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REVIEW ARTICLE

Molecular pathways of common breast cancer metastases and the distinguishing features of triple-negative breast cancer

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ABSTRACT

Breast cancer is the most common type of female cancer in Turkey, and metastasis is the most important cause of death, as in other solid organ cancers. Triple-negative tumors constitute 15-20% of breast cancer patients. Within three years after the development of the primary tumor, the tumor spreads to other organs. Breast cancer tends to spread to distant organs, such as bone, liver, brain, lung, and adrenal gland, either through regional lymph nodes or vascular channels. This condition, defined as the tendency to metastasize to specific organs, is called organotropism. Triple-negative breast cancer is a heterogeneous breast cancer subtype showing organotropism for the brain and the lungs. Identifying the molecular changes that may cause tropism for various regions and organs in non-metastatic tumors at the time of diagnosis is vital to developing targeted therapies and achieving longer overall and disease-free survival. In this review, we aimed to summarize the pathogenesis of breast cancer metastasis, the molecular changes involved in the metastatic process, and organotropism, as well as to emphasize the distinguishing features of triple-negative breast cancer in terms of metastatic organotropism.

Keywords: Breast cancer, triple-negative breast cancer, metastasis, molecular pathway, organotropism

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INTRODUCTION

Breast cancer is the most common type of female cancer in Turkey (1). Among all breast cancer subtypes, triple-negative breast cancer (TNBC) is a very heterogeneous subtype and constitutes 15-20% of breast carcinomas (2). These tumors are characterized by negativity for hormone receptors and HER2 gene amplification. Metastasis is the most important cause of death for cancer patients, including breast cancer (3). TNBCs, which are particularly predisposed to lung and brain metastases, have a lower risk for bone metastasis and differ from other breast cancer subtypes in terms of organotropism (4-6). In general, breast cancer has an aggressive clinical course in 10-15% of patients in whom the tumor spreads to other organs within three years after the development of the primary tumor (5). Metastasis, cancer's most distinguishing and challenging feature, significantly impedes treatment success (6). Although the metastatic process and associated molecular mechanisms are not fully understood, molecular studies have led to increased knowledge of the biology of metastasis and the emergence of new therapeutic targets. Metastatic breast cancer, irrespective of its subtype, tends to spread to regional lymph nodes and distant organs, such as bone, liver, brain, lungs, and adrenal glands (7). In metastatic breast cancer patients who respond less to chemotherapy, the 5-year survival rate is approximately 20% (8). It is crucial to understand metastatic organotropism, the steps of metastasis, and the molecular pathways involved in these processes to predict and prevent breast cancer metastasis and to develop more effective treatments, especially in metastatic TNBC cases where treatment options are limited.

Metastatic process in breast cancer

Metastasis is defined as the spread of cancer cells to adjacent tissues or distant organs. The heterogeneous nature of breast cancer and its distinct metastatic mechanisms make it difficult to treat (9). In general, breast cancer metastasis develops by the following processes, which are also valid for other solid organ cancers (10);

- Separation of neoplastic cells from the extracellular matrix (ECM), initiation of invasion and migration by crossing the basement membrane: The metastatic process begins with the separation of adjacent cells from each other and the basement membrane, as a result of disruption of the connection of cancer cells to ECM through cellular adhesion proteins, such as integrins. Neoplastic cells invade the surrounding tissues with proteolytic enzymes that degrade ECM.

- Intravasation: Cancer cells attach to the vessel walls, invade, and enter the lumens of lymph or blood vessels (Figure 1).

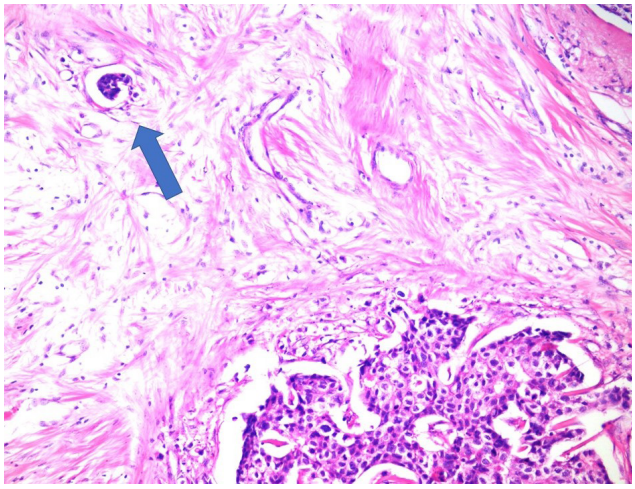

Figure 1. Lymphatic space invasion around invasive breast carcinoma (arrow, H&E, x200).

- Spread of tumor cells to other organs via blood or lymphatic circulation: Cancer cells must develop resistance to anoikis (a form of programmed cell death) to survive in circulation.

- Arrest, adhesion, and extravasation at sites of metastasis: Cancer cells, whose cell cycle stops before being extravasated at the site of metastasis, adhere to the capillary walls in target organs.

-Metastatic tumorigenesis: Since metastasis is a complex and multistep process, metastatic cells need the ability to survive, invade, and form new tumors in different conditions. In addition, cancer cells must be able to evade the immune system and apoptosis to survive. Cancer cells that gain these features can form a metastatic mass (Figure 2).

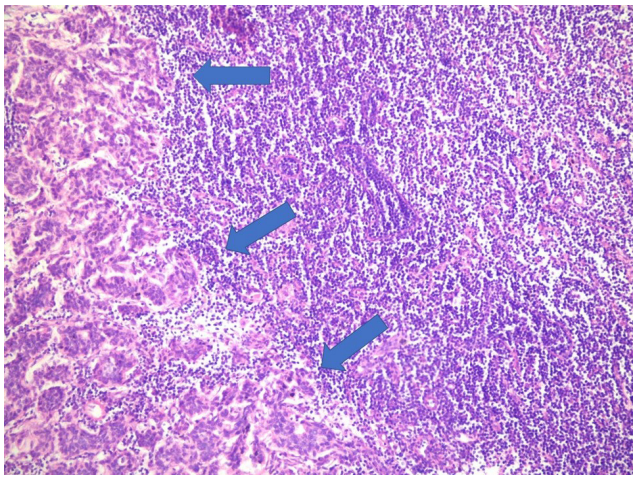

Figure 2. Invasive breast carcinoma metastasis in axillary lymph node (arrows, H&E, x200).

Molecular mechanisms involved in breast cancer metastasis

Critical proteins in cancer cell motility and survival are integrins. Cancer cells bind to the ECM through these heterodimeric proteins, which consist of α and $\alpha\beta$ subunits (11). It has been reported that integrin $\alpha 2\beta 1$ expression decreases in poorly differentiated breast cancer cells (12). Specific integrins, such as integrin $\alpha 3\beta 1$, are associated with matrix metalloproteinase (MMP)-9 activity. This relationship provides the ability to invade, metastasize, and reduce ECM components for neoplastic cells (13). E-cadherin has also been shown to play an essential role in cell-to-cell adhesion and cancer metastasis (14). It has been shown that a decrease in E-cadherin level in breast cancer is associated with increased metastatic potential, and mutation in the E-cadherin gene (CDH1) causes increased ability for invasion in lobular breast carcinoma (15). In addition, decreased expression of E-cadherin is a crucial indicator of epithelial-to-mesenchymal transition (EMT), a cellular process that plays a critical role in cancer progression and metastasis (16).

It has been found that epithelial markers, such as E-cadherin, occludin, and cytokeratin, show loss of expression. In contrast, the expression of mesenchymal markers, such as vimentin and N-cadherin, increases in the EMT process (3). The transcription factor-associated proteins, which are expressed by genes such as TWIST, SNAIL, SLUG, ZEB1, and ZEB2, increase EMT by suppressing E-cadherin expression in cancer cells (17).

TGFβ gene has also been shown to act as an EMT inducer (18). TGFβ is a tumor suppressor gene in the early stages of carcinogenesis. However, exposure of cancer cells to TGFβ protein in later stages causes the transformation of epithelial phenotype into mesenchymal-like phenotype and increases the metastatic potential (19). The TGFβ pathway has two types of signaling: SMAD-dependent and SMAD-independent signaling. It has been shown that SMAD2 and SMAD3 are up-regulated in the mammary epithelium, and EMT is induced due to SMAD-dependent signaling (20). TGFβ signaling may also occur via non-SMAD signaling pathways, such as the PI3K/AKT pathway. Both SMAD-dependent and SMAD-independent pathways have been shown to control transcription factors that mediate EMT, including TWIST, SNAIL, and SLUG (21).

Concerning tumor metastasis, angiogenesis has been investigated extensively, and it has been shown both in clinical and experimental studies that breast cancer is an angiogenesis-dependent cancer (22). Factors that increase angiogenesis have been identified, among which vascular endothelial growth factor (VEGF) shows the most effective activity (23). VEGF gene induces endothelial cell proliferation, aids in new vessel formation, and controls vascular permeability. In the process of new blood vessel formation, VEGF activates receptors VEGFR1 and VEGFR2 in endothelial cells, stimulating endothelial cell motility, vascular permeability, cell survival, and proliferation (24). The increase in vascular permeability, which VEGF causes, facilitates the metastatic spread of cancer cells (25). The activity of VEGF in inducing angiogenesis has been demonstrated and is recognized as a factor that increases the aggressiveness of breast cancer. In addition, VEGF signaling is also known to have several non-angiogenic functions. It is suggested that the VEGF pathway increases cell survival and migration by avoiding apoptosis through AKT and ERK signals in breast carcinoma cells (26).

