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Gülhane Medical Journal (GMJ) is an international, multidisciplinary and peer reviewed journal for researchers and health-care providers. It is published four times a year, and accepts submissions in English. It is the official journal of the Gülhane Faculty of Medicine, University of Health Sciences Turkey.

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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

It is my pleasure to announce the successful closing of 2021 at GMJ. In this year, GMJ has published four issues, as scheduled, with quality papers.

We have been noticing that editing support provided by our managing team before the reviewer selections promotes new submissions to GMJ. Increasing interest in submissions to GMJ makes the editorial board confident in their efforts to support the scientific community at local or international.

In the last issue of 2021, there are a number of interesting original articles, review articles, and case reports in GMJ, from a wide range of scientific disciplines.

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

Also with this opportunity, I wish our community a happy new year.

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

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Vimentin and its role as a biomarker in health and disease

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ABSTRACT

Vimentin is an intermediate filament protein responsible for maintaining cellular integrity and resistance to stress. It has a widespread distribution in many cells throughout the body where it forms a cytoskeletal framework. Vimentin plays an important role in the regulation of many cellular and tissue functions. It is overexpressed in malignancies, potentially malignant oral disorders and autoimmune conditions like rheumatoid arthritis and Crohn's disease. It is associated with cell surface binding and replication of viruses such as human immunodeficiency virus (HIV), severe acute respiratory syndrome-related Coronavirus, dengue and encephalitis. In HIV, it is associated with the viral infectivity factor which is associated with HIV replication. It can be used as a biomarker for diagnosis and prognosis and has potential as a therapeutic target in many conditions. The present review focuses on the structure, functions, clinical implications and future scope of vimentin in the management of various diseases.

Introduction

Vimentin is an important intermediate filament protein responsible for maintaining the integrity of the cell and its resistance to stress. It is expressed in mesenchymal cells and tumors and is considered as a biomarker for cellular and tissue development. It is found in many cells during initial embryogenesis and is converted to a specific intermediate filament during differentiation. Vimentin is usually present in fibroblasts, chondroblasts, smooth muscle cells, mesothelium, pericytes, melanocytes and endothelial cells (1).

Vimentin has an important role in many physiological functions such as cell adhesion and motility, maintenance of cytoskeletal structure, cell senescence, and wound healing. It is an important marker in many inflammatory conditions, potentially malignant disorders and autoimmune diseases. In malignancies, it is associated with invasion and metastasis and may have a role in prognosis. It can act as a receptor or co-receptor for many viruses, such as human immunodeficiency virus (HIV), Severe acute respiratory syndrome-related Coronavirus (SARS-CoV), dengue and encephalitis viruses, due to its implications in attachment and viral entry into cells (2).

Thus, vimentin is associated with both normal physiological functions as well as pathological conditions. The present review aims to give an overview of this biomarker which has effects on a wide range of cells and tissues and to discuss its scope in diagnosis, prognosis and therapy.

We conducted an online search in "PubMed" using the keywords "Vimentin", "Metastasis", "Epithelial Mesenchymal Transition (EMT)", "Carcinoma" and "Potentially Malignant Disorder". Only manuscripts which had full text available were evaluated and referred for the present narrative review.

History

Intermediate filaments were discovered by Buckley and Porter (3) in the late 1960's as a result of advances in microscopic techniques. Franke et al. (4), in 1978, created antibodies to a protein found in the cytoskeleton of mouse 3T3 cells and proposed the name 'vimentin' to differentiate it from proteins in other intermediate filaments. The name 'vimentin' is derived from the Latin word 'vimentum', used to describe arrays of flexible rods. The authors have discovered that vimentin forms a radial arrangement throughout the cytoplasm, more towards the nucleus, and is present in cells derived from mesenchyme.

Structure

Classified as a type 3 intermediate filament, vimentin structure includes a 310-amino-acid-long α-helical rod having acidic and basic amino acids (5). It consists of N and C terminals including amino acids which are hydrophobic in nature (6). The long α-helical rod is acidic in nature while the N-terminal domain, called head domain, is basic in nature due to the presence of 12 arginine residues in its amino acid sequence (7).

Location

In humans, vimentin is expressed in tissues like skin, lungs, kidney, bone marrow and lymph nodes (4). It is present in cells like precursor cells of the pancreas and nerves, Sertoli cells, fibroblasts, endothelial cells, renal tubular and stromal cells, macrophages, neutrophils, mesangial cells and leukocytes (8). Vimentin forms a network which extends throughout the cell cytoplasm and varies among different cells (9). Non-dividing cells show a uniform distribution while reorganization towards the nucleus occurs on exposure to factors like platelet derived growth factor, oncogenes or few viruses (10).

Functions

Epithelial to Mesenchymal Transition

EMT is the process of cellular reprogramming where epithelial cells exhibit mesenchymal phenotype with altered shape and increased motility (6). During EMT, epithelial cells are subjected to various functional and behavioral changes that lead to their differentiation into mesenchymal cells (11). These changes are needed for functions such as embryonic development, homeostasis, and tissue healing as well as in cancer metastases.

The various changes that occur during this phase include loss of epithelial cell junctions, reversal of polarity, alterations in cell shape and cytoskeletal reorganization (12). Increased expression of vimentin is an important marker for EMT as vimentin is a major protein in mesenchymal cells and its expression causes cells to become flat and elongated in shape (13-15). Vimentin also interacts with microtubules and associated motor proteins that lead to cell motility (16). The reverse of EMT is mesenchymal epithelial transition, in which cells develop a mesenchymal- to- epithelial conversion with lower motility and decreased vimentin (8). It has been suggested that increased vimentin may be due to activation of nuclear factor kappa B (nuclear factor kappa light chain enhancer of activated B cells), another regulator of the EMT process, in cancer cells (8).

Cell Proliferation, Differentiation and Apoptosis

Oncogene expression, which is responsible for higher cellular proliferation, is related to increased amounts of mRNA, protein and the soluble forms of vimentin (17). Cell apoptosis is also dependent upon vimentin since its proteolytic breakdown into a pro-apoptotic variant can trigger apoptosis (18).

Association with Various Oncogenes and Proto-oncogenes

The structural resemblance of vimentin with certain proto-oncogenes like c-fos and c-jun and oncogenes like Raf and v-mos has shown that gene expression controlled by vimentin can lead to conversion of normal cells into malignant cells (19). Thus, it plays an important role in cell proliferation (20).

Cell Motility and Adhesion

Vimentin has been found to influence the width of lamellipodium, a protein used for pseudopodial migration (21). Vimentin attaches and controls focal adhesion kinase (FAK), which can impart motile property to cells as seen at the advancing front of lung cancer. By recruiting VAV2, a Rac1 guanine nucleotide exchange factor to FAK, vimentin can regulate adhesion of cells (22).

Methods of Vimentin Assessment

Various methods have been used in vimentin assessment. These include immunohistochemistry, real-time (RT) quantitative RT-polymerase chain reaction (PCR), Western Blot test, confocal immunofluorescence microscopy and computer-assisted imaging to detect stained areas of the cell using a microscope with a charge-coupled device color camera.

Clinical Significance

Carcinoma

The transformation of epithelial cells into malignant cells causes vimentin upregulation and loss of keratin (23). Immunofluorescence, Western blot and RT-PCR analysis have shown high vimentin expression in hyperplastic and dysplasia tissues. Increased vimentin expression has been detected in sites with known carcinoma and in oral squamous cell carcinoma (OSCC), vimentin expression has been found to increase with the grade of the tumor (24). These findings are due to the role of vimentin in EMT. Vimentin regulates EMT in three ways:

I. Gene expression: Vimentin regulates expression of Axl3 which can induce EMT.

II. Protein-protein interaction: Vimentin stabilizes the cell at a protein level (25) and ensures cell polarization during cell migration.

III. Phosphorylation: Vimentin phosphorylation protects it from proteolysis, thereby enhancing migration and metastasis of cells (26-28).

Increase in vimentin expression has been noted in cancers of the prostate, breast, endometrium, central nervous system, gastrointestinal tract as well as malignant melanoma, in which it is associated with both diagnosis and prognosis. Vimentin has been used as a diagnostic marker for colorectal carcinoma (8).

Li et al. (29) found increased expression of vimentin in primary malignant melanoma with hematogenous route of metastasis as compared to those without hematogenous metastasis. Vimentin is important for EMT which, in turn, is important for migration and invasion characteristics of cells.

Metastatic Spread

Vimentin is associated with tumor invasion. Studies have shown that highly invasive breast carcinoma cells without vimentin are more pliant and have decreased tendency to metastasize (8,23). Loss of vimentin significantly has decreased the migration and invasion of cells in dense cultures while vimentin expression has been associated with longer persistence of migration (30). Vimentin is the protein most implicated in metastasis in OSCC. Increased vimentin expression is also associated with increased lymph node metastasis. Bhardwaj et al. (31) investigated vimentin in eyelid sebaceous gland carcinoma and found that overexpression was associated with lymph node metastasis and poor survival.

A study on lung adenocarcinoma found that vimentin was not associated with the development of primary tumors. However, loss of vimentin resulted in a 50% decrease in lymph node metastasis (23).

Leukoplakia

Cytoplasmic immunostaining for vimentin demonstrated higher vimentin expression for non-homogeneous leukoplakia when compared to homogenous leukoplakia where it was confined to basal and suprabasal layers of oral mucosa (24).

In leukoplakia, there is decreased expression of epithelial junctions leading to partial or total loss of cell polarity (32). This is a part of EMT, which in turn is regulated by vimentin; hence vimentin expression increases in leukoplakia.

Oral Submucous Fibrosis

Oral submucous fibrosis (OSMF) is a potentially malignant disorder affecting the oral cavity and sometimes the pharynx. Cases show weak to moderate expression of vimentin, which is confined to the suprabasal layers of oral mucosa in majority of the cases (24).

In OSMF, arecoline triggers the formation of collagen, and exhibits cytotoxicity, change in cell morphology and DNA synthesis (33). This, in turn, causes interference in cellular mitosis and transport mechanisms within the cell. Since vimentin has an important role in cell cytoskeleton, disruption of cytoskeleton in OSMF causes increased vimentin expression (34,35).

Rheumatoid Arthritis

Vimentin is a type of antigen found in synovial tissue and fluid. In rheumatoid arthritis (RA), vimentin citrullination and presentation to T cells causes formation of anti-citrullinated protein antibody, which can be demonstrated in the synovial fluid (36). This antigen-antibody reaction promotes inflammation which further aggravates the disease process (37).

Crohn's Disease

Crohn's disease is a genetically mediated inflammatory bowel disease of the gastrointestinal tract associated with increased vimentin levels. Studies have shown that the inflammatory tissue damage and the resulting intestinal fibrosis may be a result of EMT. The areas with fibrosis exhibit EMT markers like vimentin implying a role for EMT in the pathophysiology of Crohn's disease (27).

COVID-19

Vimentin is associated with viral fusion and replication and also enhances cell surface binding and viral entry. This is true for all viruses except human papilloma virus, in which it has the reverse effect. Studies have shown that interference in expression of vimentin or cell treatment using anti-vimentin antibodies can decrease few viral infections. Cell entry of SARS-CoV may be mediated through vimentin. As SARS-CoV and SARS-CoV-2 have similar spike protein sequences, vimentin may be a co-receptor for both. It may also decrease the immune response to the virus and decrease its lethal effects through its effects on the cytokine storm syndrome. Vimentin present on the surface of platelets and endothelial cells acts as a receptor for von Willebrand factor facilitating platelets to bind to subendothelial collagen and causing intravascular thrombin generation and microthrombus formation. Drugs effective against viral infection are also known to cause a decrease in vimentin. Since vimentin is involved in viral infection and lung inflammation, drugs targeting vimentin may be effective in the treatment of COVID-19. Decreased vimentin at cell surface would cause decreased viral binding, decreased intracellular vimentin would decrease viral replication and less vimentin in inflammatory cells would cause decreased lung inflammation. Melatonin, niclosamide, endogenous hydrogen sulfide and simvastatin are a few of the medications that have been suggested to decrease lung injury through their effects on vimentin (2,38).

Anti-HIV Therapy

Vimentin expressed in monocytes is important for maintaining cell structure. Since vimentin is broken down by HIV-I proteases, vimentin is postulated to have a role in HIV infection. Anti-vimentin antibody has been associated with inhibition of virus production by macrophages. It was also reported that viral infectivity factor, which is linked with the processes of viral RNA folding and packaging, had a spatial overlap with vimentin and could collapse the vimentin framework (28,39-41). Modification of endogenous vimentin levels inhibited HIV replication.

Wound Healing

Wound and tissue healing needs cell motility and adhesion. Vimentin causes an increase in fibroblast and collagen formation and helps in epithelialization of wounds (26). Vimentin acts by influencing the actin-myosin machinery, which is an important factor for wound healing but its exact mechanism of action has not yet been elucidated (26).

Atherosclerosis

Diseases of the cardiovascular system may also be associated with increased vimentin levels. Cells of the cardiovascular system have the capacity to differentiate into mesenchymal-like cells in a manner similar to the EMT observed with increased vimentin levels. Atherosclerosis has been associated with increased vimentin due to EMT. Vimentin null mice have been associated with arterial stiffness and endothelial abnormalities (42).

Cataracts

Clouding of the lens of the eye is termed as cataract. Normally, the epithelial cells in the lens show a low expression of vimentin. Cataracts are associated with misfolding and increased expression of vimentin. This increase in vimentin may be due to mesenchymal transformation of the epithelial cells of the lens. Although all cases of cataract may not be a result of EMT, EMT may be one of the causes of lens opacification. EMT suppression may thus have potential in the treatment of cataracts (20).

Aging

Vimentin has been implicated in cell senescence. Senescent cells have an increase in vimentin levels and secrete an oxidized form of vimentin. The glycated form of vimentin is seen in skin fibroblasts and is implicated as a sign in skin aging (20).

Ongoing Research

Vimentin is an important subject of ongoing research with 1,684 publications in 'PubMed' within the last one year. Recent research has focused on vimentin as a prognostic marker in various cancers, its role in tumor invasion, metastasis and as a potential target in cancer treatment (23). Anti-vimentin antibodies have been implicated in kidney transplant rejection (43). Its scope as a therapeutic target in viral infections is also being investigated (2). Most recently, vimentin has been proposed as a target in the treatment of COVID-19 (38).

Future Scope

Vimentin is an important protein in cancer metastasis. Drugs aiming at targeting vimentin could decrease cancer metastasis, thereby decreasing cancer related mortality. A multitude of factors are responsible for regulating vimentin expression in tissues. A valid drug trial therefore requires further research into the factors responsible for gene expression of vimentin (10). Due to its widespread expression in normal and abnormal tissues, it is an attractive potential therapeutic target for many disease conditions.

Conclusion

Vimentin is a crucial biomarker for the detection of many conditions with a wide range of biological functions. It is associated with increased growth, invasion and migration in cancer cells. It is used as a biomarker in both diagnosis and prognosis in various cancers. Further, overexpression of vimentin is also associated with other conditions like RA, potentially malignant oral disorders and delay in wound healing. The main process through which it regulates the above functions is through EMT. Various factors are associated with the functional diversity of vimentin and if we understand the association between various quantifiable biological phenomena, it will increase treatment options. Vimentin has potential as a drug target for many diseases. However, further research into factors affecting vimentin expression is essential prior to drug trials.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Localized dental dyschromia-causes and management

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ABSTRACT

Tooth discoloration is an important aesthetic complaint of patients. Discoloration of teeth can be either localized or generalized. It is essential to recognize the etiology and manage the discoloration accordingly. Localized discolored teeth can be managed by minimally invasive treatment options (such as resin infiltration, microabrasion, macroabrasion or bleaching) and restorative methods (such as composite or ceramic veneers or full coverage crowns). A combination of these treatments may provide a more successful outcome. An overview of the etiology of localized anterior discolored tooth, and the prevention and management of such clinical cases are discussed in this article.

Introduction

Localized discoloration of anterior teeth may present as an important aesthetic concern for many patients. The management of such teeth poses a challenge to the clinician, and obtaining an aesthetic outcome in keeping with the patient's treatment needs is often difficult (1). There are various approaches for the treatment of such discolored teeth and the selection of a particular mode of treatment may depend on factors such as the cause of discoloration, history of previous treatment performed on the teeth, amount of remaining coronal tooth structure, the patient's aesthetic needs, and financial concerns (2). Management of such teeth can range from minimally invasive treatment options such as resin infiltration, microabrasion, macroabrasion and bleaching to restorative options such as veneers or full coverage crowns (3). It is essential to carefully assess and diagnose the affected teeth in order to select a management strategy that would be best suited to the patient's needs. Certain cases would require a combination of treatment modalities in order to achieve the desired treatment outcome. This article aims to provide an overview of the etiology, diagnosis and the various treatment options available for the management of localized anterior discolored teeth.

Etiopathogenesis

An understanding of the reason for tooth discoloration will help in (i) making the correct diagnosis (ii) explaining the condition to the patient (iii) and selecting the appropriate treatment option. The causes for localized tooth discoloration have been summarized in Table 1 (4-7).

Diagnosis

History

The clinician must record a proper case history, focusing on the discolored tooth in question, in order to correctly diagnose the etiology of the discoloration. This should be done in a systematic and methodical manner. The chief complaint would provide an indication for the patient's aesthetic needs as well. The patient should be asked detailed questions regarding the duration of discoloration, any precipitating factors, history of habits such as smoking, history of pain, trauma and history of previous dental treatment (2).

If the patient provides a history of traumatic dental injury, the clinician must ask about the time of occurrence of the traumatic injury, type of trauma, and the treatment done at the time. In certain cases, a reversible pink discoloration may be noted in traumatic teeth which appear 2-3 days after the injury and do not require any treatment other than monitoring at recall appointments. If the pulp of a traumatized tooth recovers or undergoes revascularization, the discoloration can be resolved in 2-3 months. In some cases, the discoloration may only appear 2-3 months after the trauma (8).

Clinical Examination

The next step involves conducting a proper clinical examination in order to reach a correct diagnosis (2). Non-vital teeth often present with a wide variety of discolorations which can be attributed to the etiology of discoloration, as have been outlined in Table 1. Teeth with partial or complete pulp canal obliteration are usually asymptomatic. They may exhibit mild symptoms like apical periodontitis only if there is the presence of infected tissue in the pulp space (9).

Aesthetic Evaluation

The clinical examination must also include a comprehensive aesthetic evaluation. The shade of the discolored tooth and the adjacent teeth must be evaluated and recorded. The clinician must evaluate the dimensions of the affected and adjacent teeth, smile symmetry, amount of gingival display on smiling, gingival architecture and lip lines. Any additional white spot or brown spot lesions, enamel translucencies and pre-existing restorations should also be checked. The quality and quantity of the remaining coronal structure should be evaluated and the patient must be informed about his treatment options (2).

If bleaching is planned for patients with pre-existing white spot lesions, they should be informed that these may become more distinguishable in the first few days after bleaching. This phenomenon is known as the 'splotchy stage' of the treatment and it happens due to the penetration of the bleaching agent into the white spot lesion first, since it is the weakest part of the tooth (10). After bleaching is completed, the condition may resolve by itself or further treatment in the form of micro-abrasion or

Table 1. Etiopathogenesis of localized discolored tooth

Cause	Condition	Color
Traumatic causes	- Enamel hypoplasia	White or yellow-brown
	- Dentin hyper-calcification	Yellow or brown
	- Internal resorption/external cervical resorption	Pink
	- Calcific metamorphosis	
	- Intra-pulpal hemorrhage	Yellow
	- Pulp necrosis	Gray or brown
	With hemorrhage	Gray or black
	Without hemorrhage	Yellow, gray-brown
Iatrogenic causes	- Trauma during pulp extirpation	Gray or black
	- Tissue remnants in pulp chamber	Brown, gray or black
	- Inappropriate design of access cavity (traps pulp chromophore materials inside the pulp chamber)	Yellow, gray
	- Products of tissue decomposition	
	- Dental restorative materials (e.g. grey MTA, Amalgam, zinc oxide eugenol etc.)	Yellow, brown or gray
	- Endodontic materials (root canal irrigant, intracanal medicaments, sealers and gutta percha)	Brown, gray or black Gray or black
Internalized	- Caries	White spot lesion to black arrested lesion
Extrinsic causes	- Discolored restoration	Yellow or brown
Extrinsic and intrinsic causes	- Localized fluorosis	Chalky white or dark brown/black
	- Erosion	Brown
Idiopathic causes	- Molar-incisor hypo-mineralization	White, yellow or brown
Congenital causes	- Regional odontodysplasia	Yellow or yellowish brown

MTA: Mineral trioxide aggregate

resin infiltration may be indicated. Pre-existing restorations in the anterior region may need to be replaced since their shade may not match with that of the adjacent bleached teeth (2).

Radiographic Assessment and Pulp Testing

Thermal and electric pulp testing should be carried out for all discolored teeth, in order to assess their vitality. Traumatized teeth may have necrosed pulps and would thus yield a negative response to pulp vitality tests. Thermal pulp tests of teeth with pulp canal obliteration do not usually yield a positive response. Electric pulp tests for such teeth may give a normal or delayed response (9).

For teeth with calcific metamorphosis, complete or partial obliteration of the pulp chamber and pulp canal space may be seen, which occurs from the coronal to the apical direction. The periodontal ligament (PDL) space usually appears normal, intact lamina dura. If calcific metamorphosis is associated with apical periodontitis, widening of the PDL space or peri-radicular radiolucency may be noticed in chronic cases (11).

In cases of pulp necrosis associated with trauma/caries, periapical radiolucency with widening of PDL space may be observed in some cases. Periapical radiolucency with bone loss may be noted in case of periapical cysts or radicular cysts (12).

In internal resorption, round or oval radiolucent areas may be seen within the pulp chamber, with loss of dentin, and distortion of pulp space anatomy (13). Teeth with external cervical root resorption may show either asymmetric radiolucency in the cervical/proximal aspect of the tooth with irregular margins or uniform circular radiolucency centered over the root. These lesions may initially appear radiolucent, whereas advanced lesions may present with a mottled appearance due to the fibro-osseous nature of the lesion. In most cases, the root canal would remain patent and would be radiographically discernible (14).

In regional odontogenic dysplasia, the teeth exhibit a ghost like appearance radiographically, with thin enamel (15).

Prevention

Prevention of tooth discoloration, if possible, should always be the first line of management of discolored teeth. There are a few preventive measures that should be followed by the clinician in order to avoid discoloration at a later stage. To prevent intrinsic discoloration due to caries, in initial stages of the carious lesion, treatment should include topical fluoride therapy and application of pit and fissure sealants. Later, in cavitation stages, it is always better to avoid amalgam restorations especially in the aesthetic areas, and the use of tooth colored restorations, such as composite resins or ceramic restorations, is preferred.

The use of grey mineral trioxide aggregate (MTA) may cause a greyish discoloration of the tooth, hence use of white MTA is preferred. During the endodontic access cavity preparation, it is always advisable to completely remove pulp tissue and pulpal remnants and pulp horns, by adequate deroofing as well as by agitating sodium hypochlorite. The use of triple antibiotic paste as an intracanal medicament may cause discoloration since minocycline is one of the components, hence double antibiotic paste may be used (16). Irrigation with chlorhexidine immediately after using sodium hypochlorite may form brownish-red precipitates. Hence, distilled water or saline or even sodium metabisulfite have been suggested as an in between flush to prevent the formation of this precipitate (17). After root canal filling, it is mandatory to clear the obturating materials [core material and root canal sealer] from the pulp chamber to avoid coronal color change.

Management

Various factors need to be considered while developing a treatment plan for teeth with localized discoloration. A flow chart has been prepared for ease in decision making (Figure 1).

Scaling and Polishing

The first step in the treatment of tooth discoloration should be scaling and polishing, which will help in the removal of extrinsic stains, allowing the dentist to evaluate the actual underlying tooth shade (16).

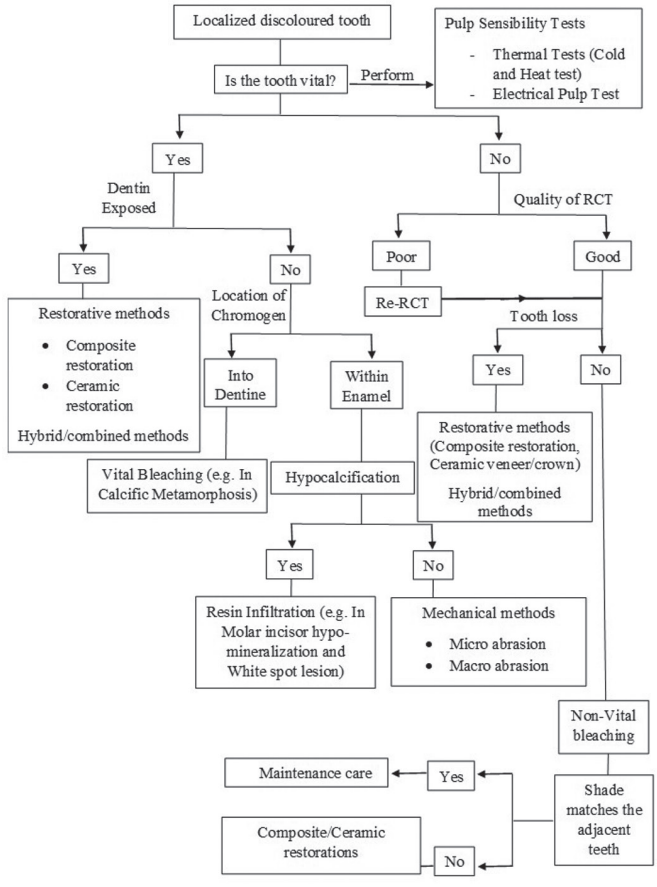

Figure 1. Treatment decision algorithm for localized dental dyschromia
RCT: Randomized clinical trial

Air Polishing

Air polishing is a procedure in which a substance like calcium carbonate, sodium bicarbonate or aluminum tri-hydrate is ejected through a mixing nozzle with air and water. This is used in the form of a controlled jet against tooth enamel. This is a rapid method for the removal of extrinsic stains (18). However, air polishing with sodium bicarbonate abrades dentine and cementum and hence it is better to avoid its usage since it can lead to increased post-operative dental sensitivity. Alternatively, bioactive glass air-polishing was shown to be more effective at desensitizing and removing stains (19).

Resin Infiltration

Resin infiltration can be used for the management of non-cavitated carious or hypomineralised lesions on the smooth or proximal surfaces of primary and permanent teeth. This preserves tooth structure, is relatively non-invasive, and can be accomplished in a single appointment (20).

The objective of resin infiltration is the occlusion of the microporosities within the body of the lesion by infiltrating light-curing resins of low viscosity (21). Scattering of light occurs due to the difference in refractive indices between the air or water inside the porosities and the enamel crystals, resulting in an opaque whitish appearance. When the microporosities are filled with resin, there is a little difference in refractive indices between the porosities and enamel and the lesions become practically indistinguishable from the surrounding sound enamel (22). Hence, resin infiltration is useful in the treatment of initial enamel caries and improvement in the appearance of white spot lesions, and mild cases of fluorosis (23).

Caries progression is inhibited by sequentially applying 15% hydrochloric acid gel for two minutes. This is followed by applying a low viscosity tri-ethylene glycol dimethacrylate resin, with a sufficiently high penetration coefficient (22).

Microabrasion

This involves simultaneous erosion and abrasion of a microscopic layer of enamel leaving behind an intact surface. This amorphous, prismless layer has a smooth and lustrous appearance. Microabrasion may be carried out along with vital bleaching or direct composite resin veneers for improving the appearance of severely discolored teeth with developmental defects or superficial stains (16). Kits for microabrasion are commercially available. An alternative is to use 37% phosphoric acid gel and pumice. Microabrasion removes about 50-200 microns of the surface layer of enamel so it should not be done repeatedly (22). If carried out properly, microabrasion should lead to only negligible loss of enamel, no damage to pulp or periodontal tissues, and produce satisfactory and permanent results in a short time period without causing any discomfort to the patient (24).

Macroabrasion

Magne was the first to describe macroabrasion or megabrasion (25). Macroabrasion is useful for the treatment of enamel opacities and yellowish-brown discolorations of enamel (16). Macroabrasion mechanically eradicates the lesion so that it can be restored with a direct composite resin. The optical appearance of the tooth is attributed to the intact underlying dentine, and the surface morphology of the tooth can be recreated by applying a neutral, translucent and slightly fluorescent resin composite. A coarse diamond instrument is used at low speeds (400-2000 rpm) to eliminate the discolored enamel in a safe and controlled manner. Sharp angles are removed with the help of coarse flexible discs. The defects can then be restored with composite resin to achieve an aesthetic appearance (16). Macroabrasion can be a possible treatment option for severe dental fluorosis without unnecessary removal of dental hard tissues (26).

Bleaching of Teeth

One of the most conservative, economical and safe treatment options for the management of a single discolored tooth is bleaching. The method of bleaching should depend on whether the tooth is vital or not, and whether the procedure would be carried out at home, or in the dentist's clinic. For vital teeth and teeth with radiographic evidence of pulp canal obliteration, an external approach may be adopted. For previously endodontically treated teeth, walking bleach technique or the inside-outside bleaching technique can be followed (1). Bleaching can be broadly categorized into vital and non-vital bleaching.

Vital Bleaching

Vital bleaching is a non-invasive procedure that can be carried out either through the use of various home-based gels or through in-office bleaching. 10% carbamide peroxide gel loaded into a tray can be used for vital tooth bleaching since it has reduced the risk of causing dentinal hypersensitivity and irritation to the adjacent soft tissues in comparison with more concentrated gels (27). It can be used for the treatment of single discolored teeth due to calcific metamorphosis, white and brown discolorations from mild fluorosis (16) or localized yellow and brown hypoplastic lesions (28). Vital bleaching is performed prior to further aesthetic treatment, such as macroabrasion, microabrasion and/or direct composite resin veneers.

Non-vital Bleaching

This is carried out to treat minimally restored teeth that have undergone discoloration as a result of necrotic pulpal remnants left behind in the pulp chamber after endodontic treatment or pulpal hemorrhage into the dentine due to dental trauma (29). In intracoronal bleaching, oxidizing agents are used in the pulp chamber of an endodontically treated tooth for the removal of discoloration (Figure 2) (2). The following methods can be employed for non-vital bleaching of teeth:

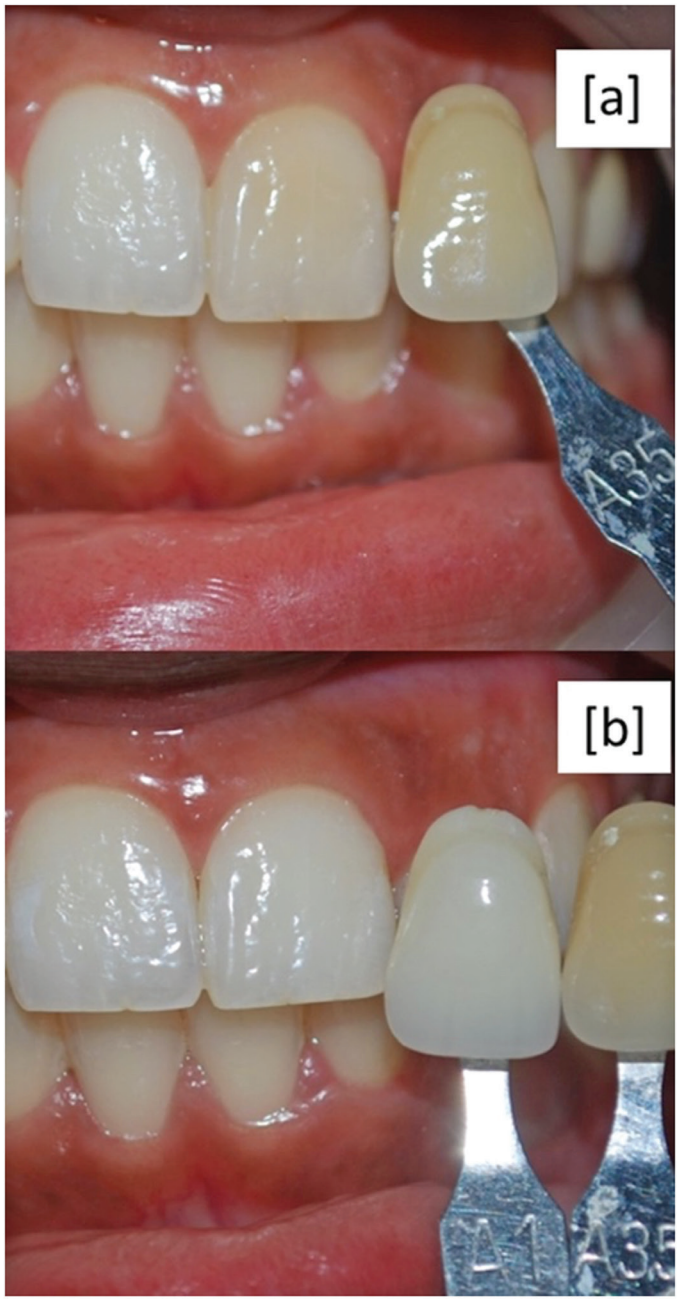

Figure 2. Non-vital walking bleach technique (a) Pre-operative image showing yellowish discoloration on tooth number #21 (b) Post-operative image after two rounds of non-vital bleaching

i. In-office bleaching: Traditionally, in-office bleaching involves internal or external application of high concentrations of hydrogen peroxide or hydrogen peroxide mixed with sodium perborate. An alternative would be supplementation of the bleaching agent with heat, known as thermocatalytic bleaching. A disadvantage of this technique is that shade matching becomes difficult due to dehydration and demineralization because of the high concentration and low pH of hydrogen peroxide used. This method also has increased tendency of side effects like external cervical root resorption due to the use of high concentrations of hydrogen peroxide and heat (2).

ii. Walking bleach technique: In the walking bleach technique, after cervical barrier placement with glass-ionomer cement, the bleaching agent is placed above the barrier in the pulp chamber. The endodontic access cavity is then restored with an interim restorative material. The patient is recalled after two weeks for re-evaluation of shade and the method is repeated as necessary until the desired shade is obtained (2).

iii. Inside/outside open technique: In this method, after cervical barrier placement, the access cavity is left open without placing a seal and the patient uses a syringe to apply the bleaching agent directly into the bleaching tray and the access cavity. The bleaching tray is then positioned carefully in the mouth such that the access cavity is covered. The patient is instructed to replace the bleaching agent at 4-6 hour intervals. The patient is recalled after 2-3 days to re-evaluate the amount of bleaching the tooth has undergone (2).

iv. Inside/outside closed technique: This technique has been modified from the inside/outside technique described above. After the placement of a cervical barrier, the bleaching agent is placed in the pulp chamber, and an interim restoration is placed to obtain a coronal seal. Then, a 'single tooth' bleaching tray is used externally to apply the bleaching agent. The patient is told to wear the bleaching tray overnight until the desired shade is obtained or until the patient is recalled for follow-up. The bleaching agent can be reapplied intracoronally, if required, at the review appointment (2,30). This technique is associated with reduced chances of microleakage, damage to the coronal seal, and it prevents food lodgment. Also, the clinician is able to control the progression of the shade (2).

Composite Resin Veneer

Thin layer of composite resin is applied directly over the entire labial surface of the discolored tooth to correct the shade and modify the contours. This is often preceded by bleaching, resin infiltration, microabrasion or macroabrasion to obtain a substrate with a more uniform shade. Direct composite veneers are indicated for young patients with minimally restored teeth since there is no unnecessary removal of tooth structure. They can be carried out in a single appointment and offer aesthetic results (31).

Indirect composite veneers are an alternative option for masking underlying tooth discoloration or simulating the discoloration of adjacent teeth. The amount of background effect and overall shade can be altered by the light absorption and internal reflection properties of the resin composite. The more the translucency of the composite resin, the more the effect of the stump shade on the final restoration (32). The shade, thickness and optical properties of the resin luting cement might also influence the final appearance of the veneer (33). CAD-CAM fabricated indirect composite veneers allow better

replication of contacts and surface morphology, especially if there is partial loss of coronal structure, hypoplasia or the tooth is a microdont (31).

Ceramic Veneers

Porcelain laminate veneers help to mask discolored teeth and replicate the natural shade of the tooth. They can be used for mildly discolored single teeth, when bleaching has not provided a suitable aesthetic result (Figure 3) (31). Ceramic veneers are more resistant to wear than composite veneers (34). Porcelain laminate veneers are also indicated when the adjacent teeth have ceramic prostheses or restorations (31). For severely discolored single tooth, a minimum thickness of 2 mm of glass-ceramic is needed in order to achieve the desired final shade (35). The underlying tooth structure affects the final appearance of the ceramic restoration. The color of the substrate (i.e. the stump shade) can be masked by increasing the thickness of the ceramic material, using opaque luting cement, using high opacity zirconia material, or using ceramic systems containing a ceramic substructure with shade-masking properties (36).

Full Coverage Crowns

These are usually indicated for heavily restored, discolored teeth or endodontically treated teeth restored with post. Crowns should only be considered when more minimally invasive treatment options have not been effective. Full coverage crowns can be either metal-ceramic or all ceramic. Discolored teeth can be restored with either translucent crowns or crowns with opaque cores. Lithium disilicate crowns are a good option for teeth with mild discolorations and can be adhesively bonded to unretentive tooth preparations, such as teeth with oblique fractures or severely worn teeth. For severely discolored teeth, opaque crowns (metal-ceramic or all-ceramic [zirconia]) are a better option since the clinician is able to better predict the final shade of the crown (Figure 3) (37).

Conclusion

Simple measures can be taken to avoid iatrogenic causes for tooth discoloration post-endodontically. For an already discolored teeth, understanding of the cause for localized tooth discoloration is important in selecting the appropriate treatment option. After recording a thorough history, conducting an oral examination and arriving at a diagnosis, clinicians are recommended to begin with minimally invasive treatment options, followed by the more invasive ones. Certain patients may even require a combination of different types of treatment in order to achieve desirable results. Based on the patient's clinical condition, the severity of discoloration and the aesthetic requirements, the clinician can opt for the appropriate treatment plan. Hence, it is essential for the dentist to possess a thorough knowledge of the treatment modalities available, in order to reach a favorable treatment outcome.

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Ethics

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Authorship Contributions

Surgical and Medical Practices: S.D., M.T., Concept: S.D., M.T., Design: I.S., Literature Search: S.D., I.S., M.T., Writing: S.D., I.S., M.T.

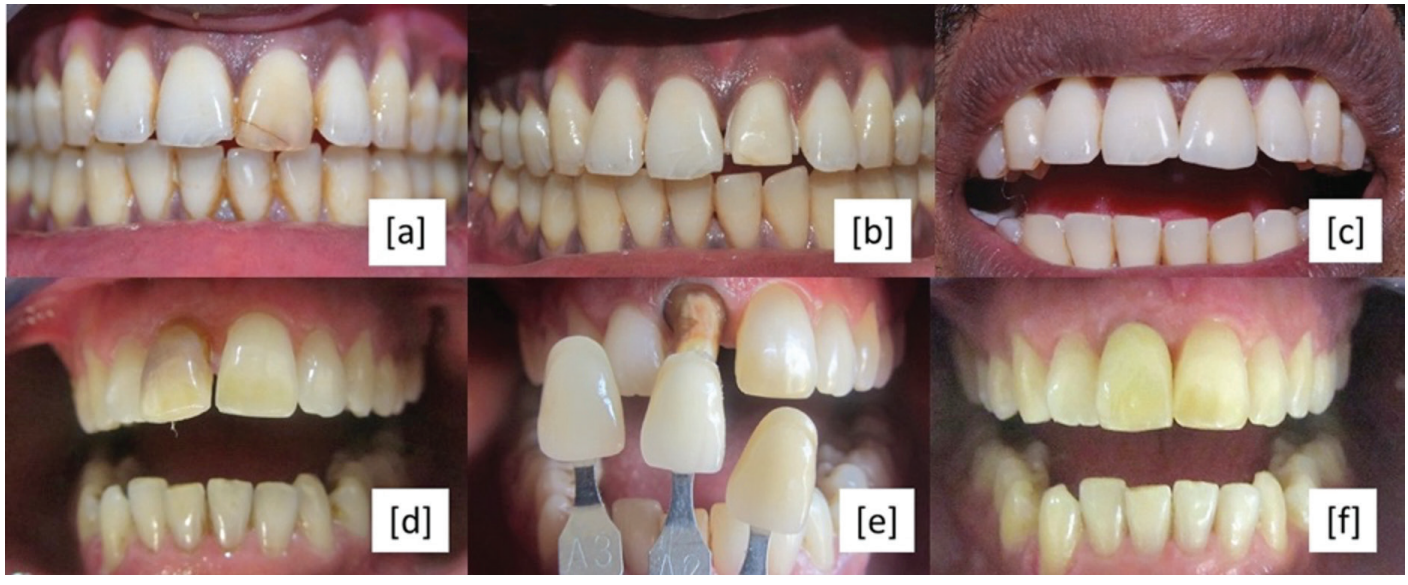

Figure 3. Restorative methods in managing localized discoloration (a to c): Lithium disilicate veneer to mask the discoloration on tooth #21; (d to e) Zirconia crown to mask the discoloration and to realign tooth #11

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The relationship between HMGB1 and immune infiltration serves as a treatment target molecule in different cancer tissues: May be used therapeutic target for possible SARS-CoV-2 infection

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ABSTRACT

Aims: To illustrate which tumor cells may express high-mobility group box 1 (HMGB1), which is also Coronavirus disease-2019 (COVID-19) target molecule as a treatment option for possible severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection.

Methods: We investigated m-RNA expression patterns of HMGB1 in 33 different cancer tissues. HMGB1 m-RNA expression profiles were compared with the Gene Expression Profiling Interactive Analysis database. Comparisons of promoter methylation levels with the UALCAN database were also performed. Finally, the correlation between HMGB1 and immune cells was investigated by using TIMER tool.

Results: High expression profile of HMGB1 was determined in 8 different cancer tissues (colon adenocarcinoma, diffuse large B-cell lymphoma, glioblastoma multiforme, brain lower grade glioma, pancreatic adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma and thymoma) when compared with the healthy tissues ($p<0.05$). The promoter methylation level of HGMB1 in different cancers was significantly lower. In addition, the level of expression and overall survival did not correlate in studied tumor samples. HMGB1 transcription level was associated with innate (monocyte, neutrophil) and adaptive immune cells (cytotoxic T lymphocyte and B cell) in tumor samples.

Conclusions: The use of agents that inhibit HMGB1 protein may offer an effective approach, not only against the cancer cell proliferation, but also as a strategy to minimize the possible SARS-CoV-2 infection in cancer patients with high HMGB1 expression.

Introduction

Cancer is a term that collectively describes a group of apparently unrelated conditions that have common immune response related pathways leading to various tissue damage (1). Although each of these cancer types has distinctive molecular background and pathophysiology, the dysregulated immune response is believed to be pivotal to diseases' pathogenesis. High-mobility group box 1 (HMGB1), a member of the HMG protein family, is one of the damage-associated molecular patterns (DAMPs) which are danger molecules released from damaged cells and activate the innate immune system components (2-4). Innate immune system is the first defense mechanism against the pathogenic microorganisms such as viruses, bacteria, and parasites. DAMPs have a dual role by contributing host's defense and promoting pathological inflammatory responses (5,6). Experimental studies have demonstrated that inflammatory

signaling pathway was induced and inflammatory cytokines including interleukin 6 (IL-6), interleukin 1 (IL-1), tumor necrosis factor (TNF) were produced by releasing of HMGB1 molecule to the extracellular region (6,7). HMGB1 is a crucial molecular target for infectious diseases, ischemia, immune diseases, neurodegenerative diseases, metabolic diseases and cancer (2,4,8). HMGB1 has many biological activities in normal and cancerous cells and regulates many basic molecular events such as transcription, replication, recombination, DNA repair, genomic stabilization and TLR4 activation (5,8,9). In the coordination of the cell's stress response, not only in intracellular functions such as chromosome protector, autophagy, and apoptotic cell death inhibitor, but also outside the cell, it plays a critical role as the DAMP prototype (6,9). The interplay of DAMP and other factors releases cytokine, chemokine, and growth factor activity and manages inflammation and immune response. The global public health is novel coronavirus [Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) – also known as 2019-nCoV], the RNA virus that causes Coronavirus disease-2019 (COVID-19) (10,11). It is important to understand the role of HMGB1 and associated cellular mechanism including transcription, methylation, immune response, prognosis in hypothetically infection with SARS-CoV-2 in various tumor tissues and healthy tissue. In humans, SARS-CoV-2 infection results in a diversity of clinical manifestations. While several genetic and epigenetic factors associated with COVID-19 have been identified, pathogenesis of COVID-19 in cancer patients is still poorly understood. There is strong evidence implicating the involvement of the immune reaction in the progression to COVID-19, tumor infiltrating immune cells playing a role (12,13). For this reason, higher HMGB1 expression in cancer patients increases the risk for developing infectious diseases. In this study, we mainly discuss our own data on the analysis of the intratumoral infiltrates in human cancers, their relationship with HMGB1 expression, the respective importance in view of their prognostic value and interaction with possible SARS-CoV-2 infection. To illustrate the general characteristic of the host HMGB1 expression-SARS-CoV-2 interactions, eight examples of the human tumors will be analyzed by using bioinformatics tools. Finally, we will propose some new insights on which tumor cells may express HMGB1, which is also COVID-19 target molecule to treatment and protection from possible SARS-CoV-2 infection.

Methods

Subjects

This study was carried out by using The Cancer Genome Atlas (TCGA) data sets of 33 different cancer types.

Expression and Correlation Analysis

Gene Expression Profiling Interactive Analysis (GEPIA) is a web server that allows the evaluation of RNA expression of normal tissue samples as a control group and tumor tissue samples on a wide scale obtained within the scope of TCGA and GTEx projects. To identify HMGB1 gene expression profile, we compared box plot from 33 different cancer tissue samples and healthy tissue samples to GEPIA database (14). In addition, overall survival analysis was performed based on the Log-rank test with a 95% confidence interval in order to create survival graphs. The p values were calculated automatically by the tool and p values under 0.05 were considered significant. HMGB1 expression profile screening was performed in 33 different cancer tissues [adrenal cancer, bladder and urothelial cancers, breast cancer, cervix cancer, colorectal cancer, lymphoma, esophageal cancer, glioblastoma, head and neck cancer, renal cancer, leukemia, liver cancer, lung cancer, ovarian cancer, stomach cancer, pancreatic cancer, prostate cancer, brain tumor, uterine cancer, mesothelioma, melanoma, sarcoma, thyroid cancer, thymoma (THYM), uterine cancer]. A statistically significant difference was found in terms of HMGB1 gene expression in 8 tumor tissues including colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), brain lower grade glioma (LGG), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), THYM among these cancer types. Further analyses were performed on these samples.

Analysis of Promoter Methylation Levels

UALCAN is an interactive open-access web page for the analysis of OMICS data (http://ualcan.Path.uab.edu/index.html). This database is built on PERL-CGI and can be used in about 6000 different gene methylation levels (15). So, the promoter methylation level of *HMGB1* gene was evaluated with this database in different cancer tissues.

Comprehensive Analysis of Tumor-infiltrating Immune Cells

TIMER is a comprehensive open access online database that can analyze immune invasion levels and differences in gene expression levels in different tumor tissues (16). Correlation between HMGB1 expression and immune infiltrating cells [B cells, CD4+ T cells, CD8+ T cells, neutrophils, macropages, and dendritic cells (DCs)] was analyzed by using the TIMER database.

Statistical Analysis

We used the GEPIA database to examine the statistical significance ($p<0.05$) by analyzing all parameters. Five statistical methods including Kaplan-Meier curves, log-rank test, Pearson test, Spearman's correlation, and Student's t-test were used in overall survival, comparison of low and high groups, correlation analyses, evaluation of HMGB1 with immune infiltration, and comparison of two independent samples, respectively.

Results

Results of Expression and Correlation Analysis

Initially, gene expression profile belonging to the 33 different cancer tissues was compared with healthy tissues and evaluated separately in terms of HMGB1 expression profiles using the GEPIA online tool in order to investigate changes in the gene expression level of HMGB1 in the cancer tissue (Figure 1). As a result of our analysis, it was determined that the expression profile of HMGB1 was statistically significant in 8 different cancer tissues (COAD, DLBC, GBM, LGG, PAAD, READ, STAD and THYM). Therefore, 8 different tumor tissues were included in all analyses made thereafter. In our study, HMGB1 was found to have a statistically significantly higher expression level in these 8 different tumor tissues when compared to healthy tissue samples (Figure 2, $p<0.05$). Then, the same datasets were used in order to analyze the overall survival of 8 different cancer patients according to HMGB1 expression (Figure 3). According to the results of survival analysis, HMGB1 high expression levels were found to be associated with good prognosis in cancer patients with THYM ($p<0.05$). No statistically significant difference was found between low and high expression levels for other types of cancer.

Analysis of the Promoter Methylation Levels

DNA methylation is an important case in the epigenetic modification of the genome and is closely related to the development process of cancer. Hypo methylation may cause genome instability and activate the related genes. It was determined that HMGB1 expression profile had high levels of expression in 8 different cancers. According to the results of our analysis, we performed using the UALCAN online tool in order to determine the level of DNA methylation, the promoter methylation level of HMGB1 was found to be lower than healthy tissues (hypo methylation) (Figure 4). GBM and LGG were not included in the analysis as the UALCAN database did not provide access to their comparison with healthy tissues.

Comprehensive Analysis of Tumor-infiltrating Immune Cells

When we analyzed the correlation between m-RNA expression level of HMGB1 and immune infiltration profile in different cancers, the correlation was detected between HMGB1 expression and immune infiltration in COAD, DLBC, GBM, LGG, PAAD, READ, THYM cancer types according to TIMER database uploaded data. HMGB1 expression was positively correlated with B cell ($r=0.11$, $p<0.05$), CD8+ T cell ($r=0.208$, $p<0.0001$), macrophage ($r=0.112$, $p<0.05$) infiltration in COAD; B cell ($r=0.688$, $p<0.05$) in DLBC; B cell ($r=0.124$, $p<0.05$), CD8+ T cell ($r=0.25$, $p<0.001$), macrophage ($r=0.144$, $p<0.001$), neutrophil ($r=0.136$, $p<0.001$) in GBM; macrophage ($r=0.109$, $p<0.05$) in LGG; B cell ($r=0.282$, $p<0.001$), CD8+ T cell ($r=0.298$, $p<0.0001$), macrophage ($r=0.298$, $p<0.00001$), neutrophil ($r=0.285$, $p<0.0001$), DCs ($r=0.351$, $p<0.0001$) in PAAD; CD8+ T

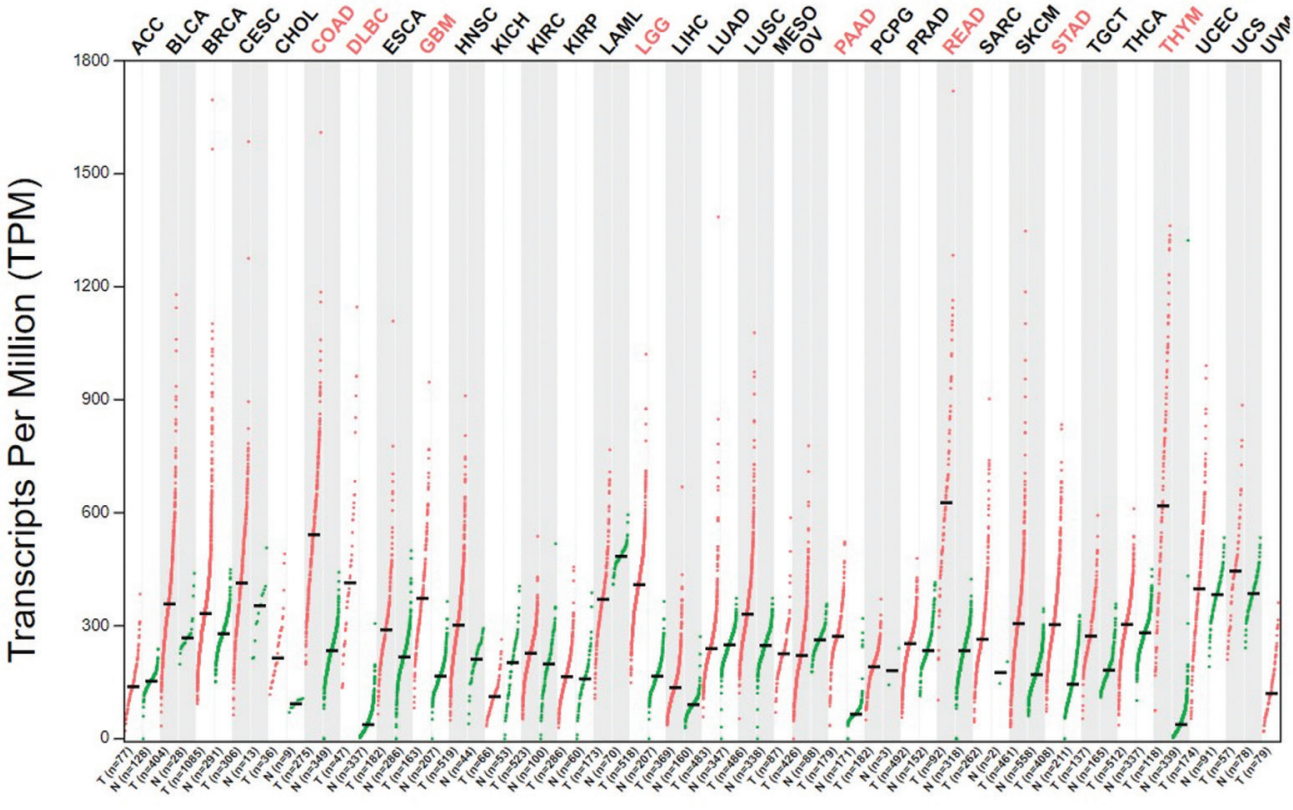

Figure 1. HMGB1 in different cancers compared to normal tissues in the Gene Expression Profiling Interactive Analysis database

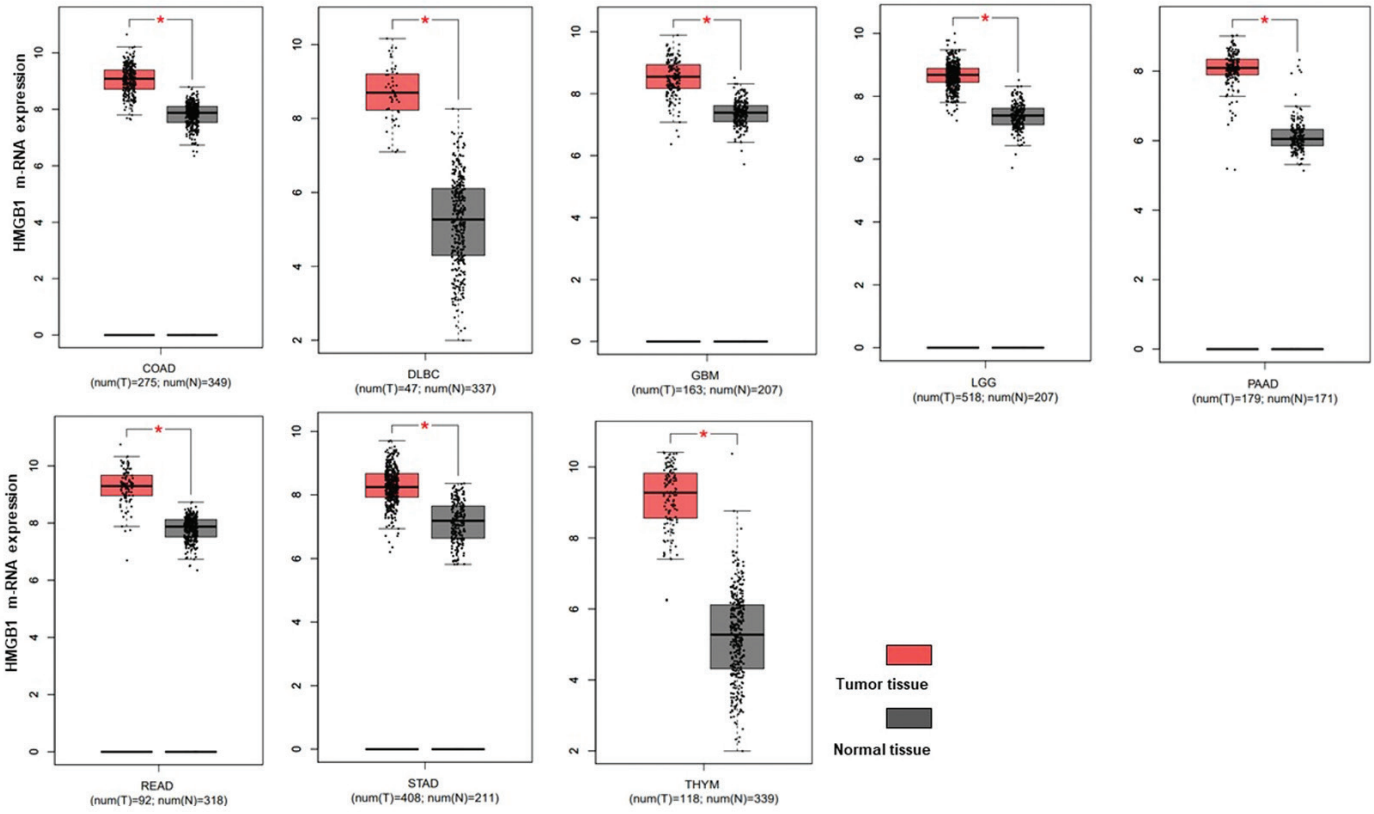

Figure 2. Comparative analysis of the tissue-specific differential expression of HMGB1 gene in different cancer tissues using GEPIA (*indicates p<0.05)

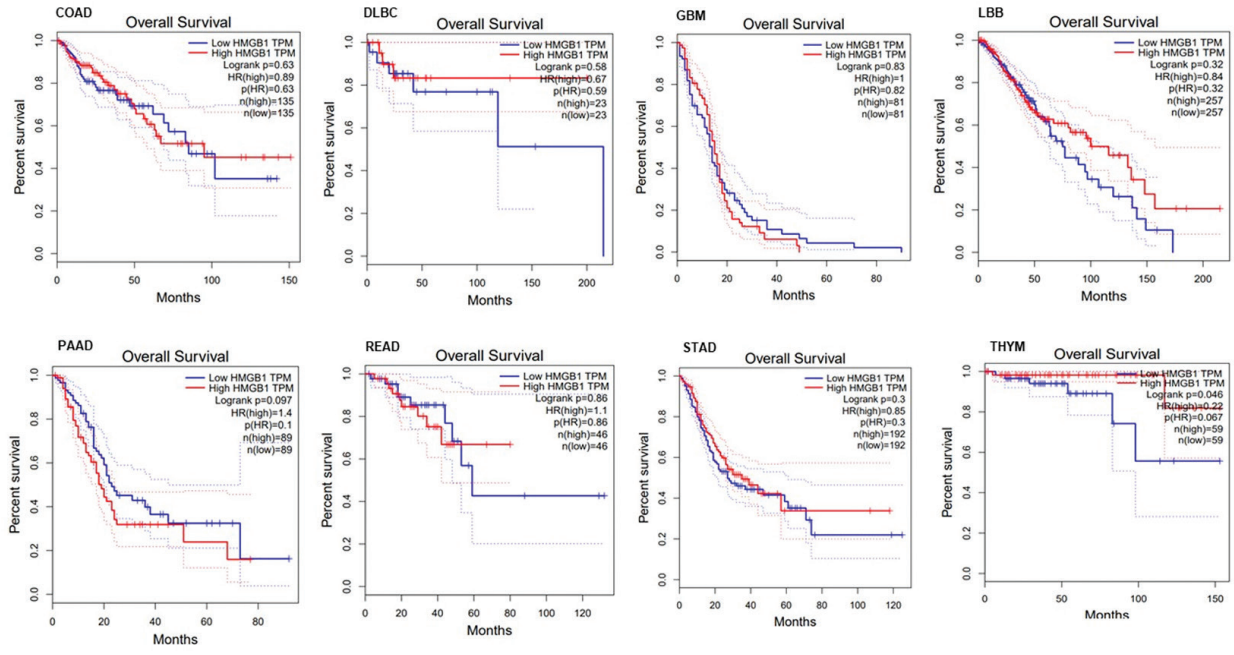

Figure 3. Comparison of Kaplan-Meier survival curves of the high and low expressions of HMGB1 in different cancer tissues

cell (r=0.191, p<0.05), neutrophil (r=0.187, p<0.05) in READ; B cell (r=0.71, p<0.0001), CD8+ T cell (r=0.566, p<0.0001), CD4+ T cell (r=0.723, p<0.0001), macrophage (r=0.6, p<0.0001), DCs (r=0.781, p<0.0001) in THYM patients' tumor samples compared to healthy samples. However, in silico analysis results showed that limited or weak correlation was detected between CD4+ T cell and HMGB1 expression level in all studied tumor tissues except THYM tumor samples (Figures 5a, 5b).

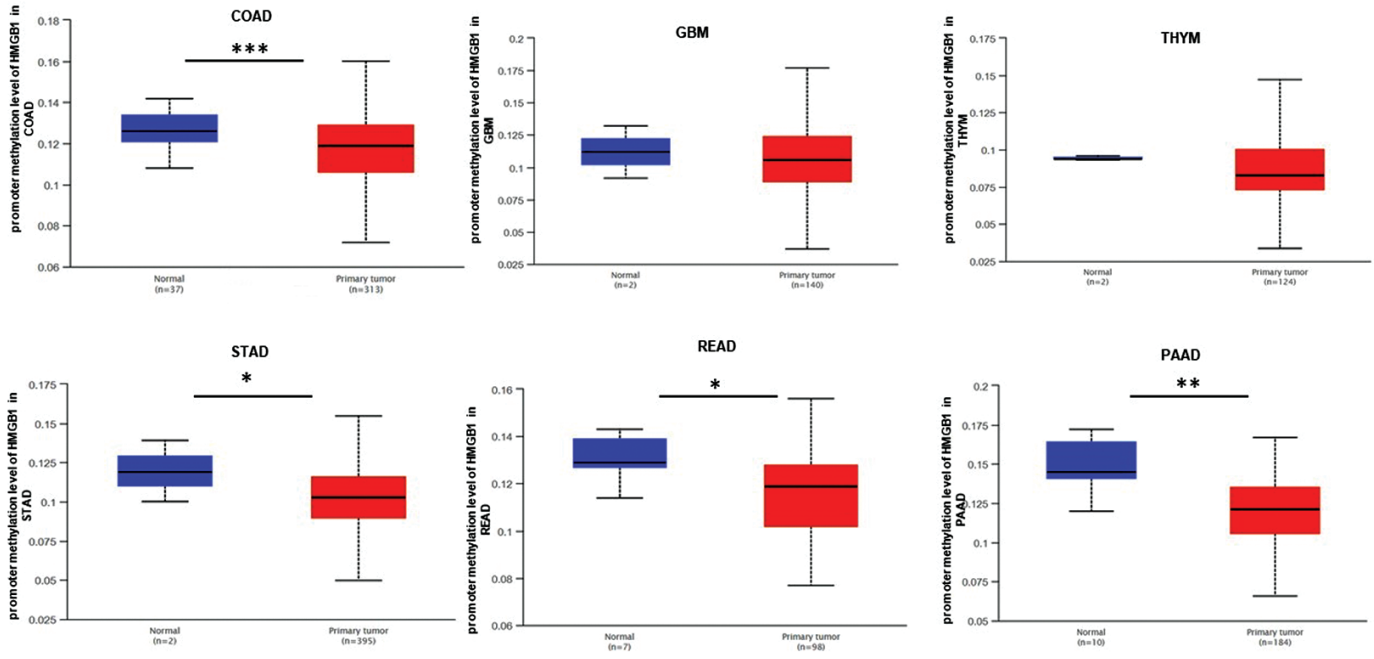

Figure 4. The promoter methylation level of HMGB1 in different cancer tissues

Discussion

It has been shown that HMGB1 may support tumor development and progression by inducing chronic inflammation and, in contrast, this molecule may inhibit tumor progression (17,18). A number of studies have reported pathophysiological roles of HMGB1 in various diseases, including infectious diseases and cancer (3,5,17,18). It is noteworthy that the up-regulation of HMGB1 in cancer is due to the fact that cancer cells are exposed to different stress parameters such as acidosis, hypoxia and inflammation. A number of studies reported anti-tumor immune stimulatory roles of extracellular-HMGB1 (19,20). Treatment based on HMGB1 can be useful against cancers and viral infection. Extracellular-HMGB1 released from damaged cells as a danger signal can stimulate DCs, followed by the release of pro-inflammatory cytokines (9,21). Moreover, some analysis results showed that expression of HMGB1 could promote the cross-presentation of peptide antigens to major histocompatibility complex class 1 in DCs, activating cytotoxic T lymphocytes (CTLs) (2,4,9,22). From the aspect of this mechanism, the roles of extracellular HMGB1 activating both innate immunity and adaptive immunity may be an important molecular mechanism in response to viral infection including SARS-CoV-2 for cancer patients (23,24). Andersson et al. (23) reported that extracellular HMGB1 might be therapeutic target in COVID-19. Based on this information, which cancer types expressed this danger molecule and what is the relationship between HMGB1 and immune cells? Here, we evaluated this in silico analysis on the role of HMGB1 in various cancer tissues including COAD, DLBC, GBM, LGG, PAAD, READ, STAD and THYM and healthy samples and the m-RNA expression of HMGB1 was higher in eight cancer types according to the results of expression analyses. We also concluded the possibility of HMGB1 as therapeutic targets for COVID-19. In cases where cancers including COAD, DLBC, GBM, LGG, PAAD, READ, STAD and THYM are high, HMGB1 is the treatment option for these cancer patients infected with SARS-CoV-2. From this study, it can be concluded that hypo methylation of HMGB1 triggers the expression of this molecule, resulting in the release of HMGB1 into extracellular region. To confirm the role of HMGB1 and SARS-CoV-2 infection with pathogenesis of cancer, further studies are required.

Immune cells have receptors such as toll like receptors (TLR) for HMGB1. Immune cells such as natural killer (NK) cells, CTLs, NKT cells which have cytotoxic functions can recognize extracellularly released HMGB1 from tumor cells (8,9,19,23,25). Elevated levels of HMGB1 have been detected in patients with COAD, DLBC, GBM, LGG, PAAD, READ, STAD and THYM cancer (17,18). We have reported that the HMGB1 expression levels were correlated with the immune infiltration in studied eight cancer types. In humans, it is well known that tumors may behave differently in terms of survival. We did a survival analysis based on the HMGB1 expression of different tumors in relation to immune infiltration through the tumor microenvironment using the Kaplan-Meier plotter. An immune control is responsible for carcinogenesis in human, and is also a research field in terms of providing novel prognostic markers as well as new therapeutic area. In this context, some studies showed the role of HMGB1 expression in various cancer such as gastric, colorectal, pancreatic, esophageal, prostate, bladder, lung cancer and hepatocellular carcinoma (17,18,26). When in silico analysis of intra tumoral immune cells became available in association with HMGB1 expression in studied 8 cancer types, our data show that HMGB1 expression was positively correlated

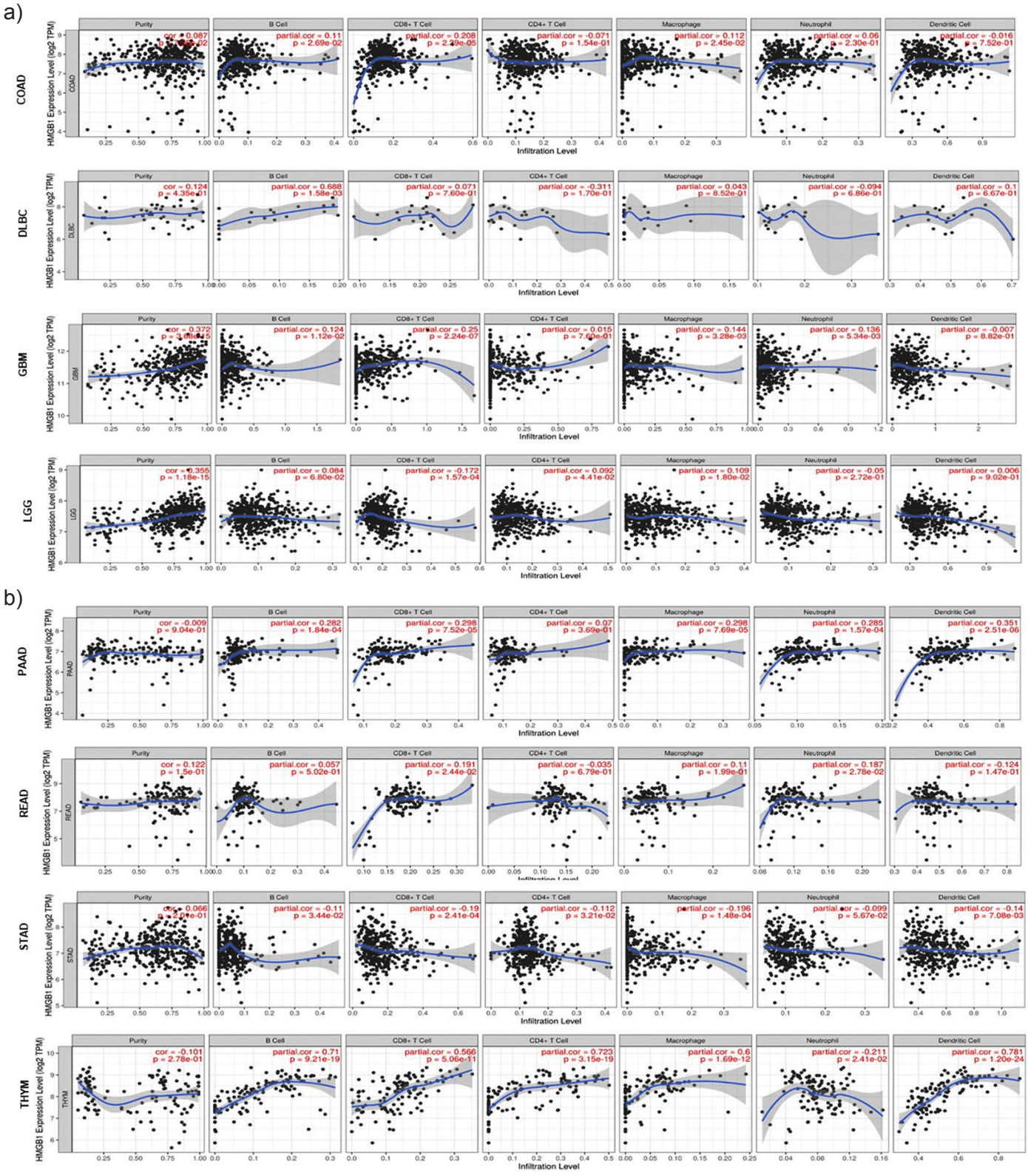

Figure 5. Correlation between HMGB1 expression and immune infiltration in different cancer tissues using TIMER algorithm (a, b)

with B cell, CD8+ T cell, macrophage infiltration in COAD; B cell and DCs in DLBC; B cell, CD8+ T cell, macrophage, neutrophil in GBM; macrophage in LGG; B cell, CD8+ T cell, macrophage, neutrophil, DCs in PAAD; CD8+ T cell, macrophage, neutrophil in READ; B cell, CD8+ T cell, CD4+ T cell, macrophage, DCs in THYM patients tumor samples compared to healthy samples. Recent studies have reported that lymphocytes infiltration in the primary tumor usually correlates with a better clinical outcome in

cancer patients (27). Our data supported that especially CD8+ cytotoxic T cell infiltration was detected in five tumors of studied eight tumor samples, neutrophil and DCs immune infiltration levels of prostate cancer. Additionally, the level of macrophage in cancer may be related to worst prognosis (28). Our data show that interplay of high expression HMGB1 and CD4+ T helper cell filtrated tumor tissue was very limited or not. The formation of lymphoid structure composing of T and B cell was widely detected in cancer patients with highly expressed HMGB1 tissue samples according to our results. Other researchers reported that T and B lymphocytes infiltration to tumor tissue was associated with their prognostic value in cancer (29).

Therefore, these results reported herein should be considered in the light of some limitations. One of these limitations is in silico design and lack of experimental study. The other limitation concerns the demographic statistics such as gender and age for healthy group, which could not be downloaded from the database.

Conclusion

In conclusion, some HMGB1-targeted anti-cancer and anti-viral drugs, inhibitors, RNA interference gene silencing are promising in both cancer therapy and also COVID-19 therapy for upregulated HMGB1 cancer types including COAD, DLBC, GBM, LGG, PAAD, READ, STAD and THYM.

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Ethics

Ethics Committee Approval: Ethics committee approval is not required for the study. Study data were obtained from The Cancer Genome Atlas, and *in silico* analysis was performed.

Informed Consent: The study is a bioinformatics study, patient consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.I.S., Design: R.I.S., Data Collection or Processing: D.F.A-B., Analysis or Interpretation: D.F.A-B., Literature Search: R.I.S., D.F.A-B., Writing: R.I.S., D.F.A-B.

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Coracoacromial ligament subligamentous intensity change on magnetic resonance images

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ABSTRACT

Aims: To describe the finding of edema-like intensity beneath the coracoacromial ligament (CAL) on shoulder magnetic resonance (MR) images of patients referred with shoulder pain and/or with the presumption of rotator cuff pathology.

Methods: A total of seven patients were included in the study group having edema-like intensity beneath the CAL on fluid sensitive sequences. None of the patients had any other additional findings such as effusion in the subacromio-subdeltoid bursa, degeneration of the acromioclavicular joint or the rotator cuff rupture. The images of the patients were obtained using two different scanners of 1,5T (six patients) and 3T (one patient). The thickness of CAL at its midportion was also measured.

Results: A total of seven patients were included. There were five women and two men with a mean age of 39,28 years. Four of the patients were referred to imaging with the suspicion of rotator cuff pathology only, one patient for having pain in the shoulder and two having both pain and suspicion for rotator cuff pathology. The best imaging plane for this finding of edema-like intensity beneath the CAL was the sagittal plane. The ligament thickness was measured with a mean value of 0.94 mm (between 0.66 mm-1.32 mm).

Conclusions: The edema-like intensity restricted beneath the CAL without thickening of the ligament can go with symptomatology as seen in subacromial impingement syndrome. Hence, it is valuable to look for and concern this intensity even if it is the sole finding on shoulder MR images, as a possible indication for symptomatology.

Introduction

Painful shoulder is one of the most common musculoskeletal complaints affecting up to 30% of individuals, which may bring them to various outpatient clinics (1-3). Different degrees of rotator cuff disorders are commonly the main etiology for shoulder pain, where impingement of the cuff plays a pivotal role. As an answer to the question why the rotator cuff impinges, two predominant etiologies, in other words "types" have been described as "intrinsic and extrinsic". Of these, extrinsic impingement is a frequent cause for the patients who seek an orthopedic or physical assessment (4). Extrinsic shoulder impingement, mainly in the form of subacromial impingement, is related to the mechanical compression of the structures forming the boundaries of the subacromial space where the rotator cuff passes thorough. The roof of this space is confined by an osteofibrous arch which is called coracoacromial arch formed by acromion, coracoid, and the coracoacromial ligament (CAL) (5,6). Diminishing the size of this space confined within the coracoacromial arch has the potential to lead the impingement symptom of persistent shoulder pain created when elevating the arm above 70°, forcing the arm above the head, and lying on the symptomatic side (3,6).

The CAL forms a bridge between the acromion and the coracoid processes of the scapula, making a restraint to superior displacement of the humeral head (7). The CAL is blamed to have a role in subacromial impingement syndrome by forming the coracoacromial arch together with the other components as mentioned earlier (8).

Degeneration of the CAL and spur formation at the attachment site of the acromion are reported to be in the spectrum of subacromial impingement syndrome (9,10). Although the histological, anatomical and biomechanical properties of the CAL in the body of the ligament have been studied before, less pertaining to the imaging features of this part of the ligament when symptomatic is published (11,12).

The aim of this study was to describe the finding of edema-like intensity beneath the CAL on fluid sensitive sequences, which is in continuation with the fascia of the supraspinatus muscle, on shoulder magnetic resonance (MR) images of the patients referred with shoulder pain or with the presumption of rotator cuff pathology. According to our research, this sole imaging finding of edema-like intensity in symptomatic patients has not been described before in the setting of impingement syndrome, hence we aimed to draw attention and discuss the potential clinical implications of this observation with literature.

Methods

This single center, retrospective study included seven patients who had edema like intensity beneath their CALs. None of the patients had any other additional findings such as effusion in the subacromio-subdeltoid bursa, degeneration of the acromioclavicular joint or the rotator cuff rupture, making the strict exclusion criteria. The referral clinics, clinical prediagnosis and the complaints of the patients were noted from the archiving system of the hospital. The examinations were performed in the period between November 2017 and June 2019. The study was approved by the Non-interventional Research Ethics Committee of University of Health Sciences Turkey (protocol number: 19/300; November 12, 2019).

All MR images were evaluated for the edema-like hyperintensity beneath the CAL by three authors (two authors with more than 15 years of experience and one author with one year of experience). Thirty eight patients having this intensity beneath their CALs were detected at first. However, according to our strict criterion, which was about the edema-like intensity beneath the CAL's being the single and the only imaging finding, we eliminated those cases with extra findings such as subacromio-subdeltoid bursitis, acromioclavicular joint arthrosis and any degree of rotator cuff pathology.

The MR images of six patients using 1.5T (Philips-Intera-Netherlands) and 1 patient using a 3T (Philips, Achieva, Netherlands) scanner were analyzed. For fluid sensitive images on 1,5 T, the acquired sequences included a combination of T2-weighted spectral presaturation with inversion recovery (SPIR) (TR range/TE range, 1392-1741/60 ms) in the oblique coronal and transverse planes, and proton density (PD) SPIR (2567-3225/30 ms) in the sagittal plane. On 3T, the acquired sequences were T2-weighted SPAIR (SPectral Attenuated Inversion Recovery) (TR range/TE range, 3563-4114/60 ms) in the oblique coronal and transverse planes, and PD SPAIR (2657/30 ms) in the sagittal plane.

Since depicting the presence of edema-like intensity beneath the CAL was forming the main purpose of the study, where this can only be carried on fluid sensitive sequences as described above, T1-weighted images were not evaluated for edema intensity. The CAL was tracked along its course from coracoid to acromion and the thickness was measured in the mid portion of the ligament.

Results

The study included seven shoulders of a patient group composed of five women and two men with age ranging from 21 to 52 years (mean age 39.3 years), only having edema-like intensity beneath the CAL. The characteristics of the subjects are summarized in the Table 1. Five of the patients were referred from the orthopedics, one from physical therapy and one from neurosurgery clinics. Four of the patients were referred with the suspicion of rotator cuff pathology only, one patient for having pain in the shoulder and two having both pain and suspicion for rotator cuff pathology.

The best imaging plane for this finding of edema-like intensity beneath the CAL was the sagittal plane; however, in all planes, the hyperintensity could be observed on fluid sensitive sequences. In these sequences, all of the patients were displaying increased signal intensity beneath the CAL, in a triangular space with the CAL forming superior, the coracohumeral ligament anterior-inferior and the fascia of the supraspinatus muscle the posterior borders (Figures 1, 2, 3). The ligament thickness was measured with a mean value of 0.94 mm (between 0.66 mm-1.32 mm) at its mid portion.

Discussion

The edema-like intensity beneath the CAL observed in our study group may be the first sign in the initiation of subacromial impingement syndrome related to this ligament. Shoulder pain is

Table 1. Demographic characteristics of seven patients with subligamantous edema-like intensity beneath the coracoacromial ligament

Case#	Gender/age	Referred for	Referral clinics
1	M/21	RCL	Physical therapy
2	F/42	RCL	Orthopedics
3	M/52	Pain, RCL	Orthopedics
4	F/28	RCL	Orthopedics
5	F/44	Pain, RCL	Orthopedics
6	F/42	Pain both in shoulder and neck	Neurosurgery
7	F/46	RCL	Orthopedics

RCL: Rotator cuff lesion, M: Male, F: Female

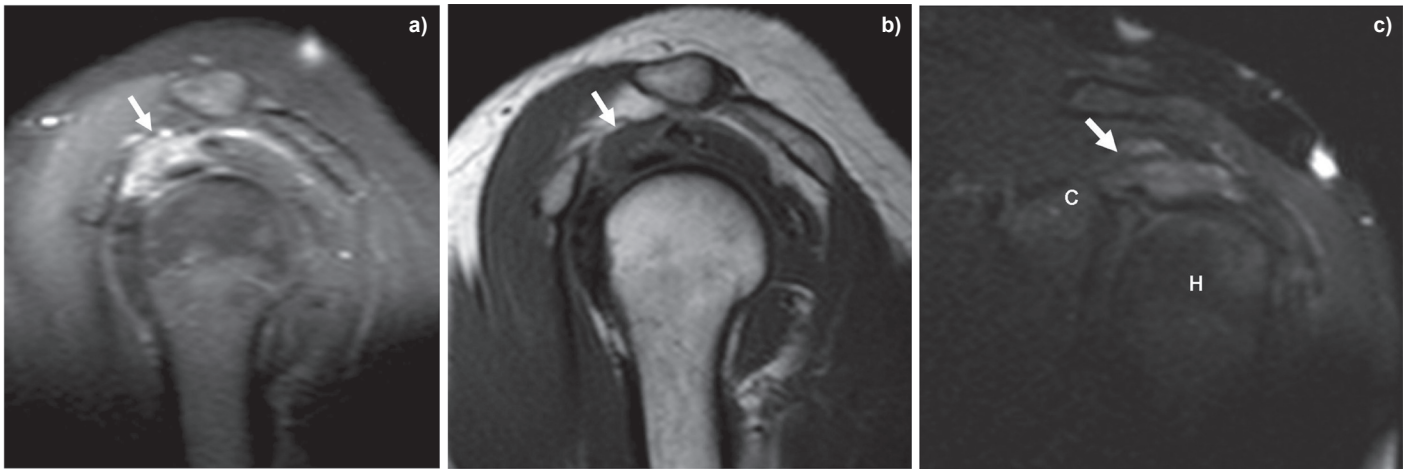

Figure 1. Case #7, 46-year-old female, referred with the suspicion of rotator cuff lesion. Sagittal proton density SPIR (a), sagittal T1-weighted (b) and oblique coronal T2-weighted SPIR images (c). Edema-like intensity beneath the CAL (arrow) is observed on fluid sensitive sequences (a and c)
C: Coracoid process, H: Humeral head

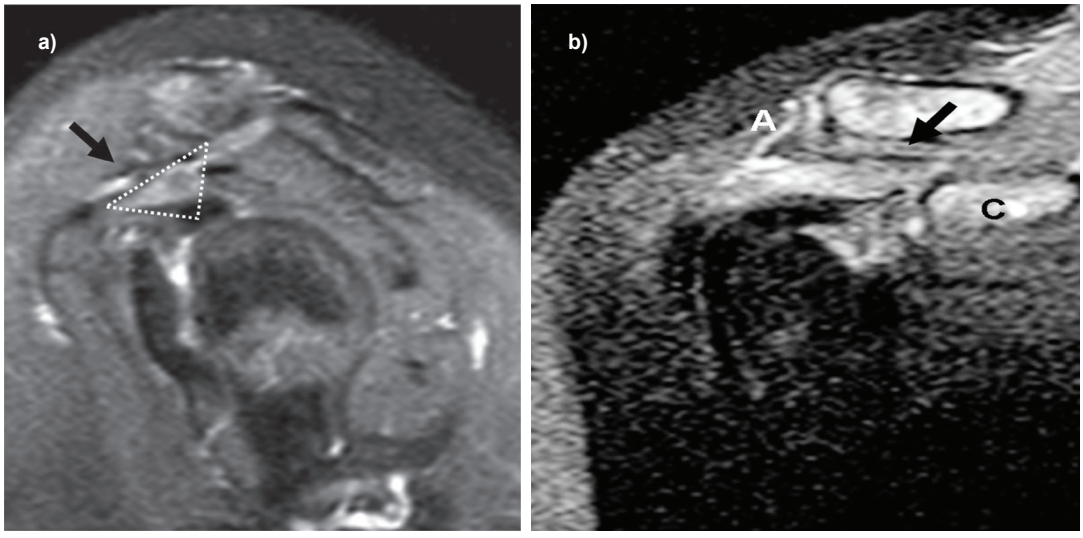

Figure 2. Case #3, 52-year-old male, referred with the suspicion of rotator cuff lesion with pain. Sagittal proton density SPIR (a), and oblique coronal T2-weighted SPIR images (b). Edema-like intensity beneath the coracoacromial ligament (CAL) (arrow) is seen in a triangle, borders formed by the CAL, the coracohumeral ligament and supraspinatus muscle fascia
C: Coracoid process, A: Acromion

a debilitating condition in which shoulder impingement syndrome is the most commonly reported disorder caused by a myriad of factors. Shoulder impingement syndrome is a compilation of symptoms and signs caused either by pathologies originating within the rotator cuff tendon substances itself (intrinsic) or structures external to the cuff (extrinsic), where occasionally both intrinsic and extrinsic pathologies may coexist (13). According to Neer (14), who described the extrinsic impingement concept first, the subacromial space, where impingement can occur, is boundaried inferiorly by the humeral head and superiorly by the coracoacromial arch which latter encompasses three structures: the undersurface of the anterior third of the acromion, CAL and the coracoid process (3).

The CAL lying between the two parts of the same bone -the scapula- acts as a tension band for the acromion and the coracoid with a significant role in transmitting forces from the surrounding musculature (8). This ligament forms the boundaries of the subacromial space with the humeral head, the acromioclavicular joint and the anterior edge and under surface of the anterior third of the acromion (14). The CAL is also an integrated part of the coracoacromial arch with the anterior third of the coracoid process, the acromion, the distal clavicle and the acromioclavicular joint (15). Narrowing of the subacromial space is one of the mechanistic theories explained for the source of subacromial impingement syndrome (16). When thickened, the CAL may diminish the space for rotator cuff tendon movements (16). A cadaveric study has demonstrated that in certain forceful

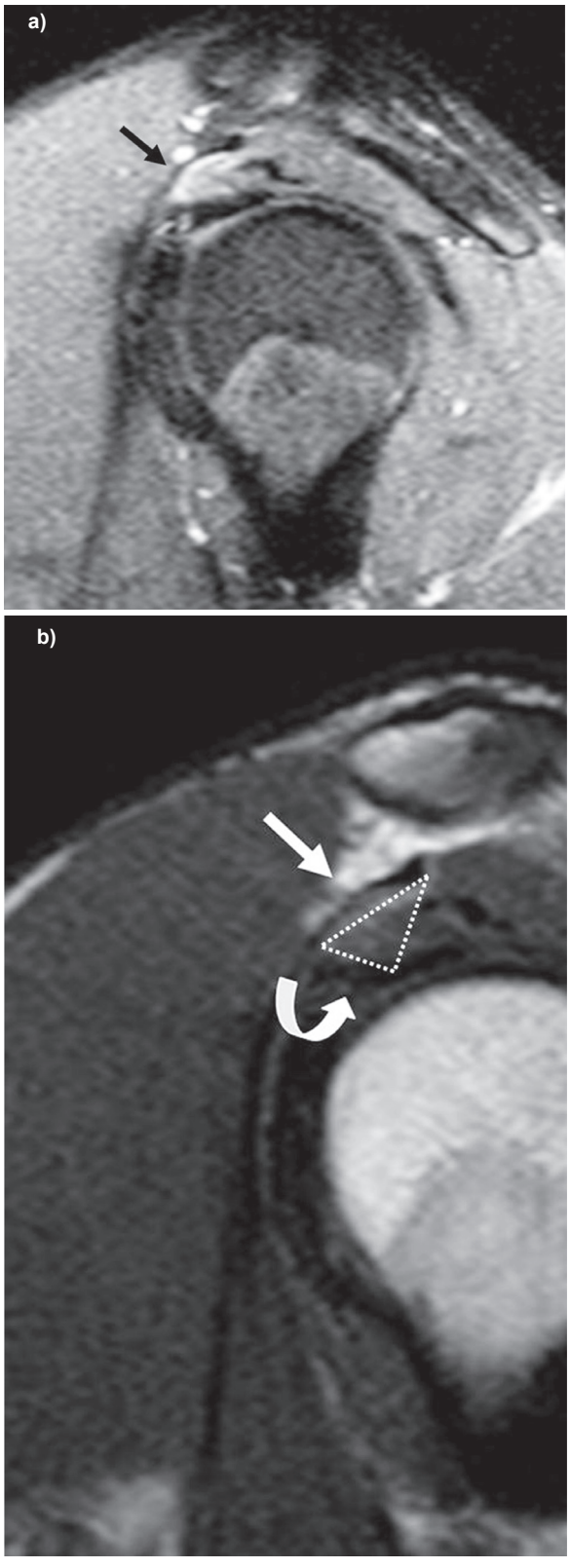

Figure 3. Case #1, 21-year-old male, referred with the suspicion of rotator cuff lesion. Sagittal proton density SPIR (a), and sagittal T1-weighted (b) images. On fluid sensitive sequence (a) the edema-like intensity beneath the coracoacromial ligament (arrow) is seen in a triangular space with anterior inferior border formed by the coracohumeral ligament (bowed arrow)

shoulder and body positions, the rotator cuff impinges at the CAL (17). There are other studies reporting a significant relationship between the existence of a thickened CAL and the incidence of rotator cuff tears (9,11,18).

Although juxtaposition between the coracoacromial arch and the rotator cuff has been shown to be a physiologic phenomenon, others have proposed that recurrent contact between the rotator cuff and the CAL may lead to degenerative changes reciprocally (19).

An adipose tissue is described to line the outermost layer of the CAL where it articulates with the subacromial bursa (7). This adipose tissue is reported to be richly innervated by both free and encapsulated receptor nerve endings, possibly contributing to mechanosensory pathways (7). A study, where the control samples composed of human CALs, showed rich perivascular innervation composed of PGP-9.5-containing nerves in the periligamentous tissue (20). The pain experienced in our patient population with edema-like intensity beneath the CAL may be a reflection of this histological finding.

Age-related changes secondary to chronic stress and cellular degradation with thickening and stiffening of the CAL are noted to contribute to a spectrum of impingement syndrome (7). This subligamantous edema-like intensity of the CAL observed in our study may be related or lead to ligamentous changes and thickening of the ligament ultimately, where it is obvious that long term follow-ups of the patients are needed.

According to a histological study with immunoperoxidase dying by Konttinen et al. (21), which was performed on the materials obtained after Neer's (14) acromioplasty in patients with chronic painful rotator cuff impingement syndrome, neither the CAL nor the periligamentous fatty and loose connective tissue displayed compilation of lymphocytes, macrophages, or other inflammatory cells. Hence, the edema-like intensity on fluid sensitive sequence in our study group is thought to be secondary mainly to reactive synovitis and edema, which needs verification by histological studies that sound practically tough. One other proposal is the frictional effect by the ligament itself. We think that, owing to the periligamentous fatty and loose connective tissue's innervation, possible reactive synovitis in the subligamentous portion of the CAL may be the source of pain in this region.

In their study, Sarkar et al. (12) reported that the CAL did not appear to be primarily responsible in the initiating process of impingement, rather it is the strain that is most likely produced by the changes beginning in the soft tissues of the subacromial space, which may be related to our proposal.

The subligamentous edema-like intensity with heterogeneity in the neighborhood fascia of the supraspinatus muscle made us think a close relationship of the CAL with this muscle fascia in such a continuum, which needs to be confirmed by histology

again. This edema-like intensity in the subligamentous fatty tissue may be alikened with other impingement syndromes around the other joints such as suprapatellar fat pad impingement syndrome about the quadriceps tendon or infrapatellar fat pad impingement syndrome around the ligamentum mucosum (22).

According to our subject group, these patients with the edema-like intensity beneath the CAL-fascial complex may have the same symptomatology with subacromial impingement syndrome. The awareness of such an intensity change about this anatomical unit may have the potential in future for targeted therapies such as needle applications to this particular space.

The best plane to evaluate the CAL on MR images has been reported to be the oblique sagittal plane in previous studies (8). This was also the same for our study that the high intensity change under the low intensity border of the CAL was well appreciated on the sagittal fluid sensitive sequences. The CAL extension from the coracoid process to the undersurface of the acromion is well studied at the acromion edge as a causative for impingement. However, the imaging information in the midsubsance and the coracoid extension of the CAL is rare in that it is lacking whether the changes about this aspect of the ligament is symptomatic. Our descriptive study raises this suspect in that the periligamentous changes at the mid-section of the CAL approaching to the coracoid process may be symptomatic as well.

The normal thickness of the CAL at the mid portion of the ligament was studied by ultrasound before. In their studies, Wang et al. (23) measured 1.97±0.49 mm (1.1-3.2) in normal healthy subjects and Dietrich et al. (24) reported 1.4±0.2 mm in asymptomatic volunteers. The ligament thickness in our study was measured with a mean value of 0.94 mm (between 0.66 mm and 1.32 mm), which is not beyond these reported values.

One of the limitations of this study is the lack of long-term follow-up of patients to observe whether this finding has the potential to lead to a structural change in CAL and ultimately to subacromial impingement syndrome. Owing to the strict exclusion criteria, the small number of the group may sound as another limitation; however, this is a descriptive study of a persistent finding, we think that the number of the cases are sufficient.

Conclusion

Advances in our understanding of the CAL through the histological and anatomical studies with the clinical and imaging perspective may further aid to the conservative treatments of the patients in the future. According to our observation in this small study group, the edema-like intensity restricted beneath the CAL without thickening of the ligament can go with symptomatology as seen in subacromial impingement syndrome, hence it is valuable to look for and concern this intensity change in the fatty tissue beneath the CAL on shoulder MR images even if it is the sole imaging finding.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey of Non-interventional Research Ethics Committee (protocol number: 19/300; November 12, 2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Parents' and healthcare professionals' views and attitudes towards anti-vaccination

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ABSTRACT

Aims: Anti-vaccination is a sign of transition from sociality to individuality, although it is a dangerous situation for public health. The aim of our study was to determine the opinions and experiences of parents and healthcare professionals about vaccination and anti-vaccination and to evaluate the ethical dimension.

Methods: This was a descriptive, cross-sectional study. The study population consisted of parents referred to the department of paediatrics of a university hospital in Ankara, Turkiye and all physicians, nurses, and midwives in the department of paediatrics. The participants were surveyed on childhood vaccinations.

Results: The sample consisted of 80 parents and 36 healthcare professionals, 56.3% of the parents were female, and 68.8% had an undergraduate degree. The average age of healthcare workers was 44 years and 72.2% of them were female. Seven parents (8.8%) rejected vaccination. The frequency of parents who wanted to know more about the vaccines was 67.4%. The frequency of healthcare professionals stating that the decision of childhood vaccination should not be left to the family was 91.7%.

Conclusions: The vast majority of parents participating in our study were concerned that vaccines could have dangerous side effects. Parents needed more information about the vaccines. Healthcare professionals, on the other hand, were against leaving the decision to the parents in a childhood vaccination program and indicated a need for precautions against anti-vaccination.

Introduction

Vaccines are biological products used to stimulate the immune system for preventing infectious diseases. People are vaccinated for two reasons; to protect from the disease and to stop community spread. Vaccination is, therefore, one of the basic preventive health measures (1,2). Vaccinating a critical number of individuals in a community reduces the risk of outbreaks of infectious diseases and therefore protects other members of the community. Thanks to those who can be vaccinated, the protection of non-vaccinated sections of society is of philosophical value. The concept of "herd immunity" refers not only to epidemiological and technical aspects but also to some sort of social solidarity. Herd immunity is the highest indicator of social solidarity against the self-centred, individualistic, selfish, and neoliberal approach to health and well-being (2).

Although childhood immunization schedules are essential, the anti-vaccination movement is a serious threat to public health. The anti-vaccination movement is parallel to the history of vaccination. Compulsory vaccination policies introduced for community health care had exerted great efforts to counter the anti-vaccination movement. However, these days, parents are influenced by the growing reach of the anti-vaccination movement on social media and news media platforms, and therefore, doubt the effectiveness and safety of compulsory vaccination. Dr. Andrew Jeremy Wakefield, a gastroenterologist, published a study (1998) in the Lancet, postulating a connection between the measles-mumps-rubella vaccine and autism, which sparked controversy all over the world. Although his study had only 12 participants and a problematic methodology, it attracted global attention in the media, resulting in a growing number of

parents refusing to get their children vaccinated. After 12 years, Wakefield was found to use some fabricated data and act unethically. Therefore, his paper was retracted by the Lancet, and his medical license was revoked in 2010 (3).

Fifteen years ago, there were no known and recorded cases of anti-vaccination in Turkey. However, the anti-vaccination movement has gained momentum since 2010. While at first there was no more than a handful of parents refusing to get their children vaccinated, the number went up to 970 in 2015, and 23,000 in 2017, which is a growing health concern (4). Turkey is a migrant-receiving and refugee-hosting country. If the number of anti-vaccination people continues to increase at this rate, immunization rates will drop further, and preventable diseases will cause an outbreak, and there will be a rise in the incidence of diseases that we thought have disappeared, which is unfortunately confirmed by the World Health Organization. While the number of cases of measles reported in Turkey was more than eight thousand in 2013, it was only 9 (nine) in 2016, which, however, increased up to 69 in 2017, 716 in 2018, and 2890 in 2019 (5).

Vaccination is still the most effective and cheapest measure to prevent infectious diseases. Therefore, adhering to the national vaccination schedule is very critical for both personal and community health, especially in Turkey, which is a country of migration (6).

Our aim in this study was to determine numerical data on the views and experiences of parents and healthcare professionals (physicians, nurses, midwives, etc.) and to discuss the ethical dimension of vaccination in light of these data.

Methods

This was a descriptive, cross-sectional study. The study population consisted of all parents referred to the polyclinic and clinic of paediatrics at a university hospital in Ankara between 11 February and 22 March 2019, and all physicians, nurses, and midwives in the department of paediatrics and childhood vaccination. No sampling was performed. Participation in the study was voluntary. Participants were informed about the study and their consent was received. Ethics committee approval was obtained for the study. The study was approved by the Medical and Health Sciences Research Board (project no: KA 19/52) and funded by the Research Fund of Baskent University Ankara.

Data were collected using two questionnaires developed. The questionnaires were created by the authors. All of the questions in the questionnaire are presented in the tables, based on the researchers' experience and literature review (3,6-9). The questionnaire for parents consisted of 12 items on sociodemographic characteristics and anti-vaccination. Item 12 investigates whether parents agree with the portrayal of vaccination in the media. The questionnaire for healthcare professionals consisted of ten items on sociodemographic characteristics and anti-vaccination.

Statistical Analysis

Number (n) and percentage (%) were used for categorical data on anti-vaccination. Data were analysed using the Statistical Package for Social Sciences (IBM, version 25.0) at a significance level of 0.05.

Results

Evaluation of Parents

Eighty parents agreed to participate in the study. Of parents, 56.3% were women, 89.9% were married, 68.8% had a bachelor's degree, 57.5% had a single child, 28.7% had two children, and 13.8% had three children. Parents were engineers and architects (24.1%), teachers (13.9%), housewives (12.7%), self-employed (11.3%), civil servants (7.6%), lawyers (6.3%), physicians (6.3%), pharmacists (5.2%), nurses (1.3%), and others (11.3%). Seven participants (8.8%) self-identified as "anti-vaccination".

Five anti-vaccination parents (71.4%) stated that they made the decision as a couple not to get their children vaccinated. Consuming organic foods (41.7%) was stated to be the most common alternative to vaccination. Anti-vaccination parents (54.5%) mostly believed that vaccinated children were more likely to contract diseases than vaccinated ones. Five of the anti-vaccination parents (83.3%) stated that they had no reaction from others about their anti-vaccination (Table 1).

Thirty parents (37.5%) stated that they knew enough about vaccines. Forty-nine parents (37.7%) stated that they were informed by healthcare professionals. Fifty-four parents (67.4%) stated that they would like to know more about vaccines (Table 2).

Parents specified that they believed that vaccination was the most effective method for preventing infectious diseases (91.2%), that their decision not to vaccinate their own children could put other children at risk for infectious diseases (87.5%), and that refusing childhood vaccination was a threat to public health (85.0%) (Table 3).

Evaluation of Healthcare Professionals

Thirty-six healthcare professionals agreed to participate in the study. The mean age of healthcare professionals was 44 years. Of healthcare professionals, 72.2% were women, 47.2% were nurses, 25% were specialist physicians, 16.7% were assistant physicians, and 11.1% were midwives, 22.9% had 21-25 years of experience, 20% had 1-5 years of experience, 5.7% had 6-10 years of experience, 11.4% had 11-15 years of experience, 20% had 16-20 years of experience, and 20% had ≥25 years of experience.

Table 1. Information on parental anti-vaccination (n=7)		
	n	%
Decision to not vaccinate		
Decision as a couple	5	71.4
My decision	1	14.3
My spouse's decision	1	14.3
Alternative measures to prevent infectious diseases*		
Organic foods	5	41.7
Keeping children away from crowds and public spaces that might have lots of germs	4	33.3
Trusting in God	2	16.7
Keeping children away from vaccinated children	1	8.3
Reasons for anti-vaccination*		
Vaccinated children are more likely to contract diseases than unvaccinated ones	6	54.5
I believe that people are always forced to get vaccinated.	2	18.2
All vaccines cause autism.	1	9.1
All vaccines cause cancer.	1	9.1
Not all vaccines are halal.	1	9.1
Others' reaction to anti-vaccination (n=6)		
None	5	83.3
Negative	1	16.7
Positive	0	0.0
Healthcare professionals' reaction to anti-vaccination		
Negative	4	57.1
Positive	2	28.6
None	1	14.3
*More than one option marked		

Table 2. Parents' knowledge of childhood vaccines (n=80)		
	n	%
Parents' level of knowledge of vaccines		
Yes, I know enough about them.	30	37.5
No, I do not know enough about them.	23	28.7
I know a little about them.	27	33.8
Sources of information about vaccines*		
Healthcare professionals	49	37.7
Internet/social media	40	30.8
Friends/relatives	40	30.8
Other	1	0.7
Willingness to know more about vaccines		
Yes	54	67.4
No	13	16.3
Not sure	13	16.3
*More than one option marked		

According to the findings from healthcare professionals, in the institution where the study was conducted, information about vaccination was provided to families by physicians at a frequency of 88.9%. Only two healthcare professionals (5.6%) stated that they encountered anti-vaccination once or twice a month. According to healthcare professionals, the main reason for parental anti-vaccination was the belief that "vaccines contain mercury, aluminium, ether, antibiotics, and many other chemicals, which cause autism and similar diseases" (15.9%). Thirteen healthcare professionals (nurses and midwives) stated that they referred anti-vaccination parents to physicians while nine stated that they found out why and tried to correct their misconceptions (Table 4).

Discussion

In this study, seven parents (8.8%) refused to get their children vaccinated. Türkay et al. (7) reported that 6.2% of parents self-identified as "anti-vaxxers." This finding is similar to our study.

Most parents (71.4%) stated that they and their spouses agreed not to get their children vaccinated, which has been reported by Çapanoğlu (3) as well. Erdem et al. (8) conducted a study on oral polio anti-vaccination and reported that it was mostly mothers (55.3%) who chose not to have their children vaccinated.

According to Türkay et al. (7), self-identified anti-vaxxers do not trust vaccine companies (2 people, 6.4%) and believe that vaccines are useless (6 people 19.4%), have side effects (20 people, 65%), contain harmful substances (8 people, 25.8%), and cause infertility (1 person, 3.2%). Our result is similar because the participating parents stated that they believed that vaccinated children were more likely to contract diseases than unvaccinated ones and that vaccines caused autism and cancer, hinting that the reason for their anti-vaccination is mostly related to the possible side effects of vaccines.

The participating anti-vaccination parents stated that they fed their children with organic foods, kept them away from crowds and dirty public spaces and other vaccinated children, and trusted in God. Studies in Australia show that anti-vaccination people use complementary and alternative medicine therapies more because they believe that they are natural, non-chemical, and reliable methods with no side effects (10). Hazır (9) reported that anti-vaccination parents believed in fate (8.7%), kept their children away from crowds and patients (8.7%), and fed them with organic and healthy food (25%) or did nothing (57.6%) to protect their children.

Thirty parents (37.5%) stated that they knew enough about vaccination while 54 parents (67.4%) stated that they would like to learn more about it. In another study, 78.3% of parents stated that they knew enough about vaccination; however, 48.7% stated that they would like to learn more about it (9). The sources of information on vaccines are healthcare professionals

Table 3. Parents' agreement with the portrayal of vaccination in the media (n=80)

Positive and negative discourses in the media concerning vaccination	Agree	Disagree
	n (%)	n (%)
I think that vaccination is the most effective method of preventing infectious diseases	73 (91.2)	7 (8.8)
I think that my decision not to vaccinate my own kid can also put other children at risk for infectious diseases.	70 (87.5)	10 (12.5)
I think that refusing childhood vaccination is a threat to public health.	68 (85.0)	12 (15.0)
Vaccine-preventable diseases are no longer a public health issue in Turkey, and therefore, I think that vaccines are unnecessary.	10 (12.5)	70 (87.5)
I think that natural infection causes better immunity than vaccines.	16 (20.0)	64 (80.0)
Good hygiene can help eliminate diseases, and therefore, I think that vaccines are unnecessary.	18 (22.5)	62 (77.5)
I think that children are vaccinated too young.	17 (21.3)	63 (78.7)
I think that babies should be breastfed rather than being vaccinated until two years of age.	18 (22.5)	62 (77.5)
Giving a baby too many vaccines (numerous and different types of antigens) disrupts its immune system and causes diseases.	20 (25.0)	60 (75.0)
I think that the primary goal of childhood vaccination companies is to make money.	16 (20.0)	64 (80.0)
I think that vaccines have many side-effects that are covered up by companies.	17 (21.3)	63 (78.7)
I think that most of the infected during an outbreak are those who have been vaccinated.	8 (10.0)	72 (90.0)
I think that vaccines have some damaging and long-term side effects that are not yet known.	19 (23.8)	61 (76.2)
I think that vaccines are unsafe and may have dangerous side effects.	16 (20.0)	64 (80.0)
I think that the preservatives in vaccines may harm children.	13 (16.3)	67 (83.7)
I think that vaccines may cause autism.	11 (13.8)	69 (86.3)
I think that vaccines may be to some extent responsible for the rise in cancer cases around the world.	7 (8.8)	73 (91.2)
I think that multiple vaccines increase the risk of side effects in children and overload their immune system.	24 (30.0)	56 (70.0)
I think that repeating vaccination more than once is unnecessary, and therefore, rapel doses are unnecessary.	15 (18.8)	65 (81.3)
Vaccine-preventable childhood diseases are an unfortunate reality of life, and therefore, I do not think that it is possible to escape this reality with vaccination.	10 (12.5)	70 (87.5)
Many unvaccinated people led long and healthy lives. Therefore, I do not think that vaccination is necessary.	8 (10.0)	72 (90.0)
I think that vaccine administrators' attitudes and approaches have a negative effect on people's willingness to get vaccinated.	13 (16.3)	67 (83.7)

(37.7%), Internet/social media (30.8%), and friends/relatives (30.8%). İncili (11) also reported that the sources of information about vaccines were doctors (82.6%), television, radio, and newspaper (11.6%), the Internet (1.9%), and neighbors (3.9%). The fact that most parents receive information from healthcare professionals about vaccination is a positive outcome. More than half (55%) of adults in the United States learn about health online. In a study on seven Internet search engines, more than 43% of the top 10 sites were revealed to contain antivaccine information (12). According to Türkay et al. (7), parents get information about vaccinations from TV (49.3%), the Internet (44.2%), and physicians (41%).

The statements "giving a baby too many vaccines (numerous and different types of antigens) disrupts its immune system and causes diseases," "I think that vaccines have some damaging and long-term side effects that are not yet known," and "I think that vaccines have some damaging and long-term side effects that are not yet known" are a sign of parents' anti-vaccination and fear about vaccinations. Despite these statements, the rate of anti-vaccination (8.8%) was low among our participants, suggesting that parents are mostly aware of their responsibility for herd immunization and solidarity, despite anti-vaccination and fear.

In the institution where this study was conducted, parents are mostly informed about vaccines by physicians (88.9%) in detail (35.4%). However, fifteen healthcare professionals (31.2%) stated that they did not do any explaining about vaccines unless parents asked for it and on the assumption that parents already knew about them (6.3%), which is not very good, because anti-vaccination is often discussed in the media and the public arena. This is supported by Çapanoğlu (3), who reported that healthcare professionals indulged in some self-criticism and

Table 4. Frequency distributions of healthcare professionals' results (n=36)		
	n	%
Getting their own children vaccinated		
I have a child, and she has all her vaccinations.	25	69.4
No, I don't have a child.	11	30.6
I have a child but I have not had her vaccinated.	-	-
I had to have my child vaccinated, even though I didn't want to.	-	-
By whom are parents informed about vaccination in the institution?		
Physicians	32	88.9
Nurses	4	11.1
Midwives	-	
Other	-	
How are parents informed about vaccination in the institution?*		
We explain to them in detail the vaccines and what diseases they prevent in children.	17	35.4
We do not do any explaining unless parents ask for it.	15	31.2
We hand out leaflets with answers to possible questions about vaccination.	7	14.6
We do not provide any detailed explanation but inform parents about the immunization schedule to hint that they should vaccinate their children.	6	12.5
Parents usually know about vaccination and so we don't provide much explanation.	3	6.3
Prevalence of anti-vaccination		
1-2 times a month	2	5.6
No answer	34	94.4
Is it wise to leave vaccination decisions up to parents?		
No, it is not.	33	91.7
Yes, it is.	3	8.3
Factors affecting parents' vaccine acceptance positively or negatively, according to healthcare professionals*		
Communication and media	22	11.1
Parents' sociodemographic characteristics (age, education, etc.)	21	10.6
The belief that breastfeeding and conventional methods are more useful than vaccination	20	10.1
Lack of knowledge	20	10.1
Concern about the side effects of vaccines	20	10.1
Influential people and pro-vaccination/anti-vaccination lobbies	15	7.5
The belief that vaccines have been developed by the pharmaceutical industry to make a fortune	15	7.5
Trust/lack of trust in the health system	13	6.5
Concern about the possible long-term damages of chemicals in vaccines	13	6.5
The belief that natural infection causes better immunity than vaccines	12	6.0
Experience with vaccines (side effects etc.)	10	5.0
Policies/laws	9	4.5
Complexity and incomprehensibility of the national immunization schedule	3	1.5
The belief that new vaccines are under-tested	3	1.5
The role of health professionals (missing or inaccurate information concerning vaccines)	3	1.5
Reasons for anti-vaccination stated by parents to healthcare professionals*		
Vaccines contain mercury, aluminum, ether, antibiotics, and many other chemicals, which cause autism and similar diseases.	23	15.9
Vaccines cause infertility.	19	13.1
Vaccines contain pork gelatin and are therefore not halal.	17	11.7
Some "prominent religious figures", "thinkers", and "doctors" claim that vaccines are harmful and do not have their children vaccinated.	16	11.1

Table 4. Continued		
Diphtheria, pertussis, tetanus, and polio vaccines cause sudden infant death syndrome.	14	9.7
Natural infection causes better immunity than vaccines.	9	6.2
Vaccines may have severe side effects that are yet unknown but will appear in the future.	8	5.5
Delivering a child more than one antigen at a time may damage her immune system and increases the risk of immune disorders.	8	5.5
Complementary and alternative medicine is more effective and has less side effects.	8	5.5
It is unwise to vaccinate children to protect them from pathogens that are not currently in Turkey.	7	4.8
Vaccinated patients are at greater risk of contracting an infection.	6	4.1
Vaccination is forced because of the greed of pharmaceutical companies.	6	4.1
In childhood, the immune system is not yet fully developed, and vaccines harm it.	4	2.8
How do healthcare professionals react to anti-vaccination?*		
I refer them to physicians (a valid option for nurses and midwives)	13	32.5
I find out why and I correct their misconceptions, if any.	9	22.5
I try to persuade them to have their children vaccinated.	8	20.0
It is their decision, and I respect that	5	12.5
I refer them to the department of infection.	4	10.0
There can be no explanation for that. I would react to them because they put both their children and others at risk.	1	2.5
*More than one option marked		

took responsibility for anti-vaccination as partly their failure to provide information to parents.

According to the participating healthcare professionals, the factors affecting parents' vaccine acceptance are communication and media tools, sociodemographic characteristics, use of conventional methods instead of vaccination, ignorance, and concern about vaccine-related side effects. Çapanoğlu (3) has also reported that according to healthcare professionals' experience and observation, parental anti-vaccination depends on sociodemographic characteristics (being a young inexperienced mother, etc.), concern about vaccines (the belief that vaccines cause infertility and autism), religion, and social media.

The participating physicians stated that they corrected the misconceptions held by anti-vaccination parents about vaccinations and tried to persuade them to have their children vaccinated, whereas nurses and midwives stated that they referred those parents to physicians. Five healthcare professionals (12.5%) stated that they respected parents' decision not to vaccinate their children. Healthcare professionals (65.2%) stated that they would advise anti-vaccination parents while 14.8% stated that they would not (13).

The great majority of our participating healthcare professionals (91.7%) were against leaving childhood vaccination decisions to parents. Arıcan (13) also reported that 93.6% of healthcare professionals were for compulsory vaccination according to the national immunization schedule. This indicates that healthcare professionals are aware that vaccination is an important and effective way for infectious disease prevention.

The study has some limitations. First, it was conducted in only one university hospital, and state-run hospitals and other university hospitals were not included. Second, the results are sample-specific and not generalizable to the whole population.

Ethical Assessment and Conclusion

Based on certain unfounded doubts and beliefs (vaccines cause autism and cancer; breastfeeding is better than vaccination, etc.), the anti-vaccination movement argues that vaccine regulations infringe upon individual autonomy and liberty. It is, of course, an important ethical value that we should respect the right of people to make choices of their own free will. However, what is generally overlooked is that limiting one's actions that harm other individuals is not the same as curtailing one's freedom (2). It should always be kept in mind that a person who causes harm, whether by acting or failing to act, is held responsible for that harm either for acting or for failing to act (14).

Given all of the above, the basic arguments of vaccination in terms of ethical values are

- Both individual autonomy and social utility should be protected,

- We should always keep in mind that vaccination is vital for the protection of children's right to life (15).

Instead of telling anti-vaccination about what they already know, we should raise their awareness of the behaviors they already exhibit for public health and get across to them that childhood vaccination is a vital public intervention that plays a

key role in controlling and eliminating infectious diseases and in protecting public health (16).

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Ethics

Ethics Committee Approval: The study was approved by the Medical and Health Sciences Research Board (project no: KA 19/52) and funded by the Research Fund of Baskent University Ankara.

Informed Consent: Participants were informed about the study and their consent was received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.D.K., Design: S.D.K., H.Y.Z., Data Collection or Processing: S.D.K., H.Y.Z., Analysis or Interpretation: S.D.K., H.Y.Z., Literature Search: S.D.K., Writing: S.D.K., H.Y.Z.

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Serum vitamin D levels in patients with oral lichen planus: A systematic review and meta-analysis

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ABSTRACT

Aims: Vitamin D is an indispensable vitamin which is actively engaged in immune upregulation. Several studies have been conducted to explore the association between vitamin D and oral lichen planus (OLP), but the results are still inconclusive. This meta-analysis was performed to understand the association of serum vitamin D levels in OLP.

Methods: A search was conducted for observational studies that inspected the association between vitamin D and OLP from inception to June 2020 on scientific databases in accordance with PRISMA guidelines. The Newcastle-Ottawa Scale was used to assess the quality of the studies. Statistical analysis of the meta-analysis was performed with Review Manager version 5.4.

Results: Five case-control studies met the selection criteria. The number of OLP cases ranged from 18 to 102. Finally, 3 case-control studies were eligible for random-effects meta analyses of the mean difference in serum 25-hydroxyvitamin D concentrations between OLP cases and controls using inverse-variance method. The pooled mean difference in random-effects meta-analysis was 5.98 and this difefrence did not reach statistical significance.

Conclusions: The current meta-analysis showed numerically lower vitamin D level in patients with OLP, which was statistically non-significant.

Introduction

Erasmus Wilson was the first person to describe lichen planus in the year 1869. It is an autoimmune disease of the chronic type that affects the skin and mucous membranes. It can affect the skin, nails, hair, oral and genital mucosa (1). Oral lichen planus (OLP) has a prevalence of 0.5 to 2.2% in the general population and affects both women and men in the ratio of 3:2. At the time of diagnosis, the mean age is 55 years (2). World Health Organization has named OLP as a "Potentially Malignant Disorder" with an unspecified risk of malignant transformation (3). Some studies have reported the malignant transformation rate of OLP as 0-12.5% (4). The etiology of OLP is multifactorial and many triggers for the disease have been identified so far: (a) cell mediated hypersensitivity, (b) autoimmune response to epithelial antigens, (c) stress, (d) viral infection (5). However, the role of the immune system in the etiopathogenesis of OLP is postulated by its histological features. The sub epithelial band of infiltration dominated by T-lymphocytes and macrophages and the liquefaction degeneration of the basal cells emphasize the role of the cell mediated arm of the immune system in the development of OLP. This is achieved by the cell mediated

cytotoxity directed against antigens expressed by the basal cell layer (2).

Vitamin D is a secosteroid that aids in metabolism of calcium and phosphorus. The role of vitamin D, i.e. 25-hydroxyvitamin D [25(OH)D], in the regulation of the immune system has been brought to light in recent literature. 25(OH)D inhibits the proliferation and differentiation of B cells and immunoglobulin secretion (6). In the T cells, it suppresses proliferation and maturation. There is a shift from Th1 to Th2 phenotype (7). This leads to a decrease in inflammatory cytokines such as interleukin (IL)-17 and IL-21 and increase in the production of anti-inflammatory cytokines such as IL-10. 25(OH)D inhibits the monocyte production from inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and tumor necrosis factor-alpha (TNF-α) (8). It also inhibits dendritic cell differentiation and maturation (9,10).

Multiple researches show that patients with autoimmune diseases like Behçet's diseases, rheumatoid arthritis, diabetes mellitus, systemic lupus erythematosus (SLE), inflammatory bowel disease and multiple sclerosis have decreased serum 25(OH)D levels compared to normal individuals. Faezi et al. (11) found that deficiency of 25(OH)D was more in patients with Behçet's disease compared to that of controls. Fakharan et al. (12) found that serum 25(OH)D levels inversely correlated with the activity of rheumatoid arthritis. Ibrahim et al. (13) conducted a study among multiple sclerosis patients and concluded that hypovitaminosis D was common among these patients. Schoindre et al. (14) found decreased levels of 25(OH)D in patients with SLE and a relationship between decreased 25(OH) D levels and increased disease process (13). Hypovitaminosis D has not yet been recognized as a biomarker of OLP. The purpose of this systematic review was to analyze and quantitatively gather evidence associated with lower levels of serum 25(OH)D concentration and OLP patients.

Methods

Search Strategy

Search was conducted in databases like PubMed, Google Scholar, Cochrane library, Wiley online library and LILACS for relevant studies published till June 2020. The keywords used were 'oral lichen planus' or 'OLP' combined with 'Vitamin D' or 'Vit D' or '25(OH)D'. References of the relevant articles were searched to avoid missing any literature. Two researchers conducted the searches independently to identify quality articles. In case of disagreement, the third author decided whether the article was included.

Selection Criteria

Articles were included in this systematic review by going through the titles, abstracts and further reviewing the full text of the articles. The PRISMA flowchart of the selection process is depicted in Figure 1. The selected studies met the following inclusion criteria: 1) cohort, case-control or cross-sectional studies; 2) studies investigating the relationship between vitamin D and OLP; 3) studies of good quality; 4) studies published in English. The exclusion criteria for the studies included 1) case reports, reviews and editorials; 2) cells or animal studies; 3) serum level of vitamin D estimated using indicators other than 25(OH)D; 4) Incomplete data. Of the 663 identified abstracts, 23 met the initial inclusion criteria. On the assessment of the full article, 17 were excluded because the outcome was not OLP and included cutaneous lichen planus and lichenoid reactions. The remaining 5 studies were included in this study (15-19).

Data Collection

The following data were extracted (Table 1): First author, publication year, study design, sample size, mean age, criteria for diagnosis of OLP, method of vitamin D estimation and serum vitamin D levels. Two authors extracted data independently, which were then reviewed by the third author. If the articles did not provide sufficient information, we tried our best to contact the authors. Since information only from existing published literature was included, no ethical committee approval or informed patient consent was acquired.

Quality Evaluation

Quality of the studies was evaluated using Newcastle-Ottawa scale (NOS) (20). It consisted of three parts: selection (0-4), comparability (0-2) and exposure/outcome (0-3). NOS scores of 1-3, 4-6 and 7-9 indicated the studies of low, medium and high quality. In this analysis, we used studies with NOS score ≥6, which were of medium methodological quality (Table 2). Any disagreement among the researchers was resolved by discussion to reach a final consensus.

Statistical Analysis

Since the measurement of serum 25(OH)D is a continuous datum, the mean difference and 95% confidence interval were calculated. Heterogeneity was calculated with chi-square and I^2 test (21). I^2 > 50% and p value <0.01 indicated substantial heterogeneity and random effects model was chosen in this circumstance. All the statistical analysis of this meta-analysis was carried out using Review Manager 5.4 (The Nordic Cochrane Centre, Copenhagen, Denmark). Results were presented as a forest plot (Figure 2).

Results

Included five studies were judged as medium quality in accordance with the NOS. Table 1 summarizes the studies included in this review (15-19). The studies were from the Northern hemisphere countries. Data collection was based on case-control studies. The number of cases ranged from 18 (17) to 102 (18) and the studies consisted of both men and women, with a women predominance. The mean age of cases ranged from 37 (15) to 51 (18) years.

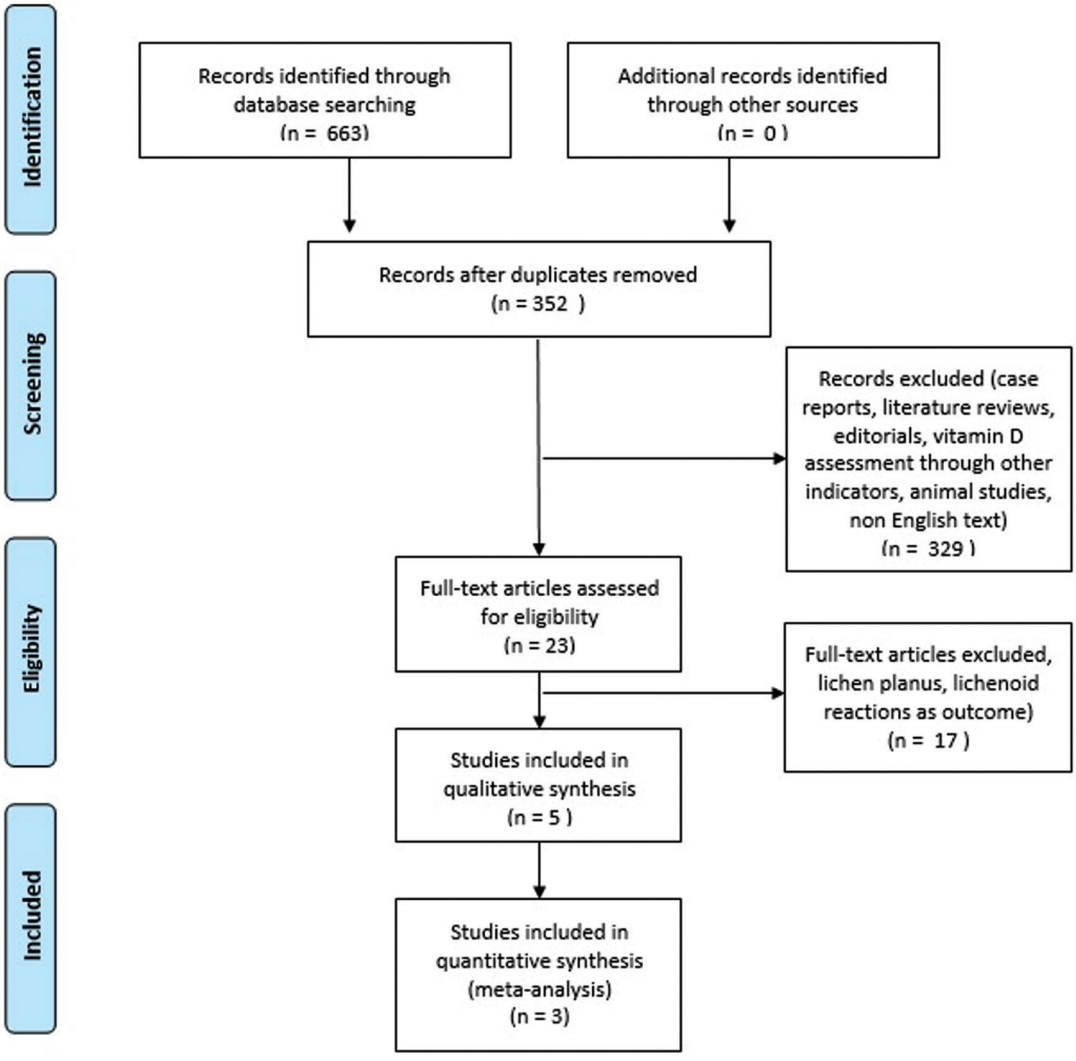

Figure 1. Prisma flow chart

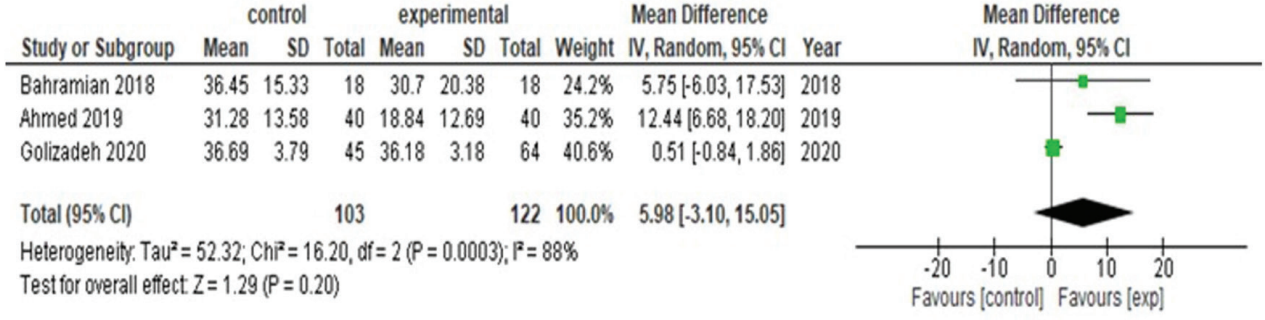

Figure 2. Meta-analysis of the included studies. Forest plot showing serum vitamin D levels in patients with oral lichen planus and controls. Box area is proportional to the sample size of each study and the horizontal line corresponds to the 95% confidence interval. Black diamond corresponds to the summary value

All the studies measured the serum concentration of 25(OH) D as a continuous variable. Different techniques were used to estimate the 25(OH)D concentration and the most commonly used one was electro chemiluminescence technique (16-18).

The diagnosis of OLP in most studies was based on clinical views/clinicopathological criteria (15,17,18). Two studies used the WHO criteria for diagnosis of OLP and oral lichenoid lesions (16,19). In all studies, controls were healthy individuals from the hospital population.

Table 1. Summary of included studies

Reference	Design	Location	Participant categorization		Vitamin D measurement method	Result
			Case	Control		
Gupta et al. (15) 2017	Case-control study	Uttar Pradesh, India (28.9° N)	102 cases - 36 males; 66 females - Based on clinical views - Mean age 37.12 y	102 age and gender matched healthy volunteers from patient population	Enzyme linked fluorescent assay (Vitek Immunodiagnostic Assay System - bioMérieux, Marcy-l'Étoile, France)	Mean of 25(OH)D in: - Cases 20.40 ng/mL - Control 32.67 ng/mL p=0.000
Tak et and Chalkoo (16) 2017	Case-control study	Jammu & Kashmir, India (34.0° N)	20 cases - Based on modified WHO diagnostic criteria of OLP and OLL (2003)	20 age and gender matched healthy controls from patient population	Electro-chemiluminescence immune assay	Cases <control p=0.0285
Bahramian et al. (17) 2018	Case-control study	Tabriz, Iran (38.0° N)	18 cases - Based on clinical views/ clinicopathological criteria - Mean age 44.16 y (range 30-71)	18 age and gender matched healthy controls - Mean age 49.9 y (range 21-76)	Electro-chemiluminescence technique	Mean±SD of 25(OH)D in: - Cases 30.7±20.38 - Controls 36.45±15.33 p=0.346
Ahmed (18) 2019	Case-control study	Erbil, Iraq (36.1° N)	40 cases - Based on biopsy and clinical or clinical pathological criteria - 9 males; 31 females - Mean age 51.7±10.8	40 age and gender matched healthy controls - 11 males; 29 females - Mean age 49.2±11.2	Electro-chemiluminescence technique	Mean±SD Of 25OHD in: - Cases 18.84±12.69 - Controls 31.28±13.58 p<0.001
Gholizadeh et al. (19) 2020	Case-control study	Tehran, Iran (35.6° N)	64 cases - Based on WHO criteria	45 age and gender matched healthy controls	ELISA (Padtan Gostar Isar Co, Tehran, Iran)	Mean±SD of 25(OH)D in: - Cases 36.18±3.18 - Controls 36.69±3.79 p=0.267

25(OH)D: 25-hydroxyvitamin D, OLP: Oral lichen planus, WHO: World Health Organization, OLL: Oral lichenoid lesions, SD: Standard deviation, ELISA: Enzyme linked immunosorbent assay

Table 2. Quality assessment of included studies using Newcastle-Ottawa scale

Reference	Selection	Comparability	Exposure/Outcome	Total
Gupta et al. (15)	3	1	2	6
Tak and Chalkoo (16)	3	1	2	6
Bahramian et al. (17)	3	1	2	6
Ahmed (18)	3	1	2	6
Gholizadeh et al. (19)	3	1	2	6

Three of five studies found significant differences between the groups illustrated by lower levels of serum 25(OH)D in OLP cases compared to controls (15,16,18). Most of the studies also showed that 25(OH)D insufficiency was more common in the controls compared to the OLP cases (15,18).

All included studies showed that females were more affected compared with males which is in accordance with previously reported literature. In the study conducted by Ahmed (18), they inferred that reticular lichen planus was more widely prevalent followed by erosive and atrophic types. They also saw that the bilateral buccal mucosa was the most commonly affected site. Gholizadeh et al. (19) assessed the salivary flow rate in patients with OLP and found no significant association between salivary flow rate and levels of 25(OH)D in the serum and saliva.

However, they observed a negative correlation between serum and saliva 25(OH)D levels and pain severity and score.

The meta-analysis was performed on 3 studies with a total of 122 cases and 103 controls (17-19). Two of the studies could not be included in the meta-analysis because the exact serum concentration of 25(OH)D with standard deviation was not provided (15,16). The obtained mean difference ranged from 0.51 to 5.75, on a scale where 0 corresponds to no difference between OLP cases and controls, and positive mean difference indicated that the OLP cases had lower vitamin D levels than controls. In two of the three studies, the CI lower limits were negative (17,21). The summary random mean difference of 5.98 indicated that serum vitamin D concentrations were overall 5.98 SD lower in OLP cases compared to controls. However, the summary value of mean difference crosses the zero line and reaches the negative scale, which indicates that this difference in the concentrations of serum 25(OH)D in OLP patients and controls holds no statistical significance.

Discussion

This study aimed to improve awareness regarding the potential role of vitamin D in the disease process of OLP and open numerous venues of future research in the same way. Very few studies which investigated vitamin D in OLP were identified in the existing literature. All included studies were cross sectional in nature.

Deficiency of vitamin D has become widespread in the general population. This may be due to various reasons like decreased intake in diet or decreased absorption, inadequate endogenous synthesis, insufficient sun exposure due to decreased outdoor activity, conservative clothing, increased use of sunscreens and intake of medications that increase metabolism of vitamin D (22). Hence researchers have been keen on investigating the link between decrease in vitamin D levels and various disease processes.

Past two decades of researches have established that 25(OH)D regulates the immune response mechanism, other than calcium homeostasis and bone metabolism. This was brought to light by the discovery that 25(OH)D could affect the production of ILs. These ILs belong to a group of cytokines, which facilitate the interaction between immune and inflammatory cells by influencing cell growth, differentiation and activation. ILs are synthesized in various cells like leukocytes, keratinocytes, trophoblasts, adipocytes, endothelial cells and other cell variants involved in specific organ related diseases. 25(OH)D has been shown to strongly influence the expression of cytokines like ILs, TNF-α, interferon-gamma (IFN-γ), growth factors toll-like receptors, C-reactive protein and enzymes like cyclo-oxygenase, 5-lipoxygenase which generate inflammatory mediators (23). These components cause apoptosis of the keratinocytes which leads to OLP. 25(OH)D induces the expression of vitamin D receptors (VDR) in the epithelial cells (24).

Many studies have studied the influence of VDR on immune cells and cascades that promote inflammation. Du et al. (24,25) and Zhao et al. (26) showed that vitamin D/VDR signaling reduces apoptosis of epithelial cells by suppressing LPS induced p53 upregulated modulator of apoptosis (PUMA) via nuclear factor-kappa B (NF-κB) pathway blockade. Zhao et al. (27), in 2019, mentioned that vitamin D/VDR signaling also suppressed LPS induced hypoxia inducible factor-1α via NF-κB pathway blockade. This reduces the production of IFN-γ and IL-1β. Ge et al. (28), in 2019, found that vitamin D/VDR signaling inhibited miR-802 expression, which causes cell apoptosis, via NF-κB pathway blockade.

In 2020, it was found that microRNA 26a/b (29) and microRNA 27a/b (30) were significantly decreased in the saliva, serum and tissue samples of patients with OLP. They identified that these microRNA had specific sites for binding VDR in their promoter region and that vitamin D/VDR signaling induced the expression of these microRNA in OLP patients. These microRNAs thereby play a protective role by inhibiting apoptosis and reducing proinflammatory cytokines. These findings show that 25(OH) D plays a vital role in inhibiting keratinocyte apoptosis which takes place in OLP and deficiency states of vitamin D can lead to increased possibility of OLP. This is substantiated by vitamin D supplementation improvement in OLP cases. Razi et al. (31) observed that premenopausal women with OLP, who were given supplements of vitamin D along with routine treatment, showed clinical improvement within the first four weeks compared to women who received routine treatment and showed improvement much later. Gupta et al. (32) found significant symptomatic improvement in patients who were given vitamin D supplementation along with topical steroid application and psychiatric consultation compared to the group of patients who did not receive vitamin D supplementation.

This systematic review showed a definite association between 25(OH)D levels and the occurence and severity of OLP. The meta-analysis also showed no statistical significance but this could be attributed to the various previously discussed factors.

Females were more affected compared with males in the current study, which is in accordance with previously reported literature. In the study conducted by Ahmed (18), they inferred that reticular lichen planus was more widely prevalent followed by erosive and atrophic types. They also observed that the bilateral buccal mucosa was the most commonly affected site. Gholizadeh et al. (19) assessed the salivary flow rate in patients with OLP and found no significant association between salivary flow rate and levels of 25(OH)D in the serum and saliva. However, they observed a negative correlation between serum and saliva 25(OH)D levels and pain severity and score.

Conclusion

Although numerous treatment modalities are available for OLP, a definitive treatment option is yet to be identified. The association of vitamin D with various autoimmune diseases and the improvement of such diseases with its supplementation has been documented in literature. The analysis we conducted has shown lower vitamin D level in patients with OLP, although statistically non-significant. Hence well-designed prospective studies with large sample sizes need to be performed. Longitudinal studies comparing variations in the serum vitamin D levels and severity of the disease and trials of supplementation with vitamin D in OLP patients with deficiency to observe improvement need to be conducted. These studies will aid in establishing the role of vitamin D in the disease process of OLP. These results reinforce the conceptualization of vitamin D as a potential biomarker and a definitive treatment modality for the enigma, which is OLP.

Ethics

Ethics Committee Approval: Since information only from existing published literature was included no ethical committee approval was acquired.

Informed Consent: Since information only from existing published literature was included no informed patient consent was acquired.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Potential of epigenetic biomarker O6-methylguanine-DNA methyltransferase gene in glioma

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ABSTRACT

Aims: Glioblastomas are the most malignant gliomas in adults with the median survival of 15 months only. O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme which overcomes the alkylating chemotherapy effect resulting in chemo-resistance. Methylation at the *MGMT* gene promoter reduces the gene expression and enhances chemo-sensitivity in cancer treatments. Therefore, this study aimed to screen *MGMT* methylation status for a potential epigenetic biomarker in glioma detection and treatmentin glioma patients at Hospital Universiti Sains Malaysia.

Methods: Forty-one glioma paraffin-embedded glioma tissue samples consisting of grade 2 (n=11), 3 (n=10) and 4 (n=20) were analyzed in this retrospective study. The extracted DNA was subjected to bisulfite treatment and the methylation status was determined via methylation-specific polymerase chain reaction targeting the *MGMT* gene promoter.

Results: It was observed that 92.7% of the glioma samples showed methylated and 7.3% unmethylated MGMT promoter. All grade 2 and grade 3 gliomas showed methylation, compared to 85% of grade 4 (p=0.183). More older glioma patients (>40 years) had methylation compared to younger patients (≤40 years) (95.8% vs 88.2%) (p=0.357). More males had methylation compared to females (96% vs 87.5%) (p=0.308).

Conclusions: *MGMT* promoter methylation was found predominant in older (>40 years) male patients with grade 2 and 3 gliomas. High percentages of gliomas, 92.7% harboring methylated *MGMT* promoter, indicate that it is a potential epigenetic biomarker for glioma detection.

Introduction

Glioma is a type of brain tumor that begins in glial cells. It is the most predominant type primary brain tumor which can be found in adults, constituting 30% to 40% of all intracranial tumors, and its utmost prevalence is between the ages of 40 and 65 years (1). In Malaysia, the annual incidence of central nervous system tumors was predicted at 2.8 per 100,000 people by Globocan 2012 (2). Grade 1 tumors are normally benign and can be treated through complete surgical excision. The median survival is 57 months for grade 2 astrocytoma and 24 months for grade 3 astrocytoma (3). About less than 5% of the grade 4

glioblastoma multiforme (GBM) patients were able to live for 5 years after diagnosis (4).

O6-methylguanine methyltransferase (*MGMT*) gene is located at the chromosome 10q26 encoding 207 amino acids that reverse the alkylation effect at the O6 position of guanine (5). Alkylating agents induce methylation at guanine that forms mismatch with thymine which subsequently leads to futile mismatch repair cycle, single-strand DNA breakage and cancer cell apoptosis (6). Removal of O6-methylguanine DNA adduct by the MGMT enzyme prevents the mismatch repair cycle and cancer cell death. Reduction of MGMT protein was able to increase chemosensitivity in high-grade gliomas (7). Another similar study reported that xeroderma pigmentosum fibroblasts transfected with DNA repair protein O6-alkylguanine-DNA alkyltransferase (ATase) were able to rescue the cells from temozolomide (TMZ)-induced damage and lead to resistance (8). Methylation at the *MGMT* promoter region spanning 1.2 kb results in *MGMT* gene silencing (6).

TMZ is an alkylating agent used to treat newly diagnosed and recurrent GBMs. Even with the current advance treatments, patients with stage 4 glioma, GBM, who completed the treatments, have 90% of recurrence rate and recurrent GBMs showed resistance towards previous treatments regimes (9). *MGMT* promoter methylation showed better survival rate in GBM patients with TMZ drug and radiotherapy as compared to radiotherapy only (21.7 vs 15.3 months, p value=0.007) (10). *MGMT* methylated GBM patients were associated with better median progression-free survival and overall survival as compared to *MGMT* unmethylated GBM patients [(8.7 vs 5.7 months, p value <0.0001) and (21.2 vs 14 months, p value <0.0001), respectively] (11).

Failure to identify the specific molecular identity of tumor can result in ineffective treatment and may worsen the prognosis (12). Response to the treatments of glioma patients is mostly dependent on the molecular characteristic of the tumors (13). Although *MGMT* promoter methylation may hold valuable diagnostic and prognostic power in gliomas, the screening test of *MGMT* promoter in gliomas is yet to be introduced in clinical setting due to lack of *MGMT* methylation data in gliomas. Therefore, we sought to determine the *MGMT* promoter methylation status of glioma patients in Hospital Universiti Sains Malaysia, as a potential epigenetic biomarker for diagnostic and treatment stratification to improve the treatment efficacy of current gliomas.

Methods

Tumor Samples

Forty-one archived formalin-fixed paraffin-embedded (FFPE) glioma samples consisting of grade 2 (n=11), grade 3 (n=10) and grade 4 (n=20) were collected from Pathology Department, Hospital Universiti Sains Malaysia. This study is a retrospective study using archived paraffin embedded tissue whereby the protocols received approval by the Human Research Ethics Committee of Universiti Sains Malaysia (JePeM) (ref. no. USM/JEPeM/17050255). Prior to DNA extraction, a neuropathologist reviewed the glioma samples to confirm the tumor types and gradings based on the 2016 World Health Organization criteria (14).

DNA Extraction

Two slices of FFPE glioma blocks, with 5 µm thickness each, were used to extract genomic DNA using GeneJET FFPE DNA Purification kit (Thermo Scientific, USA) according to the manufacturer's instructions. The DNA concentration and purity were determined using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) and kept at -20 °C.

Bisulfite DNA Treatment

Commercially available methylated and unmethylated DNA were used as positive controls in the methylation-specific polymerase chain reaction (PCR) (MSP). CpG Methylated HeLa Genomic DNA (New England Biolab, New England) and 5-Aza-dc Treated Jurkat Genomic DNA (New England Biolab, New England) were used as methylated and unmethylated positive controls, respectively. All extracted DNA and controls were subjected to bisulfite treatment using EpiMark Bisulfite Conversion Kit (New England Biolab, New England) according to the manufacturer's instructions. Approximately 10 µL of 100 ng DNA was mixed with 130 µL of bisulfite mixture and subjected to PCR. Thermocycling conditions were 95 °C for 30 seconds, followed by 40 cycles of 95 °C for 15 seconds, annealing for 30 seconds at 50 °C and extension at 68 °C for 1 minute, and finally 68 °C for 5 minutes. This treatment converted unmethylated cytosine nucleotide (C) to uracil (U) whereas methylated cytosine nucleotide (5-mC) remained unchanged.

Methylation-specific PCR

All bisulfite-treated DNAs were subjected to MSP using EpiMark HotStart Taq DNA polymerase (New England Biolab, New England) to determine the methylation status at the *MGMT* promoter. Every treated DNA samples was subjected to two different sets of primers, methylated and unmethylated primers to detect the presence of methylated and unmethylated at the *MGMT* promoter region (15). Methylated primer sequences, forward: 5'- TTTCGACGTTCGTAGGTTTTCGC-3' and reverse: 5'-GCACTCTTCCGAAAACGAAACG-3' amplifies 81 bp PCR amplicon whereas unmethylated primer sequences, forward: 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' and reverse: 5'-AACTCCACACTCTTCCAAAAACAAAACA-3' amplifies 93 bp PCR amplicon respectively.

PCR amplification was performed in a total of 25 µL reaction containing 100 ng bisulfite-treated DNA, 2.5 µL of 10X EpiMark

HotStart Taq Reaction Buffer, 2 µL of 25 mM $MgCl_2$, 0.5µL of 10 mM dNTP each, 0.5 µL of 10 µM forward and reverse primers and 0.5 µL of EpiMark HotStart Taq DNA Polymerase enzyme. Thermocycling conditions were 95 °C for 5 minutes, followed by 45 cycles of 95 °C for 30 seconds, annealing for 1 minute at 60 °C and extension at 72 °C for 30 seconds, and finally 72 °C for 5 minutes. The PCR products were analyzed using 2% agarose gel.

Normal brain cells were known to harbor unmethylated MGMT promoter only (6). Gliomas samples are often contaminated with normal tissue due to its infiltrative nature (16). Therefore, glioma samples with both methylated and unmethylated *MGMT* promoter were considered as methylated *MGMT* promoter as the unmethylated gene was from the normal brain tissue.

Statistical Analysis

The statistical analysis was carried out using GraphPad Prism software version 5 (GraphPad Software, USA). The association of *MGMT* methylation status with the clinicopathological parameters such as tumor grades, age, gender, and race of the patients was determined using the chi-square test. Statistical significance was defined as $p<0.05$.

Results

The study included 41 glioma samples (age, mean±standard deviation: 42.0±16.8 male sex: 61.0%). All glioma samples were subjected to MSP amplification as shown in Figure 1. Analysis of *MGMT* gene promoter in 41 glioma samples showed either unmethylated only or a mix of both unmethylated and methylated status. Samples with unmethylated *MGMT* promoter only showed a single MSP amplicon of 93 bp. On the other hand, glioma samples with both methylated and unmethylated *MGMT* promoter showed 81 bp (methylated) and 93 bp (unmethylated), respectively.

It was observed that 92.7% of glioma samples showed methylated and 7.3% unmethylated *MGMT* promoter. All grade 2 and grade 3 gliomas showed methylation, compared to 85% of grade 4 (p=0.183). More older glioma patients (>40 years) had methylation compared to younger patients (≤40 years) (95.8% vs 88.2%) (p=0.357). More males had methylation compared to females (96% vs 87.5%) (p=0.308). The correlation of *MGMT* promoter methylation status with the clinicopathological parameters such as tumors grading, age, gender, and the race was shown in Table 1.

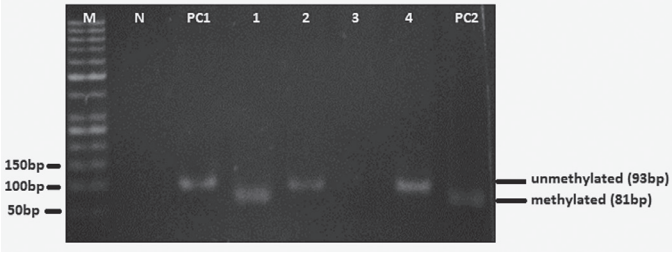

Figure 1. A representative of methylation-specific polymerase chain reaction result of MGMT promoter in gliomas. Lane M: 50 bp DNA ladder, Lane N: Negative control (without DNA), Lane PC1: Positive unmethylated 5-Aza-dc Treated Jurkat Genomic DNA control, Lane 1 and 2: Representative of methylated and unmethylated of a glioma sample, Lane 3 and 4: Representative of unmethylated of a glioma sample, Lane PC2: Positive methylated CpG Methylated HeLa Genomic DNA control

Discussion

According to the Malaysian National Cancer Registry 2007-2011, brain tumors are the second most common cancer among adults and children respectively (17). The current survival rate of stage 4 GBM is still at dismay level. The median overall survival was 6.5 months and median progression-free survival was 5.5 months only (18). Therefore, improving the current cancer therapeutic methods is important to prolong the survival rate. Cancer biomarkers have been extensively studied as it can predict responsiveness to medical treatments, patient's survival rate and disease diagnosis (19). Thus, new biomarker is needed to improve the current diagnosis and treatment stratification.

It was found that reduced MGMT protein expression via promoter methylation showed a significant improvement in the patient's survival rate and responsiveness to alkylating drug treatment (20). GBM patients with *MGMT* methylated undergone radiation showed longer median survival compared to the *MGMT* unmethylated with radiation (15.3 months vs 11.8 months). Together with TMZ and radiation, *MGMT* methylated GBM patients showed much higher median survival compared to *MGMT* unmethylated GBM (23.4 months vs 12.6 months) (21). Hence, *MGMT* promoter methylation status has a high potential for treatment stratification in glioma patients (22-24).

Lack of *MGMT* promoter methylation data in gliomas and ambiguous testing procedure in hospital has impeded the implementation of this potential biomarker in our local clinical setting. Thus, this study was conducted to determine the current methylation status of *MGMT* promoter in our local glioma samples. Besides, this study can contribute to the prevalence of *MGMT* glioma database in Malaysia and globally.

Some glioma samples that showed both methylated and unmethylated *MGMT* promoter were considered as methylated *MGMT* due to the infiltrative nature of gliomas. There is no distinct border between the glioma and normal brain tissue, thus the glioma samples are often contaminated with normal brain tissue. This leads to the presence of normal tissue's DNA in the extracted tumor genetic materials (25). Unmethylated *MGMT* gene in normal tissues produces MGMT protein to repair the damaged DNA (6).

Majority of the glioma samples (92.7%) have methylated *MGMT* promoter. Although there is no significant difference between the tumor grades and *MGMT* promoter methylation

Table 1. Association of MGMT promoter methylation status with tumor grading, age, gender and race among 41 glioma samples

Characteristics	Total no. of samples	Methylation status		p value
		Unmethylated	Methylated	
Number of samples, n (%)	41 (100)	3 (7.3)	38 (92.7)	-
Tumor grading, n (%)				
Grade 2	11 (26.8)	0	11 (100)	0.183
Grade 3	10 (24.4)	0	10 (100)	
Grade 4	20 (48.8)	3 (15)	17 (85)	
Age, years				
Mean±SD	42.0±16.8			
≤40, n (%)	17 (41.5)	2 (11.8)	15 (88.2)	0.357
>40, n (%)	24 (58.5)	1 (4.2)	23 (95.8)	
Gender, n (%)				
Male	25 (61)	1 (4)	24 (96)	0.308
Female	16 (39)	2 (12.5)	14 (87.5)	
Race, n (%)				
Malay	40 (97.6)	3 (7.5)	37 (92.5)	0.229
Chinese	1 (2.4)	0	0	

SD: Standard deviation, MGMT: Methylguanine-DNA methyltransferase

status (p=0.183), we found that all grade 2 and 3 gliomas had methylated *MGMT* promoter compared to grade 4 (85%). On contrary, a study from Europe found that approximately 44.7% of grade 4 gliomas had *MGMT* promoter methylation (10). A study from China showed that 58.6% of GBM showed *MGMT* promoter methylation (26). Based on the GBM group, most of our local sample had *MGMT* promoter methylation (85%) compared to the studies from China (58.6%) and Europe (44.7%).

Approximately 88.2% of younger glioma patients (≤40 years old) exhibited *MGMT* promoter methylation compared to unmethylated *MGMT* (11.8%). About 95.8% of older glioma patients (>40 years old) also exhibited *MGMT* promoter methylation compared to unmethylated *MGMT* (4.2%). Most of the young and old glioma patients showed *MGMT* promoter methylation but slightly more older glioma patients had methylated *MGMT* promoter compared to younger patients (95.8% vs 88.2%). There was no significant association between the patient's age and *MGMT* promoter methylation status (p=0.357). A study showed that more older GBM patients (≥50 years) were found to harbor methylated *MGMT* compared to the younger GBM patients (61.3% vs 38.7%, p=0.444) (26). However, Cancer Genome Atlas project's found that younger patients were associated with glioma CpG island methylation phenotype (G-CIMP) (27).

Our study found that more male patients had methylated *MGMT* promoter as compared to female patients (96% vs 87.5%). However, there is no significance between gender and *MGMT* promoter methylation status (p=0.308). The role of gender in determining glioma prognosis remained ambiguous. The previous study from Italy showed that female GBM patients with methylated *MGMT* promoter were found to have better survival rate compared to methylated *MGMT* males GBM patients (p=0.028) but there was no significance between the unmethylated females and males (p=0.395) (28).

One of the limitations of this study is selection bias. This was because only available FFPE glioma blocks from Pathology Department were selected for this study. This experimental setting was done at the east coast region of Malaysia and the majority race in that population was Malays (29). Thus, this may be explained why most of the samples obtained were from Malays race (97.6%) and only one from the Chinese race (2.4%). Besides, different genetic makeup between Asian and non-Asian may also contribute to the different distribution of *MGMT* promoter methylation in gliomas.

Besides, our small sample size may affect the findings as other glioma studies involve larger sample size such as 169 (28), 206 (10) and 573 (20). As the samples were collected at only one location, the sample size can be improved by collecting from different locations and equal distribution of races in Malaysia to produce more accurate information regarding this potential biomarker.

Moreover, sophisticated equipment can be used to improve the quality of the data in this study as well. Pyrosequencing can detect a very small amount of tumor DNA (5%) in the background of normal DNA. Since glioma is an infiltrative tumor with no distinct border, it would be best to use sophisticated equipment to capture the true tumor genetic nature.

Conclusion

In conclusion, the *MGMT* promoter methylation was predominant in grade 2 and 3 glioma patients, older age (>40 years) and male gender. Nevertheless, this study showed that a high number of glioma samples from Kelantan, Malaysia harbored *MGMT* promoter methylation. Therefore, this gene has great potential in diagnosis and treatment stratification to improve glioma patient's survival rate.

Ethics

Ethics Committee Approval: The study were approved by the Human Research Ethics Committee of Universiti Sains Malaysia (JePeM) (ref. no. USM/JEPeM/17050255).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.A., Design: Z.I., F.A., Data Collection or Processing: R.M., Analysis or Interpretation: S.S., B.I., Literature Search: W.C.G., Writing: W.C.G., B.I., Z.I.

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

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Development and validation of a questionnaire on knowledge and practices of disinfection and sterilization among healthcare workers

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Keywords: Disinfection, sterilization, knowledge, practices, validation, questionnaire

ABSTRACT

Aims: To develop and validate a questionnaire to measure knowledge and practices of disinfection and sterilization among healthcare workers.

Methods: A self-administered questionnaire in Bahasa Melayu, comprising 2 sections of knowledge (15 items) and practices (15 items), was developed based on available literature and expert opinions. Content validity was assessed by 9 experts while face validity was tested on 12 subjects. Psychometric properties were evaluated on 67 respondents in the pilot study, using 2 parameter logistic item response theory (2PL-IRT), exploratory factor analysis (EFA) and Cronbach's alpha. Data were analyzed with R version 3.5.1.

Results: The content validity index for knowledge and practices were 0.96 and 0.92 (after omitting 2 items), respectively, denoting good relevancy of the items. Assessment of face validity index showed the values of 0.86 for knowledge and 0.83 for practices (after dropping 1 item), indicating that the questionnaire was well-understood by respondents. In the pilot study, 2PL-IRT and EFA revealed that the questionnaire had good psychometric properties after the removal of 2 poor quality items from each section. As for internal consistency, Cronbach's alpha coefficients were determined to be acceptable; 0.72 [95% confidence interval (CI): 0.623, 0.818] for knowledge and 0.803 (95% CI: 0.733, 0.803) for practices. The validated questionnaire consisted of a total of 23 items; knowledge (13 items) and practices (10 items).

Conclusions: A self-administered questionnaire on knowledge and practices of disinfection and sterilization among healthcare workers was developed and validated. Future recommendation is for confirmatory analysis to be carried out to verify and maximize the psychometric credentials of the questionnaire.

Introduction

Despite progress and advancement in public health and hospital care, infections continue to develop in hospitalized patients and are arising tremendously every year (1). Nosocomial or hospital acquired infections are infections that are not present during the time of admission. They can manifest 48 hours after admission to the hospital and even after the discharge of the patients (2). They do not only burden the hospital workers but also affect the cost resources as they effectuate prolonged stay, persistent disability, increased antimicrobial resistance and elevated the mortality rate worldwide (3).

According to World Health Organization estimation, up to 15% of all hospitalized patients suffer from these nosocomial infections (4). In Malaysia, the prevalence was 13.9% with the most common infections including clinical sepsis and pneumonia (5). This is because during hospitalization, the patient is exposed to multiple pathogens from different sources such as the environment, healthcare providers and other infected patients (2). Transmission of these infections should be kept at bay with appropriate infection control measures (6).

Among the first actions is by maintaining the hospital environment and equipment clean with proper disinfection and

sterilization practices (7,8). The Guideline for Disinfection and Sterilization in Healthcare Facilities describes disinfection as a process that eliminates many or all pathogenic microorganisms except for bacterial spores. On the other hand, sterilization does the latter and is defined as a process that destroys all forms of microbial life through physical or chemical means (9).

Available publications addressed that there was inadequate knowledge or unsatisfactory practices or both regarding disinfection and sterilization which was despairing considering its impact on patients' safety (10-13). To the best of the authors' knowledge, all of the questionnaires used were of foreign languages and there was no detailed information on their development, which makes it difficult to be applied in the population of Malaysia. The sole local work by Keah et al. (14) was a good reference to start with but the tool could be out-dated with all the recent policies and guidelines. Thus, this study aimed to develop and validate a questionnaire on knowledge and practices of disinfection and sterilization among healthcare workers in Bahasa Melayu or the Malay language.

Methods

Phase 1: Questionnaire Development

A thorough and comprehensive review and searching of the literature was conducted to ascertain existing, as well as to identify relevant items and scales in existing questionnaires on disinfection and sterilization. The first draft of a self-administered questionnaire in Bahasa Melayu was then developed by the research team along with panel of experts based on the compilation of guidelines and scientific articles. The first phase of the study was carried out from July to December 2018.

Content Validation Using Content Validity Index

Content validation was used to assess whether the content of the questionnaire was appropriate and relevant to study purpose by the same panel of experts. Each expert independently rated the relevancy of the items using a 4-point Likert scale (1=not relevant, 2=need some amendment, 3=relevant, 4=very relevant). Content validity index (CVI) for each item (I-CVI) was computed with Microsoft Excel (15) as the number of experts giving a rating of either 3 or 4, divided by the number of experts with the cut-off point of 0.78 (16). The questionnaire was then modified based on the expert reviews to produce the second draft.

Face Validation Using Face Validity Index

Face validation on the second draft of the questionnaire was conducted on 12 respondents from the target population to evaluate the clarity and comprehensibility of the wording used in the developed questionnaire. The respondents assessed each item using a 4-point Likert scale ranging from 1 (item not clear and not understandable) to 4 (clear and understandable) (17). Face validity index (FVI) for each item (I-FVI) was calculated with Microsoft Excel (15) as the number of respondents who rated 3 or 4, divided by the number of respondents with the threshold of 0.70 (18). The revised version of the questionnaire was formulated based on the findings to be used on the later stage.

Phase 2: Validation Study

To further explore and evaluate the psychometric properties of the questionnaire, the revised version was self-administered to respondents which were healthcare workers who handled disinfectant directly in Raja Perempuan Zainab II Hospital (HRPZ II), Kelantan. Those who were not available during the data collection period from January to June 2019 due to sabbatical or maternity leave were excluded. They were first briefed about the study and informed consent was then obtained from the respondents who agreed to be involved in the study. For the pilot study, a minimum sample size of 57 was calculated using a web-based sample size calculator for reliability studies (19). The required sample size for 2-parameter logistic item response theory (2PL-IRT) and exploratory factor analysis (EFA) followed the same sample size as internal consistency and it was inflated to 66 to account for 15% drop-out rate. The data analysis was performed in R version 3.5.1 (20) using the R studio environment (21).

2PL-IRT Analysis

As the knowledge section consisted of categorical responses, it was analyzed by 2PL-IRT, using the ltm package version 1.1-1 (22). Difficulty in the range from -3 to +3 and discrimination of >0.65 were considered acceptable. Item fit was tested using the chi-square goodness-of-fit per item and unidimensionality was determined by modified parallel analysis (23).

Exploratory Factor Analysis

The practice section had ordinal responses and therefore, it was analyzed by EFA using the psych package version 2.0.9 (24). The principal axis factoring extraction method with oblimin rotation was applied in EFA (25). The items in each section were treated as continuous responses to allow the evaluation of the dimensionality of the items (25). To determine the number of extracted factors, eigenvalues >1.0, parallel analysis and scree plot examination were carried out (26). Factor loadings of $\geq$0.3 were considered acceptable (25). A Cronbach's alpha coefficient of >0.7 was considered as acceptable internal consistency reliability (27).

Ethical Approval

Permission to conduct the study at the site was obtained from the Director of HRPZ II. All subjects were remained as anonymous to ensure their privacy and confidentiality. All study

procedures were carried out in accordance with the Declaration of Helsinki and Good Clinical Practice.

Results

Phase 1: Questionnaire Development

A self-administered questionnaire in Bahasa Melayu comprising 2 sections of knowledge (15 items) and practices (15 items) was developed based on available literature and expert opinions. Items for knowledge were multiple-choice questions with "True", "False" and "Uncertain" answer options, while items for practices had a response of 5-point Likert scale with 1=always, 2=often, 3=sometimes, 4=rarely and 5=never and 5 of them were negative statements.

Content Validation Using CVI

The relevancy of the items was rated using CVI by 9 panel experts who consisted of 2 infectious disease specialists, 2 medical officers, 3 clinical pharmacists and 1 pharmacist in charge of galenical pharmacy. In the knowledge section, all of the experts evaluated 10 items as relevant, thus providing I-CVI=1.00. The remaining 5 items had calculated I-CVI=0.89. With CVI average=0.96, all items were retained.

In the practices section, only 5 items were rated as relevant by all experts; I-CVI=1.00. Another 7 items had calculated I-CVI=0.89, while 1 item scored I-CVI=0.78. The first 2 items of Q1: "I perform endotracheal tube disinfection using alcohol swab" and Q2: "I use sterile chlorhexidine 2% in alcohol 70% for multiple patients" were deleted due to I-CVI<0.78. With 13 items remaining in the practices section, I-CVI average improved from 0.89 to 0.92 (Table 1).

Face Validation Using FVI

The clarity of the items was assessed using FVI in 12 respondents. In the knowledge section, 3 items appeared to be clear and understandable by all target population (FVI=1.00). Another 4 items had FVI=0.92, while the remaining 5 items scored FVI=0.75. With FVI average of 0.86, all 15 items were retained.

For the practices section, only 1 item was clear and understandable by all target population (FVI=1.00). Out of 13 questions, 2 items obtained FVI=0.92, while the rest 9 items scored FVI=0.75-0.83. Q10 "I perform nebulizer disinfection during every treatment" had the lowest FVI=0.5 and was omitted from the questionnaire, which increased FVI average from 0.8 to 0.83 for 12 items (Table 2). Therefore, the revised version of the questionnaire consisted of a total of 27 items, with knowledge (15 items) and practices (12 items).

Phase 2: Validation Study

Evaluation of the psychometric properties was carried out using 67 respondents in the pilot study. Majority of them were female (64.2%, n=43), with mean [standard deviation (SD)] age of 35.6 (8.43) years. They were mostly Malays (95.5%, n=64), working as nurses (34.3%, n=23) with mean (SD) working experience of 11.3 (8.2) years. More than half of the respondents had never attended any infection control course (53.7%, n=36) (Table 3).

Table 1. Content validity index based on rating of the relevancy of items by 9 experts

Items	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6	Expert 7	Expert 8	Expert 9	I-CVI
Knowledge section										
Q1	4	4	4	4	4	4	4	4	4	1.00
Q2	4	4	4	4	4	4	3	4	4	1.00
Q3	4	4	4	4	4	4	4	4	4	1.00
Q4	3	4	4	4	4	4	4	4	3	1.00
Q5	4	4	4	4	4	4	4	2	4	0.89
Q6	4	4	4	4	4	2	4	4	3	0.89
Q7	4	4	4	4	4	4	4	4	4	1.00
Q8	4	4	4	4	4	4	3	4	2	0.89
Q9	4	4	4	4	4	3	4	4	4	1.00
Q10	4	4	4	4	4	4	4	4	4	1.00
Q11	4	3	4	4	4	4	4	4	2	0.89
Q12	4	4	4	4	4	4	4	4	4	1.00
Q13	4	4	4	4	4	4	3	4	4	1.00
Q14	4	3	4	4	4	4	4	4	2	0.89
Q15	4	4	4	4	4	4	4	4	4	1.00
CVI average										**0.96**
Practices section										
Q1	1	4	4	4	4	2	4	2	4	0.67
Q2	3	4	3	4	4	2	4	1	2	0.67
Q3	3	4	4	4	4	4	4	4	4	1.00
Q4	4	4	4	4	4	4	4	4	4	1.00
Q5	4	4	4	4	4	4	4	4	4	1.00
Q6	1	3	4	4	4	4	4	4	4	0.89
Q7	4	4	4	4	4	3	4	4	2	0.89
Q8	1	4	4	4	4	4	4	4	4	0.89
Q9	1	2	4	4	4	4	4	4	4	0.78
Q10	4	2	4	4	4	4	4	3	4	0.89
Q11	1	4	4	4	4	4	4	4	4	0.89
Q12	1	3	4	4	4	4	4	4	4	0.89
Q13	4	4	4	4	4	4	4	4	4	1.00
Q14	1	4	4	4	4	4	4	4	4	0.89
Q15	4	4	4	4	4	4	4	4	4	1.00
CVI average										0.89
CVI average after deletion of Q1 and Q2										0.92

I-CVI: Item content validity index, Q: Question

2PL-IRT Analysis

As shown by the 2PL-IRT analysis, the psychometric credentials of knowledge section were good. Most of knowledge items were within or close to the acceptable range from -3 to +3 for difficulty and <0.65 for discrimination. Item fit statistics showed all p values >0.05. On the assessment of fit for two-way margins, all item pairs showed good fit. Modified parallel analysis supported unidimensionality.

Table 2. Face validity index based on rating of the clarity of items by 12 target population

Items	Rater 1	Rater 2	Rater 3	Rater 4	Rater 5	Rater 6	Rater 7	Rater 8	Rater 9	Rater 10	Rater 11	Rater 12	I-FVI
Knowledge section													
Q1	4	4	3	2	3	2	3	3	3	4	3	1	0.75
Q2	4	4	4	4	4	3	4	3	3	4	4	1	0.92
Q3	4	4	4	3	4	4	4	1	4	4	4	4	0.92
Q4	4	3	3	4	3	3	1	1	3	3	4	3	0.83
Q5	4	3	4	3	3	4	4	3	4	4	4	4	1.00
Q6	3	2	3	1	3	3	3	4	3	2	3	3	0.75
Q7	4	3	3	3	1	2	3	4	3	2	3	3	0.75
Q8	4	3	3	3	1	3	4	2	2	3	3	3	0.75
Q9	3	4	3	2	3	4	3	4	4	4	2	3	0.83
Q10	4	2	4	2	3	3	3	3	3	3	1	3	0.75
Q11	4	4	4	4	3	3	3	1	3	3	1	3	0.83
Q12	4	4	4	4	4	1	3	3	3	4	3	4	0.92
Q13	4	4	4	4	3	4	4	4	4	4	4	4	1.00
Q14	4	4	4	3	4	3	4	4	3	4	4	4	1.00
Q15	4	4	4	4	2	4	3	4	3	4	4	4	0.92
FVI average													**0.86**
Practices section													
Q3	4	3	3	4	3	2	3	4	3	2	3	3	0.83
Q4	4	4	4	4	4	2	4	4	4	4	2	4	0.83
Q5	4	4	4	4	3	3	4	3	4	4	2	4	0.92
Q6	4	4	4	3	3	3	3	3	4	3	3	3	1.00
Q7	4	4	4	4	3	4	3	2	4	3	1	3	0.83
Q8	4	4	1	4	4	3	3	4	4	3	3	1	0.83
Q9	4	3	1	4	3	3	2	4	4	3	1	3	0.75
Q10	4	3	1	2	2	3	3	2	3	1	3	1	0.5
Q11	3	4	3	1	1	3	4	3	4	3	3	2	0.75
Q12	4	3	2	2	3	3	2	3	3	4	3	3	0.75
Q13	4	4	3	4	3	2	4	3	4	4	4	1	0.83
Q14	4	3	1	3	3	2	4	3	4	4	4	1	0.75
Q15	4	4	3	4	3	3	4	3	4	3	4	1	0.92
FVI average													0.81
FVI average after deletion of Q10													0.83

I-FVI: Item face validity index, Q: Question

Q3 "To avoid cross contamination, reusable medical equipment should be cleaned and sterilized" was dropped as it was too difficult with poor discrimination while Q5 "Disinfection process should be carried out using appropriate chemical disinfectant" was omitted as it was too easy with low discrimination. Q10 "Glutaraldehyde is used to disinfect heat sensitive equipment" was retained for its average difficulty and good fit despite its low discrimination.

After repeated analysis and modifications, Q13 "Example of semi-critical item is surgical instrument" was kept for its average difficulty and good discrimination even though p value was <0.05 (Table 4). Cronbach's alpha coefficients was 0.72 [95% confidence interval (CI): 0.623, 0.818], which deemed as acceptable.

EFA

In the practices section, Kaiser-Meyer-Olkin Measure of sampling adequacy=0.75 and Bartlet's test of sphericity (p<0.001) showed the items were correlated and suitable for EFA. Scree plot demonstrated the suitable factor as 1 even though parallel analysis suggested that the possible number of factors was 3. Further analysis revealed that very simple structure had the highest value of 0.65 while Velicer's minimum average partial had the smallest value of 0.037 at 1 factor.

Table 3. Demographic characteristics of respondents in the pilot study (n=67)

Demographic characteristics	n (%)
Age (years old)	35.6 (8.4)*
Gender	
Male	24 (35.8)
Female	43 (64.2)
Race	
Malay	64 (95.5)
Non-Malay	3 (4.5)
Occupation	
Medical officer	9 (13.4)
Pharmacist	15 (22.4)
Nurse	23 (34.3)
Medical assistant	20 (29.9)
Working experience (years)	11.3 (8.2)*
Education level	
Diploma	42 (62.7)
Degree	23 (34.3)
Master	2 (3.0)
Attended infection control course	
Yes	31 (46.3)
No	36 (53.7)

*: Mean (standard deviation)

Table 4. 2-parameter logistic item response theory parameter estimates and item fit statistics on knowledge section

Items	Difficulty	Discrimination	x^2 (df=13)	p value
Before modification				
Q1	0.12	1.55	8.17	0.418
Q2	-1.42	8.74	0.13	0.999
Q3	**-8.23**	**0.38**	6.75	0.564
Q4	-2.13	0.68	5.19	0.737
Q5	3.29	-1.31	4.56	0.803
Q6	-1.42	1.29	8.49	0.387
Q7	0.62	1.16	8.81	0.358
Q8	-0.09	1.80	8.04	0.429
Q9	0.84	1.74	10.20	0.252
Q10	0.72	**0.49**	9.57	0.297
Q11	-0.17	1.21	10.37	0.240
Q12	0.67	1.05	5.59	0.693
Q13	0.12	0.84	12.71	0.122
Q14	-1.42	1.80	12.87	0.116
Q15	-2.39	1.31	9.00	0.340
After modification				
Q1	0.12	1.64	4.82	0.777
Q2	-1.42	8.59	0.14	0.999
Q4	-2.17	0.67	6.91	0.546
Q6	-1.45	1.25	9.70	0.287
Q7	0.61	1.19	12.25	0.140
Q8	-0.09	1.79	7.54	0.480
Q9	0.88	1.62	4.41	0.818
Q10	0.73	**0.48**	9.08	0.336
Q11	-0.17	1.26	8.28	0.407
Q12	0.66	1.08	7.37	0.497
Q13	0.12	0.81	20.13	**0.010**
Q14	-1.42	1.80	6.13	0.633
Q15	-2.39	1.31	8.79	0.360

2-PL IRT: 2-parameter logistic item response theory model, df: Degree of freedom

Based on EFA, most of the items had acceptable factor loadings ≥0.3 and communalities ≥0.09. However, Q11 "I reuse disposable items to save cost" and Q12: "I clean blood-stained surfaces using chlorhexidine 0.5% in aqueous" were deleted due to their poor quality based on factor loading and communalities. Item Q7 "I use chlorhexidine 0.5% in aqueous for blood culture procedure" was kept as communalities value was only slightly below cut-off point.

After repeating the analysis, all remaining 10 items were determined acceptable (Table 5). Cronbach's alpha coefficients was 0.803 (95% CI: 0.733, 0.803), indicating good internal consistency. The validated version of the questionnaire had a total of 23 items; knowledge (13 items) and practices (10 items) with 3 negative statements (Appendix 1).

Table 5. Results for exploratory factor analysis on practices section

Items	Factor loadings	Communalities
Before modification		
Q3	0.5	0.28
Q4	0.3	0.10
Q5	0.6	0.39
Q6	0.4	0.13
Q7	0.3	0.07
Q8	0.5	0.24
Q9	0.8	0.59
Q11	**0.2**	**0.03**
Q12	**0.2**	**0.03**
Q13	0.8	0.61
Q14	0.7	0.46
Q15	0.7	0.46
After modification		
Q3	0.5	0.27
Q4	0.3	0.09
Q5	0.6	0.39
Q6	0.3	0.12
Q7	0.3	**0.07**
Q8	0.5	0.23
Q9	0.8	0.60
Q13	0.8	0.65
Q14	0.7	0.48
Q15	0.7	0.43

Discussion

Malaysia is a diverse country with Bahasa Melayu as the mother tongue (28). It is for this reason that this study aimed to develop and validate a questionnaire on knowledge and practices of disinfection and sterilization among healthcare workers in Bahasa Melayu. Our findings indicated that the questionnaire managed to achieve an acceptable level of response process and good internal structure. This was achieved partly because of the rigorous development and validation process that was based on standard recommendations or guidelines as well as scientific articles (29,30).

The first step of questionnaire development is the articulation of domains and item generation (29). Once the domain is delineated, the item pool can then be identified (29). An initial 30 items were prepared, including knowledge (15 items) and practices (15 items). With regard to the type of responses to these questions, items for knowledge had a polytomous answer option. A middle response of "Uncertain" was chosen to elicit the correct response. By treating it the same way as an incorrect answer would, the response was turned into dichotomous

(31). As for the practices section, a Likert-type response scale presented in an ordinal manner is used to reflect the entire measurement continuum (29).

CVI is a common reported measure of content validity and has been around for many years (32). It works by asking panel of experts to rate each scale item concerning its relevance to the underlying construct with a 4-point ordinal scale. It is recommended a minimum of 3 experts, but not more than 10. As the questionnaire was validated by 9 experts, a CVI cut-off point was set at 0.78 (16). The results showed that I-CVI values of the present questionnaire were acceptable, indicating that the items were relevant with the sections.

To establish the response process validity, FVI was used to assess the clarity of the items. FVI values were calculated from 12 target population who completed the questionnaire. This decision was according to Yusoff (20) who noted that the number of experts for content validation should not be less than 10 raters (17). Based on his other work, it was resolved that all items had FVI >0.70 since they were considered as fairly understood by participants and were able to remain in the final validated questionnaire through confirmatory analysis (18).

From 2PL-IRT results, the knowledge section showed good psychometric properties in the validation study. With regard to the difficulty parameter, all items were within the respectable range. For the discrimination parameter, all values were acceptable except for Q10. All items but one which was Q13 fitted the 2PL-IRT model with p values of >0.05. However, both were retained, given their importance in the assessment of knowledge about disinfection and sterilization.

For EFA, factor loadings ≥0.3 or 0.4 are usually considered in the interpretation (25). Communalities were calculated as sum of square of factor loading (33), which means that if factor loading of 0.3 is chosen, the threshold for communalities should be set at 0.09. Based on those values, we had to omit 2 items which were Q11 and Q12 in the practices section but decided to maintain Q7 as the communality value was only inconsiderably below 0.09.

The reliability of the questionnaire was also tested to measure the stability of the questionnaire and the consistency of the response. For this purpose, Cronbach's alpha coefficient is commonly used to reflect the internal consistency. Even though the respectable value may vary according to different literature, an instrument is often considered as reliable when the Cronbach's alpha coefficient reaches the value of 0.70 as depicted in our study (27).

The present study has several important limitations. The respondents were recruited only from a single tertiary hospital in Kelantan with moderate sample size which might not represent the whole population of healthcare workers in Malaysia. In addition to that, the use of convenience sampling could lead to sampling bias and hence, compromised the results obtained. As the questionnaire was developed in Bahasa Melayu, it was not possible to predict how well it would perform in other languages.

Conclusion

A self-administered questionnaire on knowledge and practices of disinfection and sterilization among healthcare workers was developed and validated. It consisted of 2 sections with 23 items, including knowledge (13 items) and practices (10 items). The questionnaire was proven to be psychometrically valid based on the results of 2PL-IRT and EFA. Future recommendation is for confirmatory analysis to be carried out to verify and maximize the psychometric credentials of the questionnaire.

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Ethics

Ethics Committee Approval: This research was registered with the National Medical Research Registry (NMRR-18-2624-41500) while ethical approval was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia on 19 May 2018.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The effect of anesthetic techniques on postoperative outcomes of open prostatectomy in the era of enhanced recovery after surgery

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ABSTRACT

Aims: As the enhanced recovery after surgery (ERAS) concept gains popularity in the surgical practice, the anesthetic technique has a more important effect on the postoperative course, especially in aged population. The aim of this study was to compare general anesthesia (GA) with spinal anesthesia (SA) regarding the perioperative outcomes with respect to the ERAS protocols in open prostatectomy (OP) for benign prostate hyperplasia.

Methods: This retrospective study included patients between 40 and 90 years of age who underwent elective OP between 2014 and 2020. Data were collected from hospital's database, patient files, and anesthesia charts. The exclusion criteria were malignancy, lost to follow-up, and missing data. Primary outcome measures were perioperative variables. Secondary outcome measures were factors influencing hospital discharge time.

Results: Of 105 patients (age, mean±SD: 68.3±5.7 years) included in the study, 61 patients were administered in GA (group GA) and 44 in SA (group SA). As the primary outcome measures, when compared with group SA, deliberate hypotensive anesthesia was required in more patients [26 (42.6%) vs. 15 (34.1%); p=0.027] and transfusion rate was higher (6.5% vs. 4.5%; p=0.044) in group GA. Mean visual analogue scale score (3.6±1.1 vs. 2.8±0.4; p=0.040) and opioid consumption (32.8±4.4 vs. 26.3±4.9 mg; p=0.088) were higher, and time to first rescue analgesic use was also shorter (1.1±0.9 vs. 4.7±1.3 hours; p=0.011) in group GA when compared with group SA.

Conclusions: This study showed that SA was superior to GA in maintaining hemodynamic stability, reducing blood loss, complications, postoperative analgesia requirement, and time to discharge, which are the main goals of ERAS protocols.

Introduction

As men age, benign prostate hyperplasia (BPH) becomes a common disease with a prevalence up to 60% until 9th decade (1). Although transurethral techniques such as resection, vaporization, and holmium or thulium laser enucleation of the prostate are widely used surgical treatments for BPH, open prostatectomy (OP), in other words, "simple" prostatectomy continues to be a treatment option in patients with a large prostate greater than >70 mL (2). Compared to the transurethral techniques, it has been reported that OP offers several advantages including lower re-operation rate, higher life quality and patient satisfaction, and better symptom relief in patients with large BPH (3,4). OP is performed mainly under general or spinal anesthesia (SA). The anesthetic technique is determined

primarily considering the balance of the risk and benefit, because the patients are old and have several comorbidities.

After the introduction of the enhanced recovery after surgery (ERAS) protocols in daily practice, their benefits on the outcomes are well established in major urological cancer surgeries, which provide a safer perioperative course with early hospital discharge (5). ERAS protocols include preoperative counseling, maintaining hemodynamic stability, avoiding venous thromboembolism and fluid overload, early mobilization, early feeding, and effective pain relief. The anesthetic technique plays an important role in reducing surgical stress response, complications, and length of hospital stay in the postoperative period, especially in high-risk patients with older age (6). The decision-making for an anesthetic technique is a collaborative process that is shared by the patient, anesthesiologist, and surgeon (7). However, patient's anxiety and fear for anesthesia continue to be determinant in the decision despite the risks related to the technique (8).

Contrary to the urological cancer surgery, the role of the anesthetic technique in achieving of ERAS goals is not investigated in simple prostatectomy surgery. The aim of this current study was to evaluate the effect of the anesthetic techniques on the outcome of the OP with respect to the ERAS protocols in patients with BPH.

The primary outcome measures were perioperative variables which included hemodynamic stability, blood loss, transfusion of blood products, postoperative pain scores, first analgesic requirement, rescue analgesic consumption, time to first oral intake, time to mobilization, and complications. The secondary outcome measures were to identify the length of hospital stay and the factors influencing hospital discharge time.

Methods

Study Design

This retrospective study was conducted in an university hospital and a private hospital. Data were collected from electronic medical records, patient files, and anesthesia charts to identify patients who underwent OP under general anesthesia (GA) or SA between 2014 and 2020. The patients with the American Society of Anesthesiologists physical status classification 1-3, aged between 40 and 90 years, who had BPH diagnosis and underwent elective surgery were included in the study. Patients with a history of prostate or bladder cancer, urgent surgery, lost to follow-up, and missing data were excluded. The study was approved by the hospital's ethic commitee (Gülhane Training and Research Hospital, date: 11/30/2020; protocol no: 2020/443).

Data Collection

Information about the patients were reviewed to record 1) demographic characteristics, 2) type of anesthetic technique (GA or SA), 3) intraoperative variables including operating time, hemodynamic disturbances, blood loss, blood transfusion, time to discharge from PACU, adverse events or complications, 4) postoperative variables including pain scores, analgesic consumption, time to mobilization and to oral intake (hours), and discharge time (days). Complications were classified according to the surgery surgery and anesthesia. The study followed the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines.

Anesthetic Technique

All patients were administered GA or SA. Also, a hypotensive agent (nitroglycerin) was intravenously infused to achieve a deliberate hypotension in combination with tranexamic acid to reduce blood loss during the surgery.

All patients were administered a multi-modal analgesic regimen. Pain intensity was recorded using the visual analogue scale (VAS) by the service nurses. All patients were administered pethidine as the rescue analgesic.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) program (version 21.0; IBM SPSS Inc, Chicago, IL) was used to analyze data. Continuous variables were expressed as mean±standard deviation or median (interquartile range). Dichotomous variables were presented as percentages (%). Normality of data was tested using the Kolmogorov-Smirnov test. A t-test or Mann-Whitney U test was used to compare normally or non-normally distributed variables. Categorical variables were compared using the chi-square (χ^2) and Fisher's exact test. P value of <0.05 was considered statistically significant.

Results

A total of 121 files were evaluated and 16 files were excluded due to missing data. The remaining 105 patients were divided into two groups according to the anesthetic technique, as group GA (n=61) and group SA (n=44). The demographic data are shown in Table 1. The mean age was 68.3±5.7 (54-91) years and the patients in group GA was younger than those in group SA (62.9±5.3 years vs. 74.4±4.1 years; p=0.033). The mean body mass index was similar between groups. Co-morbidity rate was 71.4% in the study population and the rate of patients with co-morbidity was higher in group SA than in group GA (79.5% vs 65.6%, p=0.021 p<0.05). The mean operative time was similar between two groups.

Primary Outcome Measure

A deliberate hypotensive anesthesia was required in more patients in group GA compared to group SA (42.6% vs. 34.1%, p=0.027). The mean blood loss and blood transfusion rate were higher in group GA than to group SA (345.9±33.0 mL vs. 290.9±18.5 mL; p=0.034 and 6.5% vs. 4.5%; p=0.044). Time to oral intake was longer in group GA (5.5±0.2 vs. 3.1±0.6 hours;

p=0.033) whereas time to mobilization was similar (6.8±1.1 vs. 6.7±0.9 hours; p=0.766). Mean VAS scores were higher (3.6±1.1 vs. 2.8±0.4; p=0.040), time to first rescue analgesic was lower (1.1±0.9 vs. 4.7±1.3 hours; p=0.011), and mean opioid consumption was higher in group GA (32.8±4.4 vs. 26.3±4.9 mg; p=0.088), especially in the first postoperative 6 hours. The complication rate was higher in group GA than group SA (37.7% vs. 20.5%, p=0.021). No fatal complication was recorded (Table 2).

Secondary Outcome Measure

The mean length of hospital stay was 2.2±0.2 (2-7) days, which was longer in group GA than in group SA (2.4±0.1 vs. 1.9±0.5 days; p=0.026). A total of 11 patients (7 in group GA and 4 in group SA) were discharged later than the mean discharge time due to the following reasons: a) atelectasis requiring therapy in 4 patients (3 patients in group GA and 1 patient in group SA), b) hemodynamic disturbance and/or arrhythmia at the postoperative 2. day (2 patients; one patient in each group), c) delirium (3 patients; 2 in group GA and 1 in group SA), d) prolonged postoperative bleeding (one patient in group GA), and e) dysregulation of the blood glucose homeostasis (one patient in group SA) (Table 3).

Difficulty in airway management was observed in two patients in group GA. Direct laryngoscopy and endotracheal intubation failed in these patients. One patient was awakened and scheduled for a further surgery under SA. The other patient was intubated using an intubating laryngeal mask airway. SA was failed in two patients in group SA.

Discussion

The results of the study showed that SA offered a more stable and safer perioperative period compared with the GA in patients who underwent OP, which is consistent with the aims of ERAS protocols due to several reasons;

I. Hypotensive anesthesia was required less often, which indicated SA maintained deliberate hypotension more efficiently compared to the GA during the intraoperative period.

II. Blood loss and transfusion rate were lower.

III. Postoperative pain relief was more effective, especially in the immediate postoperative period. Opioid consumption and opioid-related side effects were lower.

IV. Patients were discharged earlier.

Table 1. Demographic characteristics and operative time between the study groups

Parameter	General anesthesia (n=61)	Spinal anesthesia (n=44)	*p
Age (years)	62.9±5.3	74.4±4.1	0.033
ASA 1/2/3 (n, %)	21/36/4 (34.4/59.0/6.6)	9/30/5 (20.5/68.2/11.3)	0.008
Co-morbidity (n, %)	40 (65.6)	35 (79.5)	0.021
Hypertension (n, %)	23 (37.7)	19 (43.2)	0.033
Diabetes mellitus (n, %)	9 (14.8)	8 (18.2)	0.010
Coronary artery disease (n, %)	6 (9.8)	6 (13.6)	0.011
Pulmonary disease (n, %)	2 (3.3)	2 (4.5)	0.044
Mixt (n, %)	17 (27.8)	14 (31.8)	0.039
Body mass index (kgm^{-2})	26.1±1.8	27.2±2.6	0.900
Operative time (min.)	130.1±8.4	134.6±7.1	0.780

*p<0.05 was considered statistically significant.
ASA: American Society of Anesthesiologists

Table 2. Comparing primary outcome measures between the study groups

Parameter	General anesthesia (n=61)	Spinal anesthesia (n=44)	p*
Intraoperative hypotensive anesthesia requirement (n, %)	26 (42.6)	15 (34.1)	0.027
Estimated blood loss (mL)	345.9±33.0	290.9±18.5	0.034
Transfusion of blood products (n, %)	4 (6.6)	2 (4.5)	0.044
Time to oral intake (h)	5.5±0.2	3.1±0.6	0.033
Time to mobilization (h)	6.8±1.1	6.7±0.9	0.766
VAS score (0-10)	3.6±1.1	2.8±0.4	0.040
Time to first rescue analgesic (h)	1.1±0.9	4.7±1.3	0.011
Opioid consumption (mg)	32.8±4.4	26.3±4.9	0.038

*p<0.05 was considered statistically significant. h: Hours, VAS: Visual analogue scale

Table 3. Comparing secondary outcome measures between the study groups

Parameter	General anesthesia (n=61)	Spinal anesthesia (n=44)	p*
Hospital discharge time (day)	2.4±0.1	1.9±0.5	0.026
Complication rate (n, %)	23 (37.7)	9 (20.5)	0.021
Hypo/hypertension (n, %)	6 (9.83)	3 (6.8)	0.017
Arrhythmia requiring treatment (n, %)	1 (1.6)	1 (2.3)	0.388
Desaturation (SpO_2 <90 in room air) (n, %)	4 (6.6)	1 (2.3)	0.018
Atelectasis (n, %)	3 (4.92)	1 (2.3)	0.018
Nausea and vomiting (n, %)	6 (9.83)	2 (4.5)	0.039
Delirium (n, %)	3 (4.92)	1 (2.3)	0.031

*p<0.05 was considered statistically significant. h: Hours, SpO_2: Peripheral oxygen saturation

These results are consistent with previous studies that compared regional anesthesia with GA in patients undergoing radical prostatectomy and reported that a more stable and safer perioperative period was achieved with regional anesthesia, which reduced the discharge time (9,10).

This finding is indeed not surprising because the perioperative course in simple prostatectomies is expected to be less complicated compared to the radical prostatectomy, as the latter involves surgical removal of vas deferens, seminal vesicles and lymph nodes.

ERAS protocols, which aim mainly to improve patient care and reduce postoperative complications have been introduced for over 20 years in surgical procedures (5). Although ERAS concepts are applied more commonly to major urological surgery such as radical prostatectomy and cystectomy, it may also be reasonable to implement ERAS protocols in “simple“ prostate surgery because the procedure includes elderly patients with co-morbidities, has potential risks for major bleeding, transfusion requirements and complications (5,6,9,10).

Another notable finding of the current study was that GA was performed to younger and healthier patients, which suggests that those patients tended to prefer GA, or those with an advanced age and less healthy patients were directed to SA by the anesthesiologist to reduce perioperative complications. A recent study has revealed that the choice of the anesthetic technique is greatly influenced by the patient's preference, especially in favor of GA (11). In another study, it was reported that 58% of younger patients preferred GA because they did not want to receive visual and auditory stimuli during the surgical procedure (12).

On the other hand, the results of the current study showed that the complications rate was higher and discharge time was longer in the GA group. This can be interpreted as a benefit of regional anesthesia in a population at this age undergoing surgery. It is well-known that regional anesthesia offers several advantages over GA including lower complication rate, better postoperative pain relief, and reduced hospital discharge time. However, several studies have showed that a considerable number of elderly patients still preferred GA to regional anesthesia (13,14).

The reasons for refusal of regional anesthesia in patients older than 60 years were reported as limited knowledge about regional anesthesia (20.3%), fear of being awake during operation (18.7%), needle pain (7.7%), backache (11.5%), and previous negative experience with regional anesthesia (8.8%) (14). However, the most interesting finding of the present study was that the most common cause of refusal of regional anesthesia (38.5%) was the surgeon’s preference. This finding suggests that the surgeons have a major impact on patient’s preference since they are the primary care providers, whereas the anesthesiologist does not communicate with the patients until the pre-anesthetic visit. Additionally, the reason of the refusal may be that the patients are not allowed to choose their anesthesia with the anesthesiologist, or the anesthesiologist may not be involved effectively in the decision-making process. The studies indicate that patient’s satisfaction increases as they participate in the selection of the anesthetic method, but they may also need to be directed to more appropriate anesthetic options to reduce complications and to improve outcomes (14,15).

This study has several limitations. First, the retrospective design of the study may have a potential risk for bias among study groups. Although time to mobilization, an important criteria in ERAS protocols, was found similar between the groups, it may not reflect the exact mobilization time because the patients were not allowed to mobilize within postoperative six hours by the surgeons.

Conclusion

In conclusion, this study showed that, SA was superior to the GA in maintaining hemodynamic stability, reducing blood loss, complications and providing better postoperative pain relief with earlier hospital discharge, in patients who underwent OP for BPH, which are the main goals of ERAS protocols.

Ethics

Ethics Committee Approval: Approval of the Local Research Ethics Committee of our tertiary hospital was obtained before initiating the study (University of Medical Sciences Turkey, Gülhane Training and Research Hospital, project no: 2020/43, date: 11.30.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A.S., U.K., H.K., Concept: M.A.S., M.Ö.Ö., M.B.E., Design: M.A.S., U.K., H.K., Data Collection or Processing: C.Ç., H.K., U.K. Analysis or Interpretation: M.B.E., C.Ç., Literature Search: M.B.E., M.Ö.Ö., Writing: M.A.S., M.Ö.Ö., M.B.E.

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The comparison of SUV_{max} values in ^{18}F-FDG PET/CT according to cell type, stage, lymph node involvement and metastasis in lung cancers

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ABSTRACT

Aims: ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography/computerized tomography (PET/CT) provides metabolic information in addition to anatomic extension. This study aimed to compare the primary tumor maximum standardized uptake values (SUV_{max}) on PET/CT according to the histopathological type, stage, nodal involvement, and the metastasis of lung cancers.

Methods: In this retrospective study, PET/CTs of the patients with lung cancer were examined. Staging of the cancer was performed according to the eighth (8th) edition of the tumor, node and metastasis (TNM) classification system. SUV_{max} values were recorded and compared.

Results: Two hundred thirty-three patients with lung cancer (78.5% male, mean age: 67.0±9.6 years, range: 47-91 years) were analyzed. The SUV_{max} value of squamous cell carcinoma (SCC) (15.2±7.6) was higher than the SUV_{max} values of adenocarcinoma (AC) and small cell lung carcinoma (SCLC) (10.9±5.6 and 12.2±5.5, respectively, p<0.001). SUV_{max} values were not different between the stages of AC, SCC and SCLC (p=0.285, p=0.377 and p=0.061, respectively). SUV_{max} values were similar between nodal involvements (p=0.490, p=0.645 and p=0.114 for AC, SCC, and SCLC, respectively). There was no difference in SUV_{max} values of lung cancers with and without metastasis (p=0.496, p=0.209, and p=0.544 for AC, SCC, and SCLC, respectively).

Conclusions: The SUV_{max} value of SCC was highest among lung cancers. There was no significant difference between the SUV_{max} value of the primary tumor on PET/CT and the TNM stages of the tumor.

Introduction

Lung cancer ranks first both in the incidence of cancer and in cancer-related deaths in men (1). It is the second most commonly diagnosed cancer and the third most common cause of cancer-related deaths in women (1). 5-year survival has been reported as 5.2% in metastatic disease and 57.4% in localized disease (2). Staging in lung cancer is very important as it determines the prognosis. Despite the improvements in diagnosis, most lung cancers have advanced stage disease when diagnosed. Approximately, less than one third of patients with non-small cell lung cancer (NSCLC) are treated with surgery (3). Currently, eighth (8th) tumor, node and metastasis (TNM) staging system is used for lung cancer (4).

Warburg evaluated the shift in energy production from oxidative phosphorylation to glycolysis as an essential feature of the cancer cell in 1930 (5). Positron emission tomography (PET) using ^{18}F-fluoro-2-deoxy-D-glucose (FDG), a functional imaging technique utilizing this glycolytic change, is widely used and recommended as an aid in cancer diagnosis and staging (5,6). FDG PET/computed tomography (CT) provides functional and metabolic information about lung cancer and the European Society of Thoracic Surgeons guideline has reported that its

sensitivity and specificity in lymph node staging are 80-90% and 85-95%, respectively, and its negative predictive value is quite high in peripheral NSCLC (7).

Small cell lung cancer (SCLC) was classified as local and advanced disease in the past. The International Association for the Study of Lung Cancer recommended the use of the TNM staging for SCLC and NSCLC in 2007 (8). TNM classification was chosen for SCLC in this study.

Standardized uptake value (SUV) is a simple computable parameter indicating quantitative FDG uptake in tissue and tumor (9). FDG uptake in the tumor is calculated with the maximum SUV (SUV_{max}), which gives information about the activity of the disease or the aggressiveness of the tumor (10). Many factors such as blood glucose level, body weight, lesion size, respiratory movement and histological type of the lesion affect the SUV_{max} value. The SUV_{max} value varies greatly even in the same tumor type and this problem is especially detected in lung cancers and causes difficulties in diagnosis and staging (11,12). In the case of active infection, inflammation, previous lymph node sampling, sarcoidosis, anthracosis, and reactive lymph nodes, there may be false positivity in PET/CT. False negativity may occur in carcinoid tumors and adenocarcinomas (AC).

This study aimed to compare SUV_{max} values in lung cancers according to cell type, staging, lymph node involvement and metastasis. The SUV_{max} values of the cell types were also compared according to the disease stage, lymph node involvement and metastasis.

Methods

This retrospective cohort study was conducted by the Clinical Research Ethics Committee of the Umraniye Training and Research Hospital (approval no: 37, date: 20.03.2019). The study included the patients who were newly diagnosed with lung cancer. The lung cancer diagnosis was obtained with pathological examination of tissue biopsies. The diagnostic approach was decided according to the location of the lesion in the lung or the mediastinal lymph node involvement on CT or PET/CT. CT-guided needle biopsy was performed in peripheral lesions and flexible bronchoscopy was preferred in central lesions first. Convex endobronchial ultrasonography (EBUS) was performed for both diagnosis and staging in patients with mediastinal lymph nodes detected on CT or PET/CT. When EBUS was not diagnostic, patients underwent mediastinoscopy. Patients with early-stage lung cancer underwent surgery and they were diagnosed with excisional biopsy. US-guided biopsy was performed for supraclavicular lymph node or liver metastasis. 760 patients with ICD code-34, who underwent ^{18}F-FDG PET/CT between October 2016 and December 2018, were analyzed retrospectively. Five hundred twenty seven patients who underwent PET/CT for the evaluation of response to treatment, who had benign lesions in the pathology reports, who discontinued follow-up, and whose pathology reports could not be reached were excluded from the study. Two hundred thirty-three patients with lung cancer were included in the study. PET/CTs of the patients with pathologically diagnosed lung cancer were examined retrospectively and lung cancers were staged according to the 8th TNM system (13). SUV_{max} values were recorded. Lymph node involvement was classified as N0, N1, N2 and N2 (14). Metastasis (M1a: regional, M1b: solitary extrathoracic, M1c: multiple extrathoracic) was classified as present or absent.

PET/CT Procedure

FDG infusion (nearly 370 MBq of ^{18}F FDG) was applied after the patients fasted for at least six hours and the measurement of normal peripheral blood glucose or below 200 mg/dL. Approximately 60 minutes after the injection (15), ^{18}F-FDG PET/CT was performed using an integrated PET/CT scanner (Discovery ST, GE Medical Systems). Non-contrast enhanced whole body CT scans were performed using a 16-sliced helical CT scanner before the acquisition of the PET image. Images were obtained from head to mid-thigh with 6-9 bed positions (2 minutes for each bed position). The images were reconstructed in different imaging views, that is, in cross-sectional, axial, sagittal, and coronal planes. All SUV measurements were normalized for patient body weight. SUV_{max} >2.5 was considered positive. All scans were interpreted by two experienced nuclear medicine physicians.

Statistical Analysis

The patient data collected in the study were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS, Chicago, IL, USA) package program. Frequency and percentage for discrete data and mean±standard deviation for continuous data were used as descriptive values. The “independent sample t-test” was used for comparison of two groups and “ANOVA test” was used to compare three or more groups. Results were considered statistically significant when the p value was less than 0.05.

Results

The study included 233 patients with pathologically diagnosed lung cancer. Diagnoses of 115 patients were AC, 81 had squamous cell carcinoma (SCC) and 37 had SCLC (Table 1). The patients’ mean age was 67.0±9.6 years and 183 of them (78.5%) were male.

The SUV_{max} value of SCC (15.2±7.6) was higher than the SUV_{max} values of AC and SCLC (10.9±5.6 and 12.2±5.5, respectively, $p<0.001$, Table 2). Post-hoc tests suggested that it was associated with the differences between the SUV_{max} values of AC and SCC and between the SUV_{max} values of SCLC and SCC.

SUV_{max} values were not different between the stages (1, 2, 3A, 3B, 3C and 4) of AC, SCC and SCLC (p=0.285, p=0.377 and p=0.061, respectively, Table 3). There were significant differences in SUV_{max} values of histopathological types in stage 1, stage 3A and stage 4, (p=0.016, p=0.008 and p=0.001, respectively) and the SUV_{max} values of SCLC in stage 1, SCC in stage 3A and stage 4 (28.1±0.1, 14.4±6.1 and 16.9±7.9, respectively) were highest. Post-hoc test showed that the SUV_{max} values of SCLC and SCC and the SUV_{max} values of SCLC and AC types in stage 1, the SUV_{max} values of AC and SCC in stage 3A and the SUV_{max} values of SCC and AC in stage 4 were statistically different.

SUV_{max} values were similar between nodal involvements (N0, N1, N2 and N3) (p=0.490, p=0.645 and p=0.114 for AC, SCC, and SCLC, respectively, Table 4). Significant differences for SUV_{max} values were observed in N1, N2 and N3 between the cell types (p=0.007, p=0.047 and p=0.004, respectively). Post-hoc test determined that the differences were caused by AC and SCC for N1, SCLC and SCC for N2, and SCC and AC for N3.

There was no difference in SUV_{max} values of lung cancers with and without metastasis (p=0.496, p=0.209 and p=0.544 for AC, SCC and SCLC, respectively) (Table 5). SUV_{max} of SCC (16.9±7.9) was the highest among metastatic cancers (p=0.001) and SUV_{max} of AC (10.5±6.1) was the lowest among non-metastatic cancers (p=0.007). Post-hoc test determined that the difference was caused by SCC and AC in patients with and without metastasis.

Table 1. Demographic data of the patients and histopathological cell types of lung cancers

Age, years, (mean±SD)	67.0±9.6
Gender, male, n (%)	183 (78.5)
Histopathological cell type, n (%)	
Adenocarcinoma	115 (49.4)
Squamous cell carcinoma	81 (34.8)
Small cell lung carcinoma	37 (15.9)
Total	233 (100)

SD: Standard deviation, n: Number

Table 2. The comparison of SUV_{max} values according to the histopathological cell type

Cell type	SUV_{max}, (mean±SD)*
Adenocarcinoma	10.9±5.6
Squamous cell carcinoma	15.2±7.6
Small cell lung carcinoma	12.2±5.5

*p<0.001 for within group differences. SUV_{max}: Maximum standardized uptake value, SD: Standard deviation

Discussion

Lung cancer is a heterogeneous disease with highly variable prognosis and course. Staging plays an important role in guiding prognosis and treatment. TNM staging is currently the only classification method used in lung cancer. However, the different prognosis of even patients at the same stage cannot be explained by TNM staging determined according to anatomical features. PET/CT may provide additional benefit in the prediction of prognosis since it also indicates metabolic properties of the tumors (8,16). Because the TNM staging also determines the treatment of the disease in addition to prognosis, PET/CT may be included in the current staging system. The SUV_{max} value, which shows the metabolic activity of the primary tumor, has been shown to have a prognostic significance in NSCLC (17). In our study, a correlation was found between histopathologic cell types of lung cancer and SUV_{max} values. The SUV_{max} value of SCC type was significantly higher than the other lung cancer types. Similar to our study, SUV_{max} mean values were previously reported in the range of 2.5-19.1 and 0.4-28.4 in SCC and AC cell types, respectively, and the difference was statistically significant (18). In a retrospective evaluation of 176 NSCLC patients, the SUV_{max} value of SCC was statistically significantly higher than that of AC (14.8 vs. 8.6, respectively) (19).

Sahiner et al. (16) showed a positive correlation between SUV_{max} values and stages of lung cancer, without separating them according to cell types in a retrospective study including 168 patients. Due to the partial volume effect, they have stated that SUV_{max} can be detected lower than expected in small lesions, and when those with lesion size <2.5 mm was excluded from the evaluation, they reported that there was no correlation between SUVmax and stages (16). When the stages of lung cancer cell types were compared according to SUV_{max} values, no difference was found between SUV_{max} values in the present study. There was no difference in SUV_{max} values between early and advanced stage tumors in all lung cancer histopathologic cell types. In addition, comparisons were made between SUV_{max}

Table 3. The comparison of SUV_{max} values according to the TNM stage of the lung cancers

Stage	**Stage 1** (n=21)	**Stage 2** (n=31)	**Stage 3A** (n=32)	**Stage 3B** (n=27)	**Stage 3C** (n=17)	**Stage 4** (n=105)	**p**
Adenocarcinoma	8.5±3.8 (n=9)	11.6±4.9 (n=14)	8.0±3.5 (n=11)	12.4±10.9 (n=8)	12.8±5.6 (n=6)	11.2±5.3 (n=67)	0.285
Squamous cell carcinoma	12.6±3.8 (n=11)	14.3±7.5 (n=15)	14.4±6.1 (n=16)	14.6±6.1 (n=11)	21.3±15.2 (n=4)	16.9±7.9 (n=24)	0.377
Small cell lung carcinoma	28.1±0.1 (n=1)	12.8±4.3 (n=2)	10.1±2.1 (n=5)	11.1±5.2 (n=8)	11.2±3.1 (n=7)	12.9±6.2 (n=14)	0.061

SUV_{max}: Maximum standardized uptake value, n: Number

Table 4. The comparison of the SUV_{max} values according to the lymph node involvement

Lymph node involvement	N0 (n=66)	N1 (n=19)	N2 (n=53)	N3 (n=95)	p
Adenocarcinoma	11.2±5.4 (n=34)	9.6±3.9 (n=12)	12.1±8.1 (n=25)	10.3±4.3 (n=44)	0.490
Squamous cell carcinoma	13.9±7.4 (n=29)	15.5±4.0 (n=7)	16.8±7.6 (n=19)	15.5±8.7 (n=26)	0.645
Small cell lung carcinoma	17.9±9.4 (n=3)	- (n=0)	10.3±2.5 (n=9)	12.3±5.5 (n=25)	0.114

SUV_{max}: Maximum standardized uptake value, n: Number

Table 5. The comparison of SUV_{max} values according to the metastasis

Metastatic disease	No metastasis (n=128)	Metastasis (n=105)	p
Adenocarcinoma	10.5±6.1 (n=48)	11.2±5.3 (n=67)	0.496
Squamous cell carcinoma	14.5±7.5 (n=57)	16.9±7.9 (n=24)	0.209
Small cell lung carcinoma	11.8±5.1 (n=23)	12.9±6.2 (n=14)	0.544

SUV_{max}: Maximum standardized uptake value, n: Number

values of different cell types within each stage and there was a significant difference in stage 1, 3A and 4.

The size increase in the primary tumor, SUV_{max}>9, central localization and vascular invasion were reported to be associated with lymph node involvement in the study evaluating 159 patients and 1001 lymph node stations, by Billé et al. (20). In addition, when lymph node size was ≥1 cm, the sensitivity of PET/CT was 85% in the evaluation of malignant invasion (20). Although PET/CT is more useful than other imaging techniques in lymph node metastatic evaluation, it has been reported that PET findings cannot substitute for histological examination because there may be false negative and positive results (21). In a study conducted with 80 patients with NSCLC, the SUV_{max} value of the primary tumor was significantly related to lymph node involvement (22). In N0, N1, and N2 lymph node involvements, the primary tumor SUV_{max} values were 5.8±4.8, 8.1±5.1, and 8.7±3.4, respectively (p=0.036) (22). Similarly, a positive correlation was shown between the primary tumor SUVmax value and lymph node metastasis in another study (18). When the SUV_{max} value of primary tumor was ≥12, lymph node metastasis was detected in 70% of the patients; however, the frequencies of lymph node metastasis of SCC and AC tumor types were not different (18). In present study, there was no difference in SUV_{max} values between lung cancer cell types according to lymph node involvement. However, there was a statistically significant difference in the SUV_{max} values of lymph node involvement for N1, N2 and N3 according to tumor types. Especially for N1 and N3 lymph node involvement, it was determined that the SUV_{max} value of SCC was significantly higher than that of AC.

Each unit increase in the SUV_{max} value of the primary tumor was reported to increase the probability of metastasis approximately 1.5 times (23). Cerfolio et al. (24) reported that SUV_{max} was an independent marker for predicting recurrence, survival, and distant organ metastasis. When lung cancer histopathological cell types were evaluated according to metastasis status, SUV_{max} values were not different in our study; on the other hand, when metastasis was evaluated according to cell types, a positive correlation was revealed, and this was caused by the SCC cell type.

The limitations of this study were that it was a single-center, retrospective study, and parameters such as metabolic tumor volume and total lesion glycolysis, which have been reported to have prognostic significance in PET/CT except for SUV_{max} (25) were not available. Another limitation was the small sample size, limiting the generalizability of the findings to other populations.

Conclusion

This study showed that SUV_{max} value of SCC was highest among lung cancers. SUV_{max} values of the histopathological types of lung cancer were similar in terms of the stages, lymph node involvement and presence of metastasis. No relationship between the SUV_{max} value of the primary tumor on PET/CT and the TNM stages of the tumor was recorded.

Ethics

Ethics Committee Approval: We obtained permission from the Clinical Research Ethics Committee of the Umraniye Training and Research Hospital (approval no: 37, date: 20.03.2019).

Informed consent: Informed consent was waived because of the retrospective design of the study.

Peer-review: Externally peer-reviewed

Authorship Contributions

Concept: T.Ç., K.C., E.G.I., Design: T.Ç., K.C., E.G.I., Data Collection or Processing: T.Ç., K.C., Analysis or Interpretation: T.Ç., K.C., Literature Search: K.C., E.G.I., Writing: T.Ç., K.C.

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The diagnostic value of basophil activation tests in hypersensitivity reactions due to contrast media

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ABSTRACT

Aims: Current tests used for the diagnosis of hypersensitivity reactions (HRs) due to radiocontrast media (RCM) are associated with important shortcomings. The present study investigated the diagnostic value of basophil activation test (BAT) in immediate HRs to RCM.

Methods: This was a cross-sectional study with prospective enrollment. The skin test (ST) with RCM was performed in cases with suspicious immediate RCM allergy. The patients were categorized into four groups (G); G1: positive ST to RCM; G2: negative ST to RCM; G3: no RCM reaction; and G4: controls with no history of RCM use. BAT was performed using flow cytometry to analyze CD63 and CD203c expression in basophils. Activated basophil percentage (ABP), stimulation index (SI), mean fluorescence intensity (MFI), and the SI of the MFI (MFI SI) were compared across 4 groups.

Results: A total of 42 patients (female: 73.8%, age 26 to 78 years) were evaluated. SI was greater in G1 (n=7) than in G3 (n=9) (p=0.05); MFI was greater in G1 than in G4 (n=17) (p=0.03); and MFI SI was greater in G1 than in G4 (p=0.03). Four subjects (57.1%) in G1 had an ABP of >5%, 3 subjects (42.8%) had a SI ≥2, and 5 patients (71.4%) had elevated MFI; the corresponding figures in G2 (n=9) were 2 (22.2%), 1 (11.1%) and 1 (11.1%), respectively. All three criteria were positive only in 2 patients in G1.

Conclusions: This study suggests that, based on ABP, SI, and MFI assessments, BAT may represent a partially useful test in patients with a history of immediate RCM reactions.

Introduction

Adverse reactions associated with radiocontrast media (RCM) generally occur within the first hour of administration and are termed as “immediate reactions”. And delayed reactions are less frequent and occur ≥1 hour after RCM administration (1,2). Globally, each year more than 75 million imaging studies involving RCMs are performed and sometimes severe immunologic reactions may emerge (3,4). Currently, no clinical sign or laboratory test that would allow us to predict individuals who are more likely to experience such hypersensitivity reactions (HR) exists (5,6). The reported sensitivity of skin tests (ST) performed within 6 months of a hypersensitivity event for IgE-mediated immediate reactions ranges between 20% and 50%, and these figures progressively decrease with increasing duration of time after the reaction (7). Due to their chemical characteristics, RCMs may trigger a reaction by direct stimulation of the effector cells (mast cells, eosinophils, basophils) responsible for a HR. Even in patients with a history of immediate RCM reaction, the ST may not be positive, or a negative ST cannot unequivocally rule out a HR, limiting the utility of these ST for screening purposes in daily clinical practice (8,9).

Despite the identification of certain risk factors in previous large-scale studies (e.g. female gender, atopy, use of ionic RCM), no test can definitively diagnose or exclude a RCM HR (10). On the other hand, establishing a confident diagnosis of RCM HR is very important from a clinical viewpoint. Canceling a significant imaging study due to possible HR based on an inadequately investigated RCM reaction may lead to untoward consequences in terms of diagnosis and treatment of a patient. Functional in vitro tests that can predict such reactions with higher sensitivity and specificity have been developed (11,12). These tests are based on the measurement of mediators released from basophils. In this respect, the basophil activation test (BAT) is based on the detection of certain molecules produced by cell activation and presented to the cell surface. While basophils produce and release a wide range of mediators, they also express the activation markers on their surface. These alterations occurring at the cellular level are evaluated with the flow cytometry method. Major activation markers for basophils include CD63 and CD203c (13). BAT, which allows the detection of these molecules, has been shown to be valuable in the detection of reactions caused by a variety of substances including muscle relaxants, antibiotics, antiseptics, and plasma expanders (14-16). In previous studies, BAT has been used to confirm the results of ST or to assist in making clinical decisions among therapeutic options in patients. Therefore, the objective of this study was to evaluate both ST and BAT in patients presenting with immediate RCM HR history, to compare these two examinations, and to investigate the diagnostic value of BAT among patients in whom ST failed to diagnose RCM reactions.

Methods

Study Groups

This was a cross-sectional study with prospective enrollment. The participants were selected among the outpatients of the Immunology and Allergy Diseases Polyclinic of Gülhane Training and Research Hospital between October 2011 and October 2012. The main inclusion criterion was admission due to suspicious RCM allergy. The eligible patients with RCM allergy history were divided into two groups as those with an immediate or delayed type of reaction. The distinction between immediate and delayed RCM HR was made using the criteria proposed by Brockow et al. (17,18). The severity of the reaction in immediate reactions was examined using the Ring and Messmer reaction severity scale (19). ST with RCMs were performed in the immediate reaction group.

Then, the patients were categorized into four study groups based on RCM use and ST positivity with RCM. Group (G) 1 consisted of the patients with immediate RCM reaction and positive ST to RCM; G2 consisted of the patients with immediate RCM reaction and negative ST to RCM; G3 consisted of healthy controls, in whom RCM was administered with no reaction; and G4 consisted of healthy volunteers without any history of RCM use. Then BAT was performed in all these four groups using the RCM, which was associated with the highest frequency of ST positivity.

The participants in both patient and control groups were at the age of 18 years or older and those who agreed to participate in this study were included in the study. Patients younger than 18 years of age despite having a history of immediate type reaction with RCM, patients with a history of late type RCM reaction, and patients with a history of allergic reaction to other drugs despite having a history of immediate type reaction with RCM were excluded from the study. It was paid attention that the patients in the control groups did not have a history of allergic reaction to other drugs. Written informed consent was obtained from all participants in the study groups. The study was approved by the Local Ethical Committee of Gülhane Training and Research Hospital, Ankara (date: 09.08.2011, protocol no: 177).

Skin Test

Skin prick test (SPT) and intradermal test using six different RCM were performed in patients with an immediate reaction history with RCM use (17,18,20). In these tests the following RCMs were utilized: iobitridol (350 mg I/mL, Xenetix, Guebet, Villepinte, France), iodixanol (320 mg I/mL, Visipaque, Ireland), iomeprol (300 mg I/mL, Iomeron, Italy), iohexol (300 mg I/mL, Omnipaque, GE Healthcare Ireland, Cork, Ireland), ıopromid (300 mg I/mL, Ultravist, Bayer Schering Pharma, Berlin, Germany), and sodium diatrizoate (150 mg I/mL, Urografin, England). Histamine (0.01%) and physiological saline were used as positive and negative controls. SPT performed with

RCM at a dilution of 1/1 was administered on the volar surface of the forearm and the reading was performed 15 minutes after administration. SPT with a ≥3 mm induration as compared to negative control was considered positive (18,21). If SPT was negative, 0.03-0.05 mL of RCM at a dilution of 1/100 and 1/10, respectively, were administered via intradermal route on the volar surface of the forearm to form a bleb with a 3 to 5 mm diameter. The readings were performed 15 minutes after the injections. An induration of 3-5 mm, 6-10 mm, and 11-15 mm was graded 1+, 2+, and 3+, respectively (18,22).

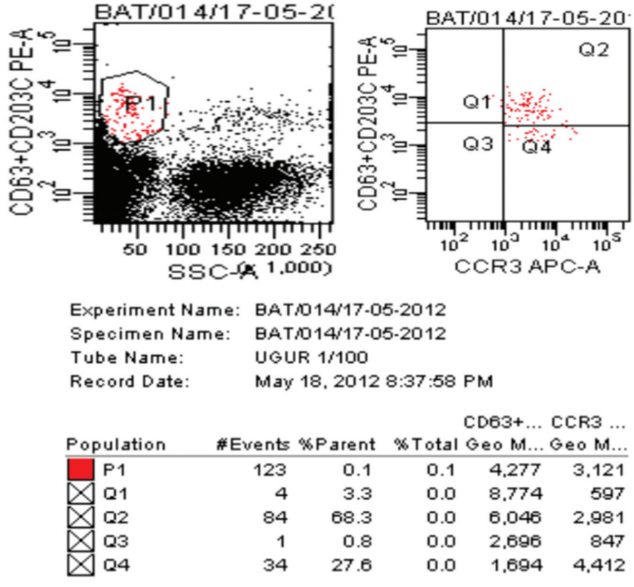
Basophil Activation Test

Iobitridol (Xenetix, Guebet, Villepinte, France) at a concentration of 1/100 was chosen for BAT testing, as this was the agent associated with the highest frequency of ST positivity and was used in most of the patients (23). Blood samples of 3-4 mL were obtained into K3 EDTA tubes from patients and healthy controls, and testing was performed within a maximum duration of 1 hour.

BAT was performed using Flow CAST^{R} Highsens (Bühlmann Laboratories, BAT, Switzerland) flow cytometry kits containing lysing reactive with stimulation buffer containing calcium and heparin; stimulation control containing anti-FcεRI monoclonal antibody (mAb); stimulation control containing N-formyl-methionyl-leucyl-phenylalanine (fMLP); and staining reactive consisting of anti-CD63-PE-DY647/anti-CD203c-PE-DY647/anti-CCR3-PE mAb mixture. For BAT, 5 flow tubes were prepared, with the delivery of 50 µL of peripheral blood in each. Fifty µL of stimulation buffer was added into PB (Patient Background tube); stimulation control containing 50 µL of anti-FcεRI mAB was added into PC 1 (patient control) tube; stimulation control containing 50 µL of fMLP was added into PC 2 tube; 50 µL of PBS was added into the basal tube, and finally, 50 µL of RCM at a dilution of 1/100 was added into the last tube. Also, 100 µL of stimulation, the buffer was added into all tubes. The antibody mixture containing anti-CD63-PE-DY647/anti-CD203c-PE-DY647/anti-CCR3-PE was dispensed into each tube at a volume of 20 µL. The tubes were gently mixed and were incubated for 15 minutes in a water bath for 37 °C with closed caps. The tubes were kept at room temperature in a dark space for erythrocyte lysis. The tubes prepared in this manner were analyzed within 1 hour using a FACS Canto flow cytometry device (BD, San Jose, USA) and FACS Diva software. For analyses, a total of 200.000 cells were counted and the entry of 300 cells into the basophil-gate was targeted. When the CD63+ CD203c expression was greater than 5% and the stimulation index (SI) was ≥2, the result was considered positive (Figure 1, 2). SI was defined as the ratio of CD63+ CD203c the expression after *in vitro* stimulation with RCM to basal CD63+ CD203c expression (23).

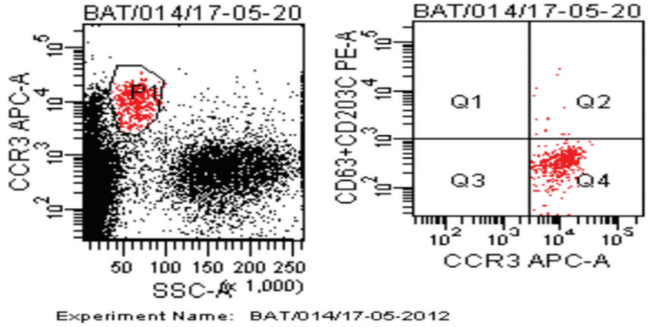
Statistical Analysis

For all statistical analyses, Statistical Package for the Social Sciences (SPSS) statistical software package (SPSS 15.0, SPSS Inc., Chicago, IL, USA) was used. Descriptive statistics were expressed as mean±standard deviation and percentage. Normality of the tests was evaluated with Kolmogorov-Smirnov test. For multiple group comparisons, one-way ANOVA with Bonferroni correction was utilized. Qualitative data were compared with chi-square analysis. P value of less than 0.05 was considered statistically significant. The sensitivity of the ST was defined as the ratio of patients with positive ST for RCM to the patients with RCM reaction history. BAT sensitivity was defined as the number of true positive cases/number of true



Population	#Events	%Parent	%Total	CD63+... Geo M...	CCR3 ... Geo M...
P1	123	0.1	0.1	4,277	3,121
Q1	4	3.3	0.0	8,774	597
Q2	84	68.3	0.0	6,046	2,981
Q3	1	0.8	0.0	2,696	847
Q4	34	27.6	0.0	1,694	4,412

Figure 1. Positive basophil activation test after *in vitro* stimulation with 1/100 radiocontrast media



Population	#Events	%Parent	%Total	CD63+... Geo M...	CCR3 ... Geo M...
P1	474	0.3	0.3	####	9,808
Q1	0	0.0	0.0	####	####
Q2	14	3.0	0.0	3,329	6,480
Q3	0	0.0	0.0	####	####
Q4	460	97.0	0.3	####	9,933

Figure 2. Negative basophil activation test after *in vitro* stimulation with 1/100 radiocontrast media

positive cases+false-negative cases, while BAT specificity was defined as the number of true negative cases/number of true negative cases+false-positive cases. The positive predictive value (PPV) for BAT was formulated as the number of true positive cases/number of true positive cases+false-positive cases, and the negative predictive value (NPV) for BAT was formulated as the number of true negative cases/number of true negative cases+false-negative cases.

Results

A total of 100 patients who were admitted to the immunology and allergy disease outpatient with suspicion of RCM allergy were included. There were 88 patients (88%) with immediate and 12 patients (12%) with delayed reactions. Patients with immediate reaction history underwent ST with six different RCMs. ST showed iobitridol positivity in 11 of 77 patients, iodixanol positivity in 1 of 36 patients, iomeprol positivity in 4 of 75 patients, iohexol positivity in 2 of 42 patients, Iopromid positivity in 1 of 5 patients, and sodium diatrizoate positivity in 1 of 9 patients (Table 1).

A total of 42 subjects [12 male (28.6%), 30 female (71.4%)] aged between 26 and 78 years formed four study groups. Group 1 consisted of 7 patients, G2 consisted of 9 patients, G3 consisted of 9 healthy controls, and G4 consisted of 17 healthy volunteers. All the participants in these groups underwent BAT using the RCM containing iobitridol. The four study groups were compared with respect to demographic characteristics. No significant differences between the groups were observed in terms of the parameters such as gender, presence of gadolinium allergy, family history of allergic disorders, recall of suspected-culprit RCM, reaction severity, and procedure during which the reaction had occurred. There were statistically significant differences between the groups related to age, and admission to the clinic but these differences did not affect the test results (Table 2). ST were performed 3 to 120 months after the RCM reaction. When subjects with a positive reaction history were compared regarding the time elapsed between reaction and RCM ST, no significant differences were detected. Patients in G1 and G2 did not exhibit significant differences with respect to symptoms occurring during the reaction.

When BAT results after *in vitro* stimulation with RCM were examined in the overall study groups, an increase in the percentage of activated basophils was found in 4 patients (57.1%) in G1, 7 patients in G2 (77.7%), 2 patients (22.2%) in G3, and

Table 1. Skin test results of patients with immediate type reaction history with radio contrast media (n=88)

RCM*	Iobitridol (n=77)	Iodixanol (n=36)	Iomeprol (n=75)	Iohexol (n=42)	Iopromid (n=5)	Sodium diatrizoate (n=9)
Positive SPT	0	0	1	1	0	0
Positive 1/10 IDT	5	0	1	0	1	1
Positive 1/100 IDT	6	1	2	1	0	0
Total positive test results, n (%)	11 (14.2)	1 (2.7)	4 (5.3)	2 (4.7)	1 (20)	1 (11.1)
Total negative test results, n (%)	66 (85.8)	35 (97.3)	71 (94.7)	40 (95.3)	4 (80)	8 (88.9)

*Each patient was not tested with all types of radio contrast media. SPT: Skin prick test, 1/10 IDT: Intradermal test with 1/10 dilution, 1/100 IDT: Intradermal test with 1/100 dilution

Table 2. Demographic characteristics of the study groups (n=42)

		G1 (n=7)	G2 (n=9)	G3 (n=9)	G4 (n=17)	p value
Age, year	Mean±SD	43.4±13.3[a]	60.7±11.4[b]	42.6±7[c]	42.9±7.7[d]	<0.001
Gender (n)	Man	1	4	1	6	0.317
	Female	6	5	8	11	
Gadolinium allergy (n)	Positive	2	0	1	1	0.072
	Negative	5	6	4	5	
	No use	0	3	4	11	
Admission type (n)	IA	2	2	9	17	<0.001
	CC	1	3	0	0	
	RC	4	4	0	0	
Family history of allergic diseases (n)	Positive	2	2	2	2	0.774
	Negative	5	7	7	15	
Suspicious RCM recall (n)	Yes	1	1	0	No reaction	0.528
	No	6	8	9	No reaction	

Between groups differences: Age, G1 vs. G2, p=0.006; G2 vs. G3, p=0.002; G2 vs. G4, p<0.001).
IA: Individual admission, CC: Clinical consultation, RC: Radiologist consultation, SD: Standard deviation

9 patients (52.9%) in G4. The sensitivity comparison between G1 and G2 based on positive BAT results showed that the sensitivity was 57.1%, specificity was 22.2%, PPV was 36.3%, and NPV was 40%. When the ST results were disregarded and patients with positive reaction history (G1 and G2) and negative reaction history (G3 and G4) were taken into consideration, the sensitivity was 68.7%, specificity was 57.6%, PPV was 50%, and NPV was 75%. BAT results after in vitro stimulation with RCM and basal BAT results were examined in the overall study groups, the number of patients with a basophil percentage >5% was 3 (42.8%) in G1, 2 (22.2%) in G2, 1 (11.1%) in G3, and 5 (29.4%) in G4. When G1 and G2 were considered with respect to this positive BAT result, the sensitivity, specificity, PPV, and NPV were 42.8%, 77.7%, 60%, and 63.6%, respectively. When the ST was not taken into consideration and the groups with (G1 and G2) and without (G3 and G4) apositive history of reaction were examined, the rates of sensitivity, specificity, PPV, and NPV were 31.2%, 76.9%, 45.4%, and 64.5%, respectively. Overall, a SI value of ≥2 was observed in 3 (42.8%), 1 (11.1%), 1 (11.1%), and 2 (11.7%) of the patients in G1, G2, G3, and G4, respectively. When SI ≥2 patients were considered to have positive BAT result in G1 and G2, the sensitivity, specificity, PPV, and NPV were 42.8%, 88.8%, 75%, and 66.6%, respectively. When the ST was excluded, the assessment among groups with a history of reaction (G1 and G2) and without history of reaction (G3 and G4) showed a sensitivity, specificity, PPV, and NPV of 25%, 88.4%, 57.1%, and 65.7%, respectively.

In the overall study groups, the mean fluorescence intensity (MFI) increased in 5 (71.4%), 1 (11.1%), 4 (44.4%), and 8 (47.05%) of the patients in G1, G2, G3, and G4, respectively, after in vitro stimulation with RCM. When this increase was considered as a positive BAT result in G1 and G2, the sensitivity, specificity, PPV, and NPV were found to be 71.4%, 88.8%, 83.3%, and 80%. When the skin test results were excluded and groups with (G1, G2) or without (G3, G4) a history of reaction were considered, the sensitivity, specificity, PPV, and NPV were 37.5%, 53.8%, 33.3%, and 58.3%, respectively. In all groups, an MFI SI ≥2 was determined in 2 patients (28.5%) in G1, 1 patient (11.1%) in G2, 2 patients (22.2%) in G3, and 2 patients (11.7%) in G4. When MFI SI values indicating a positive BAT result were taken into consideration in G1 and G2, the sensitivity, specificity, PPV, and NPV were 28.5%, 88.8%, 66.6%, and 61.5%, respectively. When the ST results were excluded, an assessment of groups with (G1 and G2) or without (G3 and G4) a history of reaction showed a sensitivity, specificity, PPV, and NPV of 18.75%, 84.6%, 42.8%, and 62.8%, respectively.

The four study groups were also compared regarding the following main laboratory results: white blood cell (WBC), eosinophil percentage, eosinophil count, basophil percentage, basophil count, total immunoglobulin E, basal BAT, BAT after RCM, SI, basal MFI, MFI after RCM, and MFI SI. There were significant differences between G1 and G3 in terms of WBC count (p=0.046), G2 and G3 in terms of eosinophil count (p=0.025), G1 and G3 in terms of SI (p=0.05), G1 and G4 in terms of MFI at 1/100 RCM (p=0.03), and G1 and G4 in terms of MFI SI (p=0.03) (Table 3).

Discussion

Although an IgE-mediated process has been implicated, the presence of specific IgE against RCM could only rarely be identified in ST (10). In this study, the potential role of BAT in diagnosing immediate HR due to RCM was investigated. The ST with several RCMs yielded a positive result in 13 (14.7%) patients and a negative result in 75 (85.3%) patients. According

Table 3. Comparison between the groups in terms of laboratory measurements and basophil activation test results (n=42)

		G1 (n=7)	G2 (n=9)	G3 (n=9)	G4 (n=17)	p value
WBC-C (x10^3 cells/μL)	Mean±SD	6700±787	6544±1005	4911±1395	5682±1434	0.019
Per. of Eos.	%	2.4	2.7	1.6	2.5	0.144
Eos-C. (x10^3 cells/μL)	Mean±SD	0.158±0.04	0.184±0.08	0.077±0.03	0.144±0.08	0.029
Per. of Bas.	%	0.9	0.8	0.8	0.9	0.739
Bas-C. (x10^3 cells /μL)	Mean±SD	0.06±0.01	0.05±0.02	0.04±0.01	0.05±0.02	0.196
Total. IgE (IU/mL)	Mean±SD	17.8±18.2	29.7±15.8	31.08±22.6	22.2±20.9	0.474
Percentage of BAT basal	%	4.5	3.01	6.3	5.5	0.415
Percentage of BAT 1/100 RCM	%	7.2	3.5	3.4	3.9	0.395
SI	Mean±SD	2.1±2.2	1.3±0.5	0.6±0.4	0.9±0.5	0.043
MFI basal	Mean±SD	2977±1906	2981±1316	4061±2489	4403±3014	0.511
MFI 1/100 RCM	Mean±SD	4734±3001	2535±904	2626±965	2359±767	0.024
MFI SI	Mean±SD	2.04±1.7	0.94±0.47	0.8±0.42	0.68±0.28	0.026

Between group differences: WBC-C., G1 vs. G3, p=0.046; Eos-C., G2 vs. G3, p=0.025; SI, G1 vs. G3, p=0.05; MFI 1/100 RCM, G1 vs. G4, p=0.03; MFI SI, G1 vs. G4, p=0.03. WBC-C: White blood cell count, Per. of Eos.: Percentage of eosinophils, Eos-C.: Eosinophil count, Per. of Bas.: Percentage of Basophils, Bas-C.: Basophil count, IgE: Immunoglobulin E, BAT: Basophil activation test, BAT 1/100: BAT achieved with using 1/100 diluted RCM, RCM: Radio contrast media, MFI: Mean fluorescence intensity, MFI 1/100 RCM: MFI achieved with using 1/100 diluted RCM, MFI SI: Stimulation index of the mean fluorescence intensity.

to these data, the sensitivity observed in our study is lower than the previously reported figure of 20% (7,10,21,22,24). In another study, 270 patients with a history of reaction to RCM also underwent skin testing, and the predictive value and sensitivity of ST for ionic RCMs were reported to be 1.2% and 3.7%, respectively (25). When this latter piece of evidence is taken into consideration, the observed sensitivity of ST in our study was higher. BAT has also been introduced as an alternative or complementary method to skin testing in the assessment of immediate HRs due to RCM (26). Although some case reports have suggested that BAT may have a significant role in the identification of early RCM reactions, published clinical data regarding its predictive value is very scarce (15,23,27).

On the other hand, the presence of a patient group with contrast media use but no reaction history (i.e., G3) may be considered as a distinguishing aspect of our study. Patient and control groups described above facilitate the assessment of BAT results in the context of the study design. However, one disadvantage of our study was the inclusion of a smaller patient population in G1, as compared to previous similar studies (7<11) (23). In general, the stimulant doses used in BAT have been partially established thanks to previous investigations. However, BAT testing for RCM has been relatively limited. In one previous study, 1/10 and 1/100 dilutions of 5 separate RCM were used irrespective of the results of the ST, and *in vitro* stimulation with 1/100 RCM was found to be associated with high sensitivity and specificity for BAT, correlating well with clinical manifestations (23). In the current study, since the culprit RCM could not be generally recalled, 1/100 concentration of the RCM associated with the highest frequency of positive results in ST was used as a stimulant for BAT.

The basophilic activation marker utilized in BAT has crucial importance with regard to test results (28). CD63 was generally used as the basophilic activation marker in previous BAT applications used for the diagnostic examination of drug HRs (29). However, the use of CD63 alone is associated with certain shortcomings. For instance, CD63 can be hardly identified in resting cells and in addition to basophils, may also be expressed by some other cell types, such as platelets. Also, it may take up to 15-25 minutes before the expression reaches the maximum level. Challenges associated with CD63 expression may also lead to false-negative results. On the other hand, CD203c is the only marker that is not expressed by other peripheral blood cells and that is expressed by basophils both in the resting and active state. Expression reaches the maximum more rapidly, i.e., within 5 to 10 minutes, as compared to CD63. On the other hand, CD203c expression in basophils, which is not as strong as that of CD63, may be affected by many substances other than the real stimulus, and thus may easily be associated with false-positive results. For these reasons, a BAT kit containing both CD63 and CD203c activation markers has been utilized in the current study to improve the reliability and accuracy of our results. To the best of our knowledge, this study is the first of its kind in using both CD63 and CD203c as basophilic activation markers for a BAT procedure aiming at detecting RCM hypersensitivity.

Basal active basophil percentage (ABP) after *in vitro* stimulation with RCM, SI; basal MFI, MFI after *in vitro* stimulation with RCM, and MFI SI values were obtained with BAT. Also, several other laboratory parameters that could assist in interpreting HR, including the WBC, eosinophil count, eosinophil percentage, basophil count, basophil percentage, and total immunoglobulin E, were examined. Comparisons between the groups showed higher WBC count in G1 than in G3, higher eosinophil count in G2 than in G3, higher SI in G1 than in G3, higher MFI after RCM in G1 than in G4, and higher MFI SI in G1 than in G4. The higher parameters were detected in G1 and G2 groups. A higher number of parameters that were significantly elevated (e.g., WBC count, eosinophil count) suggests that the HR may persist in these individuals. On the other hand, SI BAT, MFI, and MFI SI after in vitro stimulation with RCM, which are responsible for the difference between the groups of G1 and G3 and G4, have a major significance in terms of confirming the RCM reaction history with BAT. In other studies that utilized BAT, the criteria for positivity were generally not fully standardized. In one study utilizing RCMs for stimulation, expression of CD63+ basophils >5% and a SI ≥2 was considered as a positive result (23). In our study, the test results were interpreted using the same criteria, although additional factors that might have an impact in terms of the outcome were evaluated, including an increase in BAT expression after *in vitro* stimulation with RCM (no upper limit defined), increase in MFI, and an MFI SI of ≥2. The sensitivity, specificity, and predictive value of the BAT were first compared between the groups with a history of reaction on the basis of the ST (i.e., G1, and G2), and then between all groups regardless of the history of a reaction.

A high level of sensitivity, specificity, PPV, and NPV values was achieved when parameters such as an ABP >5% and SI ≥2 were considered as the criteria for positivity in the assessments in G1 and G2. However, in contrast with some previous studies, the highest degree of sensitivity, specificity, PPV and NPV were obtained when the increase in MFI compared to baseline after stimulation with RCM was considered (sensitivity 71.4%, specificity 88.8%, PPV 83.3%, and NPV 80%). These results are in line with previously reported numbers such as a sensitivity of 50% and a specificity of 90.7% (23). Regarding the reliability of the tests of our study, the highest specificity and NPV were obtained when the criteria consisting of elevated MFI and SI ≥2 were utilized. Also, when the overall study groups were compared, SI, MFI, and MFI SI values were significantly higher in the patient groups (Table 1). When the results of this comparison and the resultant sensitivity, specificity, PPV, and

NPV were collectively considered, it may be assumed that a SI ≥2 may be considered to show a positive result if BAT testing is performed in subjects with suspected RCM reaction. When an assessment among patients in G1 and G2 was made regarding ABP ≥5%, a SI ≥2, and MFI elevation as compared to baseline after stimulation with RCM, it was found that 4 patients (57.1%) had ABP ≥5%, 3 patients (42.8%) had SI ≥2, and 5 patients (71.4%) had elevated MFI in G1. All three positivity criteria were present in only 2 patients (28.5%) in G1. On the other hand, 2 patients (22.2%) had an ABP >5%, 1 patient (11.1%) had SI ≥2, and 1 patient (11.1%) had elevated MFI in G2. All three positivity criteria were not present in G2.

Based on the percentage of activated basophils, SI, and MFI results, we may suggest that BAT alone does not seem to be as useful as skin tests in patients with a history of immediate RCM reaction history. When a good level of specificity for BAT is considered between 88.4% and 100% based on the use of all allergens, the elevations in SI and MFI in our study may partly provide this specificity. However, our results also showed that BAT could confirm only a fraction of the patients with immediate RCM reactions, in whom ST was positive, while it did not seem to provide meaningful contributions in those with negative ST reaction to RCM. When patients with a history of reaction were considered, an ABP >5%, SI ≥2, and MFI elevation after RCM stimulation provided higher sensitivity, specificity, PPD, and NPD as compared to other parameters, and therefore, their combined use may be important for the reliability of the test. The failure to achieve BAT positivity using these parameters in some patients may be accounted for by the prolonged duration between the reaction and testing time, or by the fact that certain reactions may not be mediated by the degranulation of basophils. While this is the second study systematically analyzing the accuracy of BAT in immediate RCM reactions, it is the first to utilize two different basophilic activation markers for RCM reactions to obtain complementary and more accurate results. When the findings of our study are taken into consideration, we may suggest that our study was partially successful in showing a consistency between ST results and BAT, and that BAT may potentially be used as a diagnostic tool, and particularly a confirmatory test in some cases of RCM related immediate HRs. It appears from these results that combined use of both ST and BAT may be more useful in the diagnosis of RCM related immediate HRs. Combined use of ST/BAT may represent an alternative option for the identification of a safe RCM in patients with a previous history of RCM and also for screening RCM related immediate HRs prior to RCM administration in high-risk patients. In the light of the currently published data, it appears that two separate activation markers have never been measured simultaneously in studies examining the diagnostic accuracy of BAT in RCM reactions. This situation gives a privilege to our study.

On the other hand, this study has some limitations. One of them is the low number of patients in the groups. The other important limitation of the study is that BAT test was performed only with one RCM (iobitridol). Moreover, since it was not ethically possible to carry out skin testing with RCMs in control subjects, the specificity and predictive value of our skin testing could not be estimated.

Conclusion

BAT represents a novel and promising diagnostic tool for immediate-type drug HRs involving IgE-mediated mast cell and basophil activation. Although BAT was successful only in a certain proportion of our patients with a history of RCM, the results of the current study seem to be supportive of this hypothesis. BAT may also provide a safe alternative testing tool in cases where routine *in vitro* diagnostic methods do not allow alternative testing, or in situations where clinical suspicions need to be confirmed (despite negative test results) in patients for whom ST or drug provocation tests are potentially dangerous, particularly if several drugs need to be tested altogether. However, since BAT is mostly an investigational method, further clinical studies are warranted to extrapolate its use into daily clinical practice. On the other hand, since BAT studies on RCM are relatively few and mostly involve case reports, studies with a larger sample size are required to obtain more accurate sensitivity, specificity, PPV, and NPV data and to confirm these observations.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethical Committee of Gülhane Training and Research Hospital, Ankara (date: 09.08.2011, protocol no: 177).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.D., Ö.K., A.B., O.Ş., Design: F.D., U.H.M., M.G., Ö.K., S.Y., Data Collection or Processing: F.D., S,Y., A.B., Analysis or Interpretation: U.H.M., M.G., A.A.P., R.I.S., Literature Search: F.D., Writing: F.D.

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

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Determination of awareness, knowledge and behavior of a preclinical dentistry student group about the COVID-19 pandemic

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ABSTRACT

Aims: Severe acute respiratory syndrome Coronavirus-2 is usually transmitted by direct contact such as coughing and sneezing. In this survey, we aim to evaluate the knowledge of a group of preclinical dental students, who have not yet started working in the clinic, about the pandemic, protective measures, and the risks of infection in dental clinics.

Methods: In this descriptive study the criterion sampling method, one of the qualitative sampling techniques, was used to evaluate students with no clinical experience. The respondents were the preclinical dental students (1st, 2nd and 3rd classes). The survey questionnaire consisted of four domains: demographic information; general information about Coronavirus disease-2019 (COVID-19); preventive behaviors applied during the pandemic; and relationship of COVID-19 with dentistry.

Results: Of the students surveyed (n=95, age between 18 and 23 years, female 67.4%), 47 were from the 1st class, 24 from the 2nd class, and 24 from the 3rd class. The least applied item among the preventive behaviors was the item "I have increased the frequency of cleaning the places that are in contact with the hands (e.g., door handle)" by 90.5%. The majority of the responders (97.9%) thought there was a high risk of transmission from the patient to the dentist. There were small differences in the direction of responses across student classes.

Conclusions: Dentistry students who participated in this study showed significant awareness of COVID-19, and were highly adapted to the preventive behaviors. However, the results also suggested some deficiencies in the knowledge level of students about the risk of viral transmission during dentistry procedures.

Introduction

A type of virus causing pneumonia in patients and rapidly spreading was detected in Wuhan, the Republic of China, in late December 2019 (1). On January 8, 2020, this virus was reported to be a coronavirus type by the Chinese Center for Disease Control and Prevention (2). The virus was called Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) (3). The disease caused by this virus, Coronavirus disease-2019 (COVID-19), was officially announced as an international emergency on January 30, 2020 and with the spread of this disease to dozens of countries, COVID-19 disease was declared by the World Health Organization (WHO) as a pandemic (4).

The first case in Turkey was diagnosed on 11th March 2020, andas of 23 December 2020 and 2.082.610 cases have been diagnosed with COVID-19 and 18.860 people have died due to coronavirus (5). The most important of the previous coronavirus types, including SARS and Middle East Respiratory syndrome outbreaks, had higher mortality rates, although they had a lower contagious rate compared to COVID-19 disease (6).

The most important symptoms of COVID-19 disease are dry cough, fever and shortness of breath (7). In addition, patients with a severe condition may develop complications such as pneumonia, pulmonary edema, Acute respiratory distress syndrome, multiple organ failure or death (8). WHO has informed the public that the duration of clinical symptoms to arise after contact with this virus is an average of 5 days and the incubation time is up to 14 days (9).

The virus is usually transmitted by direct contact such as coughing and sneezing. Although the main source for transmission is symptomatic people, carrier individuals who are asymptomatic have also been reported to be effective in spreading this virus (10). It has been shown that SARS-CoV-2 virus can remain viable in aerosols emerging during dental procedures approximately for 3 hours. Therefore, dental clinics are considered among the places of a higher risk of cross infection for patients and the dental team (11,12).

In order to prevent spread of the COVID-19 outbreak, it is important that the patients going to the dental clinics and the dentistry staff implement the protective measures recommended by the Ministry of Health in Turkey during the COVID-19 outbreak. In this survey study, we aimed to evaluate the knowledge of dentistry students, who have not yet started working in the clinics, about this pandemic, their protective measures, and the risks of cross infection in dental clinics.

Methods

Study Design, Participants and Measures

This was a descriptive, cross-sectional study. Criterion sampling method, which is one of the qualitative sampling techniques, was used to evaluate students with no clinical experience. This survey study was applied to volunteer preclinical dental students (1st, 2nd and 3rd classes) at the Department of Dentomaxillofacial Radiology, Gülhane University Faculty of Dentistry, University of Health Sciences Turkey, after the COVID-19 pandemic started. The questionnaire was prepared using the "Google Forms" application. In preparing the questions of the survey, a previous study on COVID-19 on Iranian Students was taken as a reference, and the questions adapted from the study by Taghrir et al. (13) (Table 1, 2). Some new questions about cross-infection in dentistry were also added to the survey (Table 3). Since it was a study conducted in Turkish society, the questions were prepared in Turkish.

In the first step, the pre-test of the prepared Google survey was conducted on approximately 10 people with similar characteristics to the target audience before starting this study. Then, the necessary corrections were made by considering the criticism and opinions of the participants in the pre-test questionnaire form, and the final form of the questionnaire was created after the duration of the questionnaire (about 5 minutes) was determined. The link of the form was sent through appliacations WhatsApp and Instagram to the students.

The questionnaire consisted of 4 main sections: demographic information such as gender, age, smoking; true/false questions for general information about COVID-19; questions about preventive behaviors applied during the COVID-19 pandemic; and questions about the relationship of COVID-19 disease with dentistry.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 22.0; SPSS Inc, Chicago, IL, USA) and Microsoft Excel 2016. Descriptive statistical methods and the criterion sampling method were employed.

The study was conducted in accordance with the Declaration of Helsinki. The questionnaire forms were delivered to the participants after approval by the Gülhane Scientific Research Ethics Committee of the University of Health Sciences Turkey, on 24.09.2020 (no. 2020-370). Informed consent was obtained from the participants.

Results

Of the students surveyed (n=95, age between 18 and 23 years, female 67.4%), 47 were from the 1st class, 24 from the 2nd class, and 24 from the 3rd class The youngest participant was 18 years old, the oldest participant was 23 years old, and the average age of the participants was 20.05 years. The majority of the students (67.4%) were female (n=64, 67.4%). Sixteen (16.8%) students were smoker. Smoking was more common among male participants (35.5% vs. 7.8%).

Only 1 student answered "no" to the question "Do you know about the COVID-19 pandemic?". For the participants who answered "yes" to this question, another question was asked, "From which source did you get this information?". The answer options for this question were "International articles", "Media tools such as television, newspapers, magazines", "Social media", and "Other". The majority of the participants (76.8%/73 people) answered this question as "media tools such as television, newspapers and magazines" (Figure 1).

During the period when the questions were answered by the participants, only the persons diagnosed with the disease were recommended to wear a mask by the Ministry of Health, but as the pandemic progressed over time, it was recommended that everyone wore a mask. For this reason, since the answer to the item "All people should wear a mask" changed in time, this question was excluded from during the statistical analysis. This question was lilnked to the options of "true", "wrong", and "do not know". The average rate of correct answers given to the remaining 12 questions was 85.7%. The two items with the lowest correct response rate were "N95 mask is required only during intubation, bronchoscopy and cardiopulmonary resuscitation (T) (38.9%)" and "It can be treated with normal antiviral drugs (F) (54.7%)". In addition, these 2 items included an "I don't know" option which was marked at the highest rate. Regarding the inter class comparisons, , the correct response rates were highest for the 3rd grade students, while the were lowest for the 1st grade students (84.72%>83.68%>80.14%) (Table 1).

Table 1. The questions about knowledge of COVID-19, adapted from the study by Taghrir et al. (13) including "true (T)/false (F)/I don't know" options

Options	1st grade (n=47)				2nd grade (n=24)				3rd grade (n=24)				
	Correct option		False option		Correct option		False option		Correct option		False option		Total correct option
	n	%	n	%	n	%	n	%	n	%	n	%	n (%)
Q1. It is a respiratory infection caused by a new strain of coronavirus family. (T)	45	95.74	2	4.26	23	95.83	1	4.17	21	87.5	3	12.5	89 (93.68)
Q2. The general symptoms of the disease are fever, cough and shortness of breath. (T)	47	100	0	0	24	100	0	0	24	100	0	0	95 (100)
Q3. Incubation period is between 5 and 14 days. (T)	43	91.49	4	8.51	24	100	0	0	23	95.83	1	4.17	90 (94.74)
Q4. The virus can survive on surfaces for a few hours or up to a few days, depending on the surface type, temperature or humidity of the environment. (T)	39	82.98	8	17.02	23	95.83	1	4.17	23	95.83	1	4.17	85 (89.47)
Q5. It is diagnosed by PCR test from swab or sputum taken from the nasopharyngeal and oropharyngeal region. (T)	31	65.96	16	34.04	18	75	6	25	22	91.67	2	8.33	71 (74.74)
Q6. It is transmitted by respiration with droplets formed as a result of coughing and sneezing. (T)	43	91.49	4	0	24	100	0	0	24	100	0	0	91 (95.79)
Q7. It is transmitted by contact with an infected person. (T)	36	76.6	11	23.4	23	95.83	1	4.17	20	83.33	4	16.67	79 (83.16)
Q8. Use of medical masks is useful to prevent contamination. (T)	41	87.23	6	12.77	22	91.67	2	8.33	21	87.5	3	12.5	84 (88.42)
Q9. Contamination can be prevented by decreasing close contacts such as shaking hands, kissing and paying attention to hand disinfection. (T)	47	100	0	0	22	91.67	2	8.33	24	100	0	0	93 (97.89)
Q10. It is necessary to wear a n95 mask only during intubation, bronchoscopy and cardiopulmonary resuscitation. (T)	16	34.04	31	65.96	11	45.83	13	54.17	10	41.67	14	58.33	37 (38.95)
Q11. It can be treated with normal antiviral drugs. (F)	31	65.96	16	34.04	10	41.67	14	58.33	11	45.83	13	54.17	52 (54.74)
Q12. If symptoms occur within 14 days after contact with the suspected case, a public health center should be visited. (T)	33	70.21	14	29.79	17	70.83	7	29.17	21	87.5	3	12.5	71 (74.74)

COVID-19: Coronavirus disease-2019

According to the answers to questions about preventive behavior, the proportion of people who performed these behaviors was 96%. The least applied item among these preventive behaviors was "I have increased the frequency of cleaning the places that are in contact with the hands (door handle, etc.)" with a rate of 90.5%.

The items in the risk perception section were "I argued with my family and friends about preventing COVID-19", "I can be infected more easily than people with COVID-19 disease", and "I am afraid of being infected with COVID-19 disease". The ratio of the students who positively responded to these items were 95.8%, 18.9%, and 60%. Responses to the preventive behaviors and perceptions are shown in Table 2.

Considering the responses to the questions about the spread of COVID-19 disease in dental clinics (Table 3), 97.9% of the students thought that dental clinics were environments

Table 2. Items about practicing preventive behaviors and the risk perception, adapted from the study by Taghrir et al. (13)

Items	1st grade (n=47)				2nd grade (n=24)				3rd grade (n=24)			
	Yes		No		Yes		No		Yes		No	
	n	%	n	%	n	%	n	%	n	%	n	%
I1. I reduced the use of public transport.	47	100	0	0	24	100	0	0	24	100	0	0
I2. I decreased the frequency of going shopping.	46	97.9	1	2.1	24	100	0	0	24	100	0	0
I3. I spend as little time as possible in closed places.	45	95.7	2	4.3	20	83.3	4	16.7	23	95.8	1	4.2
I4. I avoid coughing around people as much as possible.	47	100	0	0	23	95.8	1	4.2	23	95.8	1	4.2
I5. I have increased the frequency of cleaning and disinfecting items that can be easily touched with hands (i.e. door handles and surfaces).	41	87.2	6	12.8	22	91.7	2	8.3	23	95.8	1	4.2
I6. I wash my hands more often than usual.	46	97.9	1	2.1	23	95.8	1	4.2	24	100	0	0
I1. I argued with my family and friends about preventing COVID-19.	47	100	0	0	22	91.7	2	8.3	22	91.7	2	8.3
I2. I can be infected more easily than people with COVID-19 disease.	8	17	39	83	6	25	18	75	4	16.7	20	83.3
I3. I am afraid of being infected with COVID-19 disease	31	66	16	34	12	50	12	50	14	58.3	10	41.7

COVID-19: Coronavirus disease-2019

with a high risk of cross-infection between dentists and patients; 96.8% of the students thought that not to apply to dental clinics for procedures other than emergency treatments.

By the 87.4% of the participants high-speed hand tools used during dental treatment were thought to be effective in reducing the spread of the COVID-19 virus and 77.9% of the participants thought that standard preventive measures in dental clinics were adequately effective in the prevention of spread.

Of the participants, 81.1% positevly responded to the question “Does it pose a risk for the spread of the COVID-19 when the patient shows his aching tooth with his finger during the examination?”. The number of students who answered “Yes” to the question “Is panoramic radiography or computed tomography a riskier imaging method than intraoral radiography in terms of the spread of the virus?” was only 5, while the majority (61.1%) chose the answer “I don’t know”,. For the question "Is the use of high volume saliva absorbers effective in reducing the production of droplets and aerosols?”, 36.8% of the participants chose the “I don’t know” option, while 44.2% of the students answered “Yes” to this question,.

Regarding the answers to the multiple choice questions related to the procedures within the scope of emergency dental treatments, only 13 students marked the options (“Short-term tooth sensitivity”, “Severe toothache caused by pulpal inflammation”, “Tooth abscess” and “Trauma-related dental avulsion/luxation”). Responses to the options of emergency dental treatments are shown in Figure 2.

Of the students, 84.4% stated that the dentist who performed emergency dental treatment during the COVID-19 pandemic

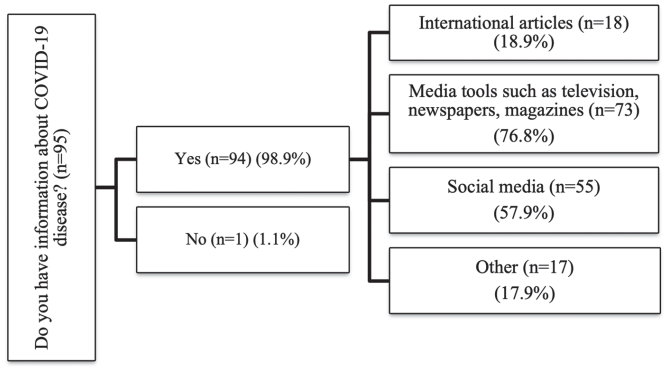

Figure 1. Information source about Coronavirus disease-2019

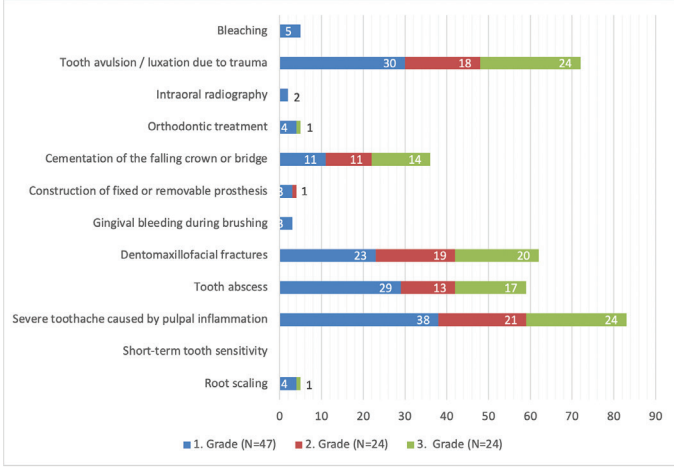

Figure 2. Emergency dental procedures

Table 3. The questions about the spread of COVID-19 disease in dental clinics

Questions	Yes		No		I don't know	
	n	%	n	%	n	%
Q1. Do you think dental clinics are places with a high risk of cross-infection between dentists and patients?	93	97.9	1	1.1	1	1.1
Q2. Should dentist clinics be visited other than emergency treatments?	3	3.2	92	96.8	0	0
Q3. Do you think high-speed hand tools used during dental treatment are effective in spreading the COVID-19 virus?	83	87.4	3	3.2	9	9.5
Q4. Do you think "panoramic radiography" or "computed tomography" is a more risky imaging method than intraoral radiography in terms of the spread of the virus?	5	5.3	32	33.7	58	61.1
Q5. Do you think standard preventive measures in dental clinics prevent the spread of the COVID-19 virus?	5	5.3	74	77.9	16	16.8
Q6. Do you think using of high-volume saliva aspirator is effective in reducing the production of droplets and aerosols?	42	44.2	18	18.9	35	36.8
Q7. Do you think a patient showing her/his aching tooth with her/his finger during the examination poses a risk for the spread of the COVID-19 disease?	77	81.1	6	6.3	12	12.6

COVID-19: Coronavirus disease-2019

should use "Gloves, glasses, mouth mask, face mask, sterile disposable dress" together. For the question "Which one or ones are sufficient for the patient who will apply to the dental clinic during the COVID-19 outbreak?", 24% of the students chose the answer of "glove and mouth mask". The rate of students who marked only the "mouth mask" option was 15.6%, and the rate of students who marked all options (Gloves, glasses, mouth masks, face masks, sterile disposable clothes) was 15.6%.

For the question "Which one or ones would be appropriate when the patient returns home after dental treatment?", 98.9% of the participants thought that their hands should be washed. The rate of those who marked "Should wash my hands, put my clothes into dirty, take a shower" options together was 86.5%.

Discussion

Healthcare workers and students are always at higher risk than other people concerning the infectious diseases. In order for health policy makers to make appropriate planning, it is important to evaluate knowledge of preventive behaviors and to understand the risk perception and anxiety level of health students.

The protocol of this survey had to be modified in a single item during the collection of data, indicating the dynamic changes and updates during the worst pandemic of the last centruy. The item that "All people should wear masks", which is one of the 13 items containing general information about COVID-19, was not included in the survey results because, in the period when the surveys were answered (March-April 2020), health managers stated that it would be sufficient for people diagnosed with COVID-19 to wear a mask, while it was stated that everyone should wear a mask during the period when the statistics of the survey results were made.The majority of the students correctly answered the questions with "true/false/do not know" options containing general information about COVID-19, in similar to a study previously which was conducted on medical students and reported this rate as 86.96% (13). In this context, the 2 items with the lowest rate should cautiously be evaluated. Less than half of the students answered the item "It is necessary to wear an N95 mask only during intubation, bronchoscopy and cardiopulmonary resuscitation" and slightlu more than half of the students correctly answered the item "COVID-19 disease can be treated with normal antiviral drugs". More training about the N95 and other similar standard masks and drugs should be provided to 1st, 2nd and 3rd gradestudents of the dentistry faculty, who start clinical internships and treat patients soon. This could be the primary measure to protect them form infectious diseases both during their internships and their professional life.

When the classes are compared, the correct response rates increased, as we expected, from the first grade to the third grade students (80.14%<83.68%<84.72%). However, although there were slight differences between the rates, they are very close to each other. The reason for this can be explained by the fact that none of the students had clinical experience when surveyed.

In a similar study conducted on healthcare professionals (doctors, nurses, and pharmacists) in Pakistan, 88.7% of the participants stated that they performed preventive measures during the COVID-19 period. On the other hand, this rate was higher in our study, almost all the respondents chose to perform preventive behaviors (14). The item with the lowest rate of response was the item "I have increased the frequency of cleaning the places that are in contact with hands (door handle, etc.)", though still with a rate of 90.53%. The outcome to be drawn from this finding is that students should be given more information about frequently contacted areas and surface disinfection.

Around one-fifth of the students thought that they would be more easily infected with the COVID-19 virus than other people. As we expected, the students in our study did not place themselves in a different stiuation from the other people because they had not yet treated patients and these results were lower compared to the similar previous two studies. In the survey study conducted by Ataş and Talo Yildirim (15) on dentistry students, 77.6% of preclinical students stated that they were afraid of being infected with COVID-19 disease. In another survey (16) on dentists, 54.5% of the participants strongly agreed that there was a high risk of contracting the COVID-19 in dental practice.

Aerosols that occur during dental treatments are considered a risk factor for cross-infection in the dental clinics, due to long-term dental treatments and close contact between the dentist and patient. In addition, the aerosols that occur during dental treatments pose a risk for the other people in the dental clinic because of their suspension in the air for a long time (17). In a previous survey study conducted on the parents of children (aged: 0-14) who visited the dental department, 91.89% of the participants stated that the virus could be transmitted to their children during an intervention (18). The result in our study was slightly higher, 97.9% of the participants thought that dental clinics were environments with a high risk of cross-infection.

Acute infections in the oral cavity, severe toothaches and traumas affecting the jaw require urgent intervention. Accordingly, it is recommended to dental clinics by healthcare professionals not to apply for aesthetic dental treatments and painless dental caries treatments during the COVID-19 pandemic. In accordance with these recommendations, a vast majority of the students thought that people should not apply to the dental clinic during the pandemic period. According to the report of the Republic of Turkey Ministry of Health, the procedures are considered as emergency dental treatment. The relevant emergency treatment limitations adapted from the study by Falahchai et al. (19) and the study by Oral et al. (20) are as follows:

a. Severe toothache caused by pulpal inflammation

b. Severe pain caused by pericoronitis or third molar

c. Postoperative osteitis or alveolitis

d. Abscess or bacterial infection that causing localized pain and swelling

e. Tooth fracture causing pain or soft tissue trauma

f. Tooth avulsion/luxation caused by trauma

g. Dentomaxillafacial traumas

h. Pain and/or infection due to injury in soft tissue as a result of breakage of brackets and wires of patients undergoing orthodontic treatment

i. Feeding plate applications of patients with newborn cleft plate

j. Temporamandibular joint

k. Biopsy (in cases of suspected malignancy) (19)

l. Acute and painful lesions/ulcerations of the oral mucosa,

m. Life-threatening or uncontrolled bleeding

n. Intraoral/extraoral infections that threaten the patient's airway patency

o. Treatment of patients who are undergoing radiotherapy and/or organ transplants

p. Dental consultation for medical problems

q. Taking sutures

r. Treatment so as not to form an aerosol for temporary restoration loss/fractures injuries preventing the use of removable dentures (20).

In the current study, four urgent procedures were given as the right choice for the 12-choice questions in which emergency procedures in dental practices were questioned; “Severe toothache caused by pulpal inflammation, tooth abscess, dentomaxillafacial traumas, tooth avulsion/luxation caused by trauma”. Less than one-fifth of the participants in the survey correctly answered the emergency treatments in dentistry. As expected, the highest scores were “Severe toothache caused by pulpal inflammation”,,“Tooth avulsion/luxation caused by trauma”, “Dentomaxillafacial traumas” and “Tooth abscess”, with responses higher than 50%. In a study performed by Oral et al. (20) 17.4% of the participants answered the options of “severe toothache, tooth abscess, jaw and facial fractures and tooth dislocation as a result of trauma” for a similar question, by correctly marking their options.

Close contact of dentists and nurses with the patient’s oral area during dental treatments, aerosols and saliva scattered into the air with high-speed hand tools used during dental treatments increase the risk of the spread of the virus. The use of high-volume saliva absorbers reduces the spread of the virus by reducing the scattering of droplets and aerosols into the air (21). Similarly, up to 90% of the students in our study thought that high-speed hand tools used during dental treatments were effective in the spread of COVID-19 virus and a low percentage of participants thought that high-volume saliva absorbers used during dental treatments were useful to reduce the droplets and aerosols. Therefore, to prevent the spread of COVID-19 virus and other droplet-borne microorganisms, the frequency of use of high-speed hand tools should be reduced and high-volume saliva absorbers should be used during dental treatments.

The majority of the students stated that the dentists who performed emergency dental treatment during the COVID-19 epidemic chose the option to use “gloves, glasses, mouth masks, face masks, sterile disposable clothes”, and around one-fifth of the students found it sufficient for patients admitted to the clinic to use “gloves and mouth masks”. In a study conducted by

Ahmed et al. (22) on patients admitted to a dental clinic, 60% of participants stated that the dentist and patient should wear "gloves, face masks and protective clothes".

This study has some limitations. Firstly, it was difficult to reach more participants because the clinics were closed due to the pandemic at the time of the study. Secondly, this was an online survey and the responses depended on judgement.

Conclusion

The current study suggest that dentistry students were closely following the pandemic measures, and were highly adapted to the preventive behaviors about COVID-19. On the other hand, the results revealed that some proportion of students showed confusions at the level of knowledge about the risk of cross-infection in dentistry procedures.

Ethics

Ethics Committee Approval: The questionnaire forms were delivered to the participants after the approval of the Gülhane Scientific Research Ethics Committee of the University of Health Sciences Turkey, on 24.09.2020 (no. 2020-370).

Informed Consent: Informed consent was obtained from all participants before the questionnaire was administered.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.O., A.A.B., Design: N.O., A.A.B., H.P.Ö., Data Collection or Processing: A.A.B., İ.H.A., Analysis or Interpretation: N.O., A.A.B., H.P.Ö., İ.H.A., Literature Search: A.A.B., İ.H.A., Writing: N.O., A.A.B., H.P.Ö., İ.H.A.

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Malignant phyllodes tumor in a young woman with multiple recurrences

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Keywords: Malignant phyllodes tumor, young woman, surgery, recurrence

ABSTRACT

Phyllodes tumor (PT) is an uncommon tumor of primary breast origin, constituting about 1% of the breast tumors. Most of the PTs are benign, but some have malignant potential. A 22-year-old single female was referred with a history of left breast mass since she was 13 years old. The diagnosis was fibroadenoma and she had a history of 2 surgeries (three and six years ago). Left breast mass had recurred since last year. Ultrasound examination showed 3 mass-like hypoechoic lesions without calcification. On core needle biopsy, the pathologist reported borderline PT. On mastectomy, the report changed to malignant PT with 10 mitoses/10 HPF. The greatest tumor diameter was measured as 10+8+6 cm. The PT has unpredictable clinical behavior and has a high risk of recurrence regardless of histologic subtype. Pathologists and clinicians should be aware of this tumor type in younger individuals for a better diagnosis and treatment.

Introduction

"Fibroepithelial lesions" consist of fibroadenoma (FA) and phyllodes tumor (PT). FAs are more common in young females but PTs are often seen in the fourth and fifth decades of life. The PT is uncommon tumor of primary breast origin, constituting about 1% of the breast tumors (1,2). Breast lesion is rare in adolescents and young population but FAs are the most common tumors at this age. Only 20% of PTs in adolescents and less than 10% of PTs before the age of 20 years are reported (1,3,4). Large and rapidly growing mass in the breast is a key feature of PT (4). Most of the PTs are benign, but some have malignant potential and can behave like sarcoma with blood born metastasis to various organs (1). The World Health Organization has defined several histological criteria to differentiate FA from PT and for PT classification (5). Here we report a rare case of malignant PT in a young patient with multiple recurrences.

Case Presentation

A 22-year-old single female patient was referred on August 1st, 2019 with a history of left breast mass since she was 13. The diagnosis was FA and she had a history of 2 surgeries (6 and 3 years ago). Left breast mass had recurred since last year. Drug

and family histories were unremarkable and vital signs were stable. Physical examination was unremarkable except for left breast mass. The patient had the report of core needle biopsy on July 2nd, which showed a spindle cell tumor compatible with PT. Mitotic figures were about 4 mitoses/10 HPF and mild atypia without necrosis was present. The pathologist suggested low grade (borderline) PT on core needle biopsy (Figure 1) and recommended for complete excision and immunohistochemistry with CD34, CD31, CK, SMA, and Ki-67. Ultrasound examination showed 3 mass-like hypoechoic lesions without calcification and dimensions of 75x41, 75x55, and 66x34 mm with lobulated margin. The radiologist commented on further evaluation due to large size and edema to rule out malignancy and PT. The patient underwent a total mastectomy on August 3rd. She experienced pruritus and urticaria after blood transfusion which were managed with hydrocortisone and diphenhydramine. The specimen that was sent for the pathologist consisted of breast tissue including skin measured 17x15x9 cm with 3 well-defined masses on cut surface measured 10, 8, and 6 cm. The pathologist reported as compatible with malignant PT (Figure 2). Mitotic figures were 10 mitoses/10 HPF. The greatest tumor diameter was measured as 10+8+6 cm (24 cm, multiple). Inferior and medial margins were involved by tumor. On 26th August, the patient was readmitted for the surgery of remnants of the tumor. Physical examination showed a surgical scar at the mastectomy site with a palpable 1cm mass. The specimen consisted of one piece of breast tissue measured 17x3x2 cm including skin (17x0.3 cm). The pathologist found no remnant of the tumor with unremarkable surgical margins. On 19th May 2020, the computed tomography was done with markers for planning of radiation therapy. Written informed consent was obtained from the patient for the report.

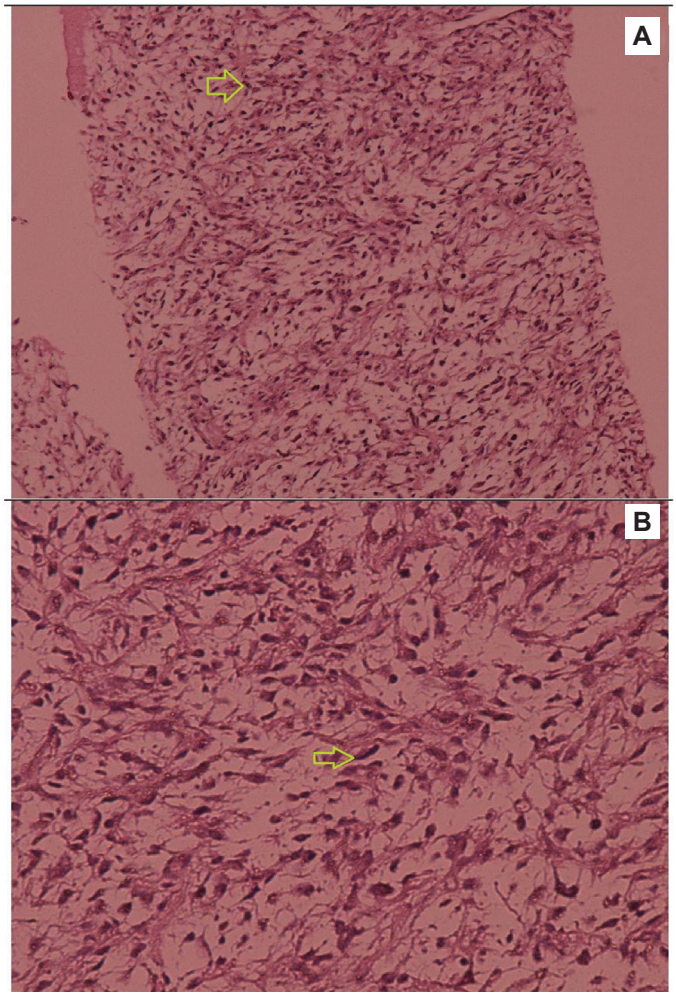

Figure 1. Borderline phyllodes tumor diagnosed on core needle biopsy. Tumor cells with spindle cell morphology (arrow). Hematoxylin-eosin stain: A) x100 and B) x200 magnifications

Discussion

The differential diagnosis of breast lesion in young and adolescent population includes giant FA, juvenile FA and PT. Clinical features of PT are the same as FA and include well-defined, movable and painless lesion. The only difference is more rapid growth in PTs (2,3). PT's size has been reported from 1 to 40 cm; about 20% of them are larger than 10 cm (6). Etiologic causes and risk factors of PTs are unknown but more reported in Asian races (1).

Diagnosis of PT is based on clinical symptoms, radiological findings and histological examination (6). Preoperative diagnosis is important but there is no standard protocol for diagnosis (6,7). Ultrasonography and mammography cannot differentiate PT from other benign breast tumors (1). The use of cytologic examination in the diagnosis of PT has been controversial but core needle biopsy has sensitivity of about 70% (1,7,8). PT in core needle biopsy may be mistaken for low grade myofibromatosis, solitary fibrous tumor, FA and leiomyosarcoma (9). PT is often benign (35-64%), while malignant PT is larger than borderline and benign PTs and it accounts for 6.5-27%

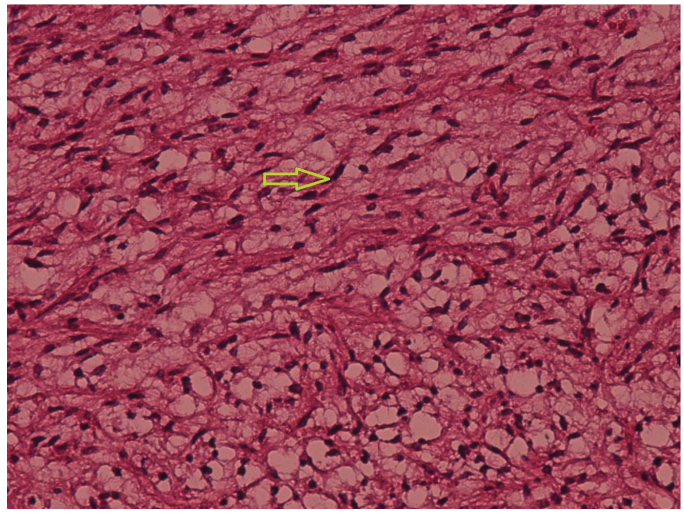

Figure 2. Malignant phyllodes tumor diagnosed on mastectomy specimen. Tumor cells with spindle cell morphology (arrow). Hematoxylin-eosin stain: x200 magnification

of cases (1,4). It is difficult to differentiate between malignant PT and pure sarcoma. In breast, rhabdomyosarcoma and liposarcoma are more common than fibrosarcoma (1). A clonal analysis showed monoclonal stromal cells and polyclonal epithelial cells. The study of the profile can help the evaluation of malignant transformation (6).

Thirty percent of malignant PTs are due to FA transformation and cases of transformation from benign into malignant PTs have been reported (4,6,10). Decreased expression of beta catenin and ER with increased expression of EGFR, P53, C-kit, CD34, and Ki-67 are associated with malignant PTs. Among these markers, P53 expression is the most accepted one (6). MED-12 (Mediator of RNA polymerase 2 transcription) expression is associated with long time survival and its absence is related to high recurrence rate of PTs (5,10). The relationship between malignant PTs and a further recurrence of the tumor is unknown. About 20% of malignant PTs are associated with distant metastasis and more metastases are without local recurrence of the PT (8). A research has reported malignant PTs with six episodes of local relapse without evidence of distant metastasis (11). One study has reported that stromal cells pleomorphism and histologic grade are associated with local recurrence rate and distant metastasis respectively (8). Malignant PT is more common than benign PT in adolescent population. Adolescent and young females have more recurrence rate compared to adult population (4). Free margins and adjuvant radiotherapy are the best treatment to control local recurrence in borderline and malignant PT and patients need to be closely followed up with breast examination and ultrasonography at least for 30 months because average recurrence time is less than 24 months (8). Multiple PTs are rare (7). This study reported three separated malignant PTs.

Conclusion

PT is a mysterious tumor with unpredictable clinical behavior and has a high risk of recurrence regardless of histologic subtype. Pathologist and clinician must be aware of this tumor in young patients for better diagnosis and treatment.

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Ethics

Informed Consent: Written consent was obtained from the patient for reporting the case report.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.R., Design: Z.A., Data Collection or Processing: M.R., Analysis or Interpretation: Z.A., M.S., Literature Search: M.S., M.R., Writing: M.R.

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