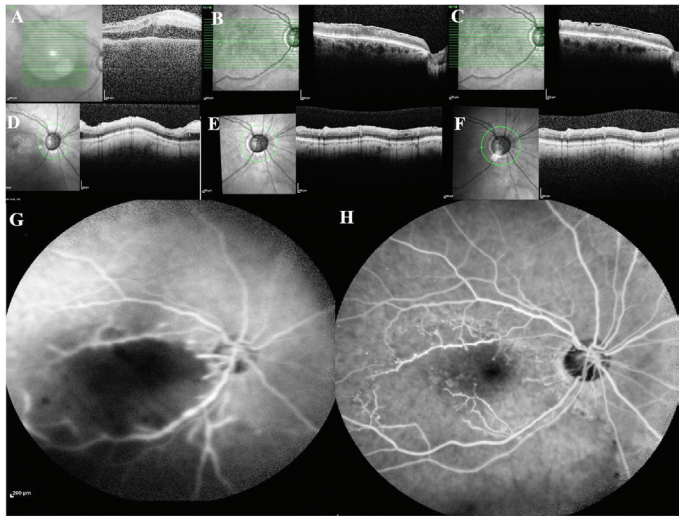

Figure 2. Macular thickness and retinal nerve fiber layer thickness by time. A) Image at the time of diagnosis following pars plana vitrectomy. Macular thickness is 448 µm. B) One year later follow-up exam, the macular thickness is 185 µm. C) Three-year follow-up exam, the macular thickness is 135 µm. D) Optic disc image at the time of diagnosis. E) One-year follow-up exam. F) Three-year follow-up exam. G) Macular ischemia at the time of diagnosis following pars plana vitrectomy. H) Areas of capillary drop out one year later

through the blood-ocular barrier. These patients are usually immunocompromised related to their systemic problems like diabetes mellitus, renal insufficiency, and malignancy. A major source for *Klebsiella pneumoniae* endogenous endophthalmitis (KPEE) is a liver abscess with septic metastasis to the eye (10). Clinical manifestation for KPEE is not unique though accompanying previous systemic health problems with or without *Klebsiella pneumoniae* infection should remind us a possible KPEE. Our case had only diabetes mellitus as a possible predisposing factor, and serotype K1 and K2 are reported with increased pathogenicity in diabetic patients employing their virulence factors (11). Unfortunately, visual prognosis in KPEE is very poor though macular infarction is not reported previously. Probably macular infarction was the main reason for this poor prognosis and missed because FA is not routinely performed in endophthalmitis cases.

Conclusion

Our case is unique with no possible endogenous focus of the causative agent and unusual presentation of *Klebsiella pneumoniae* endophthalmitis with macular infarction. In this regard, endophthalmitis with macular infarction should remind us *Klebsiella pneumoniae* as causative agents, even predisposing systemic factors are lacking.

Ethics

Informed Consent: The patient gave informed consent to publish the case report without revealing her identity.

Peer-review: Externally peer-reviewed.

Surgical and Medical Practices: M.K., A.H.D., Concept: D.A., Design: D.A., A.H.D., Data Collection or Processing: D.A., A.K.S., Analysis or Interpretation: D.A., M.K., A.K.S., A.H.D., Literature Search: D.A., M.K., A.K.S., Writing: D.A., A.K.S.

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

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The mistake has been made inadvertently. The correction of the error in the article has been demonstrated below:

Ayla Demirtaş, Gülten Güvenç, Özlem Aslan, Emine Öksüz, Ayşe Kılıç Uçar
University of Health Sciences Turkey, Gülhane Faculty of Nursing, Ankara, Turkey

On the first page, the expression of the affiliations of the authors' has been corrected as;

Ayla Demirtaş[1], Gülten Güvenç[1], Özlem Aslan[2], Emine Öksüz[1], Ayşe Kılıç Uçar[3]
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[3]Istanbul Bilim University, Florence Nightingale School of Nursing, Istanbul, Turkey

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