Organotropism in breast cancer metastasis

Primary tumors tend to metastasize to specific sites and organs. The tendency to spread to particular organs is a nonrandom process known as metastatic organotropism (27). In recent years, the mechanism of metastatic organotropism has been defined. Organotropism is determined partially by cancer cell-specific pathways. Genetic mechanisms that mediate organ-specific metastasis and autonomous mechanisms have also been identified in organ tropism. For example, chemoattractants in metastatic organs can recognize cognate chemokine receptors expressed in cancer

cells (28). Organotropism is regulated by many factors, such as subtypes of related cancer, molecular characteristics of cancer cells, host immune system, microenvironment, and interactions with local cells (7).

The host microenvironment can be modified to create a pre-metastatic niche (PMN), a supportive environment for metastatic tumor growth before a tumor spreads to that area. PMN is regulated by factors and exosomes secreted from the tumor cell, aggregation of cells that do not belong to this region, and host cells (29). In addition, tumor cells can interact with the ECM of the host tissue to facilitate metastasis to specific sites. This theory, known as the "seed and soil" theory, has been proposed by Steven Paget to describe site-specific metastasis. According to this theory, the ability of tumor cells to initiate growth largely depends on the communication between metastatic tumor cells and the host microenvironment (30).

There are many factors affecting organotropism in breast cancer metastasis. These include histologic and molecular subtypes of breast cancer, genetic alterations, gene expression features, micro-RNAs, exosomes, stem cell-like molecular features, circulating tumor cells, circulating cancer stem cells, and the immune system (7).

Bone metastasis

Bone is a region where 70% of breast carcinomas metastasize, and metastases frequently occur as osteolytic type (7). Although all molecular subtypes of breast cancer are prone to bone metastases, luminal tumors (>80%) develop bone metastases at a higher rate (31). The group with the lowest risk for bone metastasis is TNBC, which significantly overlaps with the molecular basal-like subtype (32).

Among molecular changes that contribute to the tendency of breast cancer to form bone metastases, integrin complexes play significant roles, such as tumor cell adhesion and osteolytic tumor growth (7). With the effect of the TGFβ-SMAD4-IL11 signaling pathway and HIF1α, both VEGF activation and CXC chemokine receptor 4 (CXCR4) activation occur, which causes a predisposition for the development of bone metastasis (33). Growth factors such as IGF1, PGE2, PDGF, and FGF2, interleukins such as IL1 and IL-6, PTHrP, OPN, Heparanase, RANKL-RANK pathway, and Src-dependent pathways are also associated with the development of bone metastasis (7).

Liver metastasis

The most common site of metastasis for all solid organ cancers is the liver. It is also the second most common site (30%) where breast cancer metastasizes (34). Metastatic masses formed in the liver by breast cancer are larger and more numerous than lung cancer. This suggests that the liver has a favorable microenvironment for breast cancer metastasis (35).

Liver metastasis was found to be associated with ER expression, high Ki-67 proliferation index, and luminal B subtype (34). It has also been shown that the beta-catenin-independent WNT signaling pathway plays a role in the development of liver metastases in breast cancer patients (35). In addition, the downregulation of ECM genes is an essential factor for liver metastasis of breast carcinoma. The other molecular pathways include CXCR4/CXCL12 chemokine and chemokine receptor interaction, integrin complexes such as IL-6, α2β1 and α5β1, N-cadherin, HIF-regulated LOX, OPN, VEGF, and TWIST genes (7).

Brain metastasis

Brain metastases develop in 10-30% of breast cancer patients (36). Younger age, poorly differentiated tumors, HER2-enriched subtype, and luminal B subtype are associated with an increased risk of brain metastasis. Still, the subtype that most commonly metastasizes to the brain is TNBC.

Molecular changes that play a role in the tendency of breast cancer to form brain metastases include the effect of ST6 N-Acetylgalactosaminide alpha-2,6-sialyltransferase (the protein product of ST6GALNAC5 gene) in crossing the blood-brain barrier and expression of cancer stem cell markers, Nestin, CD133, and CD44 in tumor cells. Cytokines, such as MMP-1 and MMP-9, are also crucial as they act in transendothelial migration. In addition, growth factors VEGF and HBEGF, CXCR4 chemokine and its receptor, CK5, IL-8, Ang-2, COX2, and L1CAM are associated with developing brain metastasis (7).

Lung metastasis

Considering the molecular subtypes of breast cancer, basal-like tumors that make up the majority of TNBCs, as well as luminal B tumors, have a more aggressive clinical course and a higher rate of metastasis to the lung (38). Similarly, when histological subtypes are considered, infiltrating ductal carcinoma with a triple-negative phenotype is associated with a higher risk for lung metastasis (39).

One of the molecular changes suggested to be responsible for the tendency of breast cancer to metastasize to the lung is the attachment of tumor cells to the lung capillaries via MMP-1, MMP-2, and COX2 as a result of the effects of TGFβ, EGFR, EREG, and VEGF gene products and their receptors (7). Lung-derived bone morphogenetic proteins (BMP) are known as the source of the lung's antimetastatic signal. GALNT and Coco, which are BMP inhibitors, neutralize these signals and allow metastatic breast cancer cells to colonize in the lung (40).

Regional lymph node metastasis

Lymph node metastasis is a predictive factor for distant organ metastasis and is a poor prognostic feature (41). Among breast cancer subtypes, luminal and HER2-enriched subtypes show a higher correlation with lymph node metastasis (42). The presence of lymphovascular invasion and a high Ki-67 proliferation index are essential indicators of the metastatic potential of neoplastic cells.

It has been shown that four members of the kallikrein (KLK) family (KLK10, KLK11, KLK12, and KLK13) are up-regulated, and the B cell receptor signaling pathway is down-regulated in breast cancer cases with lymph node metastasis (43).

The distinctive molecular features of triple-negative breast carcinomas

The heterogeneity of TNBC has been explored by Lehmann et al. (4), who subdivided these tumors into four molecular subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (M), and luminal androgen receptor (LAR). Among these subtypes, BL1 represents the majority of TNBCs, having TP53 mutations in more than 90% of cases and a high frequency of homologous recombination DNA repair deficiency (HRD) related mutations. The BL2 subtype also shows a high mutation rate in TP53 and HRD-associated signatures. BL1 and BL2 subtypes constitute most tumors with germline and somatic BRCA1 mutations. On the other hand, the mesenchymal subtype is characterized by activation of the PI3K pathway and the LAR subtype is characterized by mutations in PIK3CA, AKT1, NF1, GATA3, and CDH1 genes (44).

CONCLUSION

Metastatic tumors show mutational similarities with their primary site and may contain different mutations. This phenomenon indicates that new mutations can develop during the metastatic process (45). In terms of targeted therapy, detecting mutations in metastatic tumors is considered as a more rational approach. Although it is known that the probability of pathological complete response after neoadjuvant therapy is high in TNBCs, the probability of brain and lung metastases within three years after the diagnosis is higher than in other subtypes (5, 6). Nevertheless, the identification of molecular changes that can cause tropism to develop for various regions and organs in non-metastatic tumors at the time of diagnosis will help us to develop targeted therapies and achieve longer survival for breast cancer patients.

Declarations

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Ethical committe approval is not required because of this article is a review article

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ORIGINAL ARTICLE

The prevalence of immunodeficiency in a special population: intern doctors

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ABSTRACT

Background: This study analyzes the immune system parameters of intern doctors working actively during the COVID-19 pandemic. When an intern doctor failed to respond to the treatment during a severe COVID-19 condition, the immune system panel was examined and as a result, it revealed an underlying immunodeficiency. The death of the intern doctor caused several concerns among other intern doctors, and for this reason, their immune systems were also considered to be examined. This study aims to demonstrate that immunodeficiencies might be more common than is known among the general population.

Methods: In this single-center study, the demographic characteristics and European Society for Immunodeficiencies (ESID) diagnostic criteria of 92 intern doctors have been examined retrospectively. For the study, immune system parameters (complete blood count, serum immunoglobulins and subgroup levels, specific vaccine responses, isohemagglutinin titers, lymphocyte subgroups, and class-switched memory B cell (cSMB) levels have been evaluated.

Results: When the demographic characteristics have been analyzed it is seen that the median age is 23.6 (21-28) years, and 64 (70%) of the intern doctors are female. In immune system parameters, one or more are found to be low in 51.08% of the doctors. Among the immunoglobulin subgroups, low IgG4 has been the most common. Selective IgA deficiency has been detected in 2.17% and selective IgM deficiency has been detected in also 2.17% of them. Low B cells (CD19+) are detected in 10.9% and low levels of class-switched memory B cells are found in 35.7% of them.

Conclusion: This study reveals that deficient immunological parameters, especially selective Ig A, selective IgM deficiency, and low IgG4, might be more frequent than known. Depending on the data, it can be concluded that immunodeficiency might be more common than it is known among the general population; however, low immunological parameters alone do not lead to immunodeficiency.

Keywords: Intern doctor, immune system, immunodeficiency, immunoglobulins, lymphocyte subgroup

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INTRODUCTION

Immunity means the protection from diseases. The immune system consists of the cells, tissues, and molecules that provide this protection. The importance of the immune system is based on the susceptibility of individuals with immune system damage to serious and life-threatening infections. Innate immunity and adaptive immunity form the host defense mechanism (1).

Inborn errors of immunity (IEI), which are also called primary immunodeficiency (PID), are a heterogeneous group of disorders caused by damage to germline variants in single genes. In such conditions, an increased susceptibility to infections, autoimmunity, autoinflammatory diseases, allergies, bone marrow diseases, and/or malignancies has been observed. Although it is a rare group of diseases, it represents an important health burden (2). It is estimated that approximately 6 million people worldwide suffer from IEI. However, the number of reported cases is lower. Several studies suggest that the lack of awareness by physicians for IEI might delay the diagnosis and the treatment of these patients (3).

It is known that a pandemic was declared by the World Health Organization on March 11, 2020, due to Coronavirus Disease 2019 (COVID-19), which was caused by Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in 2019. During the pandemic, more than 600 million cases of COVID-19 were followed up and it was reported that the pandemic caused more than 6 million deaths (4). In addition to the physiological effects of COVID-19, there has also been a psychological impact on global society. Health crises such as the COVID-19 pandemic can have a psychological impact on both society and health workers by causing fear, anxiety, depression, or insecurity (5).

This study aims to analyze the data obtained after the evaluation of the immune system parameters of the intern doctors who had severe concerns after the death of an intern doctor during the COVID-19 pandemic.

MATERIALS AND METHODS

Study Design

This retrospective cohort study was conducted at Necmettin Erbakan University Faculty of Medicine Department of Internal Medicine Division of Allergy and Immunology clinic.. The Local Ethics Committee approved the study protocol (Decision no: 2023/4212, Date: 03.03.2023), which complied with the Declaration of Helsinki (1975) tenets. As the data in this study was scanned retrospectively, it was optional to obtain informed consent after the approval of the ethics committee. This study was approved by the Necmettin Erbakan University of Medical Faculty Ethics Committee (Dated: 03.03.2023; Approval Number: 2023/4212). Therefore, informed consent was not obtained from the patients. The study protocol included 92 intern doctors (≥18 years of age, working actively in clinics) who worked during the COVID-19 pandemic between 2020 and 2022. The study did not include those who met the exclusion criteria (Figure 1).

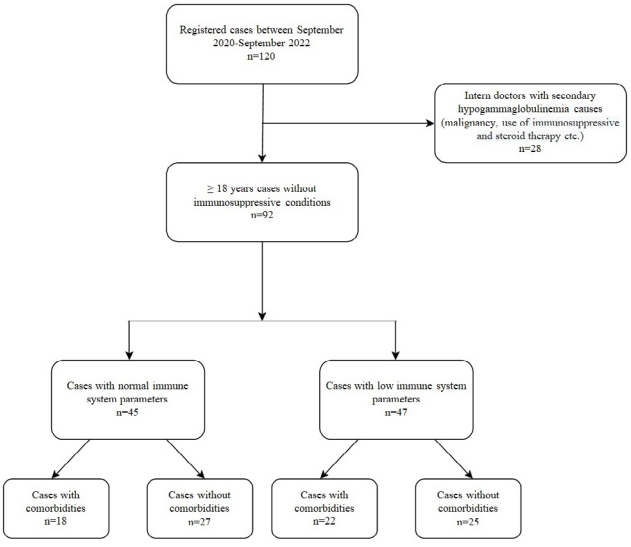

Figure 1. Flow chart of the study protocol

Data collection

The age, sex, medical history information, and laboratory values of 92 intern doctors were obtained from the electronic medical record and archive system of the hospital. Their symptoms, background information, parents' consanguinity, immunodeficiency history, European Society for Immunodeficiencies (ESID) criteria, and comorbidities

were also recorded. Besides, serum immunoglobulin (Ig) values (IgG, IgA, IgM), IgG subgroups (IgG1, IgG2, IgG3, IgG4), CD3+ T cell, CD3+CD4+ T cell (T helper), CD3+CD8+ T cell (T cytotoxic), CD4/CD8 ratio, CD19+ B cell, CD19+CD27+IgD- class-switched memory B (cSMB) cell and CD16+CD56+ Natural Killer (NK) cell counts, specific antibody responses to protein and polysaccharide vaccines (tetanus and pneumococci) and isohemagglutinin titers (Anti-A, Anti-B) were recorded.

Those with one or more of the six warning signs recommended by ESID for adult immunodeficiency were considered to be important (6):

1. Four or more infections requiring antibiotics within one year (otitis, bronchitis, sinusitis, pneumonia).

2. Recurrent infections or infections requiring prolonged antibiotic therapy.

3. Two or more serious bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis).

4. Two or more radiologically proven pneumonias within 3 years.

5. Infection with unusual site or unusual pathogen.

6. PID in the family.

Serum immunoglobulin measurements

Serum immunoglobulin levels were determined by nephelometric methods (Siemens BNII System, Erlangen, Germany).

Specific antibody responses

Pneumococcal polysaccharide antibody titers were measured by using a multiplex immunoassay (Elizen, Angleur, Belgium). An impaired response to Pneumovax-23 vaccine was considered if the post-vaccination titer was <250 mU/mL or less than a twofold increase from the pre-vaccination titer. Tetanus antitoxin IgG ELISA kits (Novalisa, Vienna, Austria) were used to detect tetanus antibodies. Tetanus antitoxin IgG was recognized as an antibody at a protective level ≥ 0.1 IU/mL.

Isohemagglutinin titer

To measure isohemagglutinin titer, blood samples in ethylenediaminetetraacetic acid (EDTA) tubes were centrifuged at 5000 rpm for 1 minute. After the centrifugation, the separated plasma was diluted with saline for titration. Titers ≥ 1:8 were considered to be normal.

Flow cytometric analysis

Peripheral blood samples (2 mL) were collected in an EDTA anticoagulant tube and tested within 6 hours. Peripheral blood lymphocyte subsets were measured by multicolor flow cytometry using a key panel. Cells were analyzed on a BD FACS Canto II Flow Cytometry System (BD Biosciences).

Statistical Analysis

The statistical analysis of this study has been performed by using the SPSS statistical package program (V.22.0). Descriptive statistics have been calculated for each variable. Continuous variables with normal distribution are presented as mean ± standard deviation, continuous variables without normal distribution are presented as median with range (min-max), and categorical variables are presented as numbers and percentages in each category and applied to the whole analyses.

RESULTS

This study has included 92 intern doctors who applied to our outpatient clinic after the death of an intern doctor who was unaware of her illness and had an underlying immunodeficiency during the COVID-19 pandemic. In our study, the median age is 23.6 (21-28) years, and 64 (70%) of the intern doctors are female. When they applied to the clinic, there were no complaints in 52 (56%) of them (Table 1).

According to the analyses, the complete blood counts showed normal lymphocyte values. In addition, immune system parameters were within the normal range in 48.9% of the participants. Comorbid conditions accompanied 40.4% of them with normal values. The most common comorbid disease in this group was allergic rhinitis. It is seen that one or more immune system parameters were low in 51.08% of them. Of these, 51.06% had comorbid conditions, and the most common condition was allergic rhinitis. Although immunological parameters were low in two of the three individuals with ESID criteria, it did not lead to a diagnosis of immunodeficiency (Table 1).

Table 1. General characteristics and comorbidities of the intern doctors included in the study

Number of intern doctors	92
Gender, n (%)	
Female	64 (70)
Male	28 (30)
Age, years, mean ± sd (range)	23.6 ± 1.4 (21-28)
Consanguinity between parents, n (%)	9 (9.8)
Smoking, n (%)	10 (10.9)
Family history of immunodeficiency	0 (0)
Complaints and history at admission, n (%)	
None	52 (56.5)
Allergic rhinitis symptoms and history	32 (34.7)
History or suspicion of frequent infections*	8 (8.6)
History of itchy skin lesions	3 (3.2)
Recurrent oral aphthae	2 (2.1)
Comorbidity, n (%)	
Allergic rhinitis	21 (22.8)
Skin diseases**	8 (8.6)
Asthma	7 (7.6)
Gilbert's Disease	4 (4.3)
Anemia	4 (4.3)
Other***	18 (19.5)
ESID criteria, n (%)	3 (3.2)

**Upper respiratory tract infections that do not require hospitalization, conjunctivitis, herpes labialis, urinary tract infection, cryptic tonsillitis, etc.*

***Acne vulgaris, urticaria, atopic dermatitis, lichen planus*

****Hyperthyroidism, chronic sinusitis, peptic ulcer, Wilson's disease, familial mediterranean fever, irritable bowel syndrome, polycystic ovary syndrome, hirsutism, anxiety disorder*

Abbreviations: *ESID, European Society for Immunodeficiencies*

When serum immunoglobulin (Ig) values and IgG subgroups were examined, it was seen that while IgG2 deficiency was not detected in all intern doctors, IgG4 deficiency was the most common (7.6%). Besides, while low IgA was detected in 4.3% of them, it was observed that 2.17% of the participants met the diagnosis of selective IgA deficiency. While 6.5% of them were found to have low IgM, 2.17% were found to have selective IgM deficiency.

When peripheral lymphocyte subgroups were evaluated, low CD19+ B cells were detected in 10.9% of them. cSMB cell levels were able to be measured in 30.4% of them, and a low level was observed in 35.7% of the participants. It was observed that there was low CD3+ in 1.1% and low CD4+ in 6.5% of them. On the other hand, low CD8+ was not detected. The CD4/CD8 ratio was found to be low at 18.5% of the intern doctors. Also, low NK cells were detected in 4.3% of them (Table 2 and Table 3). Finally, deficiency in immunological parameters was found to be more frequent in those with parental consanguinity (Table 4).

Table 2. Laboratory findings of intern doctors at the time of admission

Parameter	Normal range	Value, median (range)
Lymphocyte count, 103/uL	0.8 – 5.5	2.1 (0.9 – 3.8)
Immunoglobulins, g/L		
IgA	0.07 – 4	1.5 (0.02 – 4.4)
IgM	0.46 – 3.04	1 (0.1 – 4.7)
IgG	7 – 16	11.7 (6.7 – 20)
Immunoglobulin G subgroups , g/L		
G1	4.05 – 10.1	(3.7 – 15.8)
G2	1.69 – 7.86	4.2 (2.1 – 7.9)
G3	0.11 – 0.85	0.36 (0.07 – 1.47)
G4	0.03 – 2.01	0.52 (0.008 – 4.02)
Surface markers,%		
$CD3^+$ T cells	57 – 85	75 (56 – 88)
$CD3^+CD4^+$ T cells	30 – 61	40 (25 – 58)
$CD3^+CD8^+$ T cells	12 – 42	32 (16 – 48)
$CD4^+/CD8^+$ ratio	>0.9	1.3 (0.6 – 3.2)
$CD19^+$ B cells	6 – 29	10 (2.9 – 21)
$CD19^+27^+IgD^-$	9.2-18.9	11.7 (4.5 – 21)
$CD16^+56^+$ NK cells	4 – 25	11 (3 – 31)

Abbreviations: *CD, cluster of differentiation; Ig, immunoglobulin; NK, natural killer*

Table 3. Distribution of immunological parameters according to normal limits

Parameter	Detected low, n (%)	Normal detected, n (%)
Lymphocyte count	0 (0)	92 (100)
Ig A	4 (4.3)	88 (95.6)
Ig M	6 (6.5)	86 (93.5)
Ig G	1 (1.1)	91 (98.9)
G1	2 (2.2)	90 (97.8)
G2	0 (0)	92 (100)
G3	1 (1.1)	91 (98.9)
G4	7 (7.6)	85 (92.4)
$CD3^+$ T cells	1 (1.1)	91 (98.9)
$CD3^+CD4^+$ T cells	6 (6.5)	86 (93.5)
$CD3^+$ $CD8^+$ T cells	0 (0)	92 (100)
CD4/CD8 ratio	17 (18.5)	75 (81.5)
$CD19^+$ B cells	10 (10.9)	82 (89.1)
$CD19^+27^+IgD^-$ (n:28)	10 (35.7)	18 (64.2)
$CD16^+56^+$ NK cells	4 (4.3)	88 (95.7)
Tetanus antibody response	3 (3.2)	89 (96.7)
Pneumococcal antibody response	2 (2.2)	90 (97.8)
Isohemagglutinin#	3 (3.3)	86 (93.3)

 # The blood group of 3 patients was AB.

Abbreviations: *CD, cluster of differentiation; Ig, immunoglobulin; NK, natural killer*

Table 4. Distribution of immunological parameters according to the characteristics of intern doctors

	ESID criteria (n:3)	Family history of consanguinity (n:9)	Symptom Yes (n:40)	Symptom No (n:52)	Comorbidity Yes (n:43)	Comorbidity No (n:49)
Individuals with low immune parameters n=47	2	6	22	25	24	23
IgG, n=1	0	0	0	1	1	0
IgG1, n=2	0	1	1	1	2	0
IgG2, n=0	0	0	0	0	0	0
IgG3, n=1	0	1	1	0	1	0
IgG4, n=7	0	0	5	2	1	6
IgA, n=4	0	2	2	2	3	1
IgM, n=6	1	0	3	3	4	2
$CD3^+$ T cells, n=1	0	0	1	0	1	0
$CD3^+CD4^+$ T cells, n=6	1	1	2	4	1	5
$CD3^+CD8^+$ T cells, n=0	0	0	0	0	0	0
CD4/CD8, n=17	1	2	8	9	9	8
$CD19^+$ B cells n=10	0	0	5	5	5	5
$CD19^+27^+IgD^-$, n=10	0	1	3	7	5	5
$CD16^+56^+$ NK cells, n=4	0	1	0	4	2	2
Tetanus antibody response, n=3	0	0	3	0	1	2
Pneumococcal antibody response, n=2	0	0	1	0	1	0
Isohemagglutinin, n=3	1	0	2	1	1	2

Abbreviations: *CD, cluster of differentiation; ESID, European Society for Immunodeficiencies; Ig, immunoglobulin; NK, natural killer*

DISCUSSION

This study has examined the immune systems of intern doctors who were working actively during the COVID-19 pandemic. An intern doctor who had an underlying immunodeficiency and was unaware of her condition died of severe COVID-19 disease during the pandemic which heightened the fears of other intern doctors. For this reason, those who did not even have symptoms suggesting immunodeficiency went to immunology and allergy polyclinics voluntarily or on the orders of the units where they worked. Low immune system parameters, especially selective IgA, selective IgM, and IgG4 deficiency, were higher than usual among this educated and well-informed group.

IEIs are considered to be rare diseases, affecting one in 10,000 to 50,000 births. However, 70 to 90 percent of them remain undiagnosed (7). Delayed diagnosis of adult patients with IEI remains a challenge for clinicians worldwide. This can be explained by the lack of immunologic studies required to diagnose IEI due to a lack of awareness (8). The International Union of Immunological Societies (IUIS) has increased the number of IEIs to 485 in 2022. The purpose of the IUIS-IEI Expert Committee is to increase awareness, facilitate diagnosis, promote optimal treatment, and support research in clinical immunology (9). A study showed that the awareness of IEI was limited to 32% of the physicians, and awareness was higher among pediatricians (10). The lack of awareness among physicians explains the limited number of reported cases of IEI and delayed diagnosis. The starting point of our study is this lack of awareness.

It is reported that at least 7,000 healthcare workers worldwide have died from COVID-19 (11). The emergence of new pathogens with the COVID-19 pandemic continues to pose potential health risks to the general population due to the lack of immune memory. Individuals with known and unknown IEIs might be at higher risk for a more severe illness following the infection with SARS-CoV-2 (9). In addition to the physiological effects of the disease, it also had psychological effects on the global community. A systematic meta-analysis has shown that the COVID-19 pandemic had a high psychological impact on healthcare workers and people with chronic diseases (12). Another study has also reported that there was an association between higher levels of education and increased anxiety, depression, and stress during the COVID-19 pandemic (5). The population in our study has also consisted of health care workers, which is a group with high levels of education and anxiety at the same time.

ESID suggests 6 warning signs in those with suspected PID and recommends evaluating these drawings more than their owners (6). However, a study has claimed that the ESID criteria are not sufficient to identify immunocompromised patients, and more infection-related questions are required (13). In our study, it has been observed that participants with ESID criteria did not meet any diagnosis of immunodeficiency.

As the spectrum of immunodeficiencies and diagnostic capabilities expand, it is increasingly recognized that IEI is becoming more common. The burden of this disease has been increasing in low and middle-income countries with high consanguinity rates and poor access to diagnosis and treatment (14). Family studies of blood donors have demonstrated that first-degree relatives have a prevalence of selective IgA deficiency of 7.5%, which is 38 times higher than unrelated donors (15). Several other studies have reported that the diagnosis of selective IgA deficiency is more common in families with a consanguineous history (16). In this study, it has been determined that 9.8% of the individuals had consanguinity between their parents. In addition, selective IgA deficiency was observed in 11.1% of those with a family history. It is better to highlight that the high rate of consanguineous marriages in Turkey can explain one of the reasons for the high rate of selective IgA deficiency in our study. In general, selective IgA deficiency ranges from 1:143 to 1:965 in different regions. In a study conducted in Turkey, the rate of selective IgA deficiency was found to be 1/188 (0.53%), and similar results were obtained in other European countries (17). It is considered that the high rate (2.17%) in our study might be due to the high rate of consanguineous marriages in our country and the fact that the population in the study were health professionals with a high level of education and awareness. However, it is argued that the true rate would be higher due to the lack of routine immunodeficiency screening programs and many patients with selective IgA deficiency are asymptomatic (18). The results obtained from the analyses for this study also support this hypothesis.

A method for determining the prevalence of common immunoglobulin deficiencies was the prevalence of selective IgA and IgM deficiencies of 0.097% and 0.03%, respectively. Isolated IgG deficiency was not detected

(19). In another study with adults, selective IgM deficiency was found to be more common than previously thought, with a rate of 0.26% (20). In this study, isolated low IgG was found to be 1.1%, selective IgA deficiency was detected in 2.17%, and selective IgM deficiency was seen in 2.17% of the participants. The rates are found to be higher in this study than similar studies in adults.

IgG, which is one of the most abundant proteins in human serum, is evaluated in four subgroups. Selective subgroup deficiencies are generally not harmful to the individuals and sometimes result in an increased susceptibility to certain pathogens. Generally, one or more IgG subgroup levels (usually IgG2 and/or IgG4) are low in healthy individuals (21). In our study, IgG4 deficiency was seen as the most common, while G2 deficiency was not detected. It is also believed that IgG4 deficiency is common in the general population and most of them are considered to be asymptomatic (22). The results of our study also support this statement.

Deficiencies of lymphocyte subsets are rare and their prevalence is not completely known, especially among the healthy population (23). While low CD8+ was not detected in our study, 1.1% of the participants had low CD3+ and 6.5% of them had low CD4+. However, immunodeficiency was not detected.

A low CD4/CD8 ratio is associated with aging, comorbidities, and mortality. In addition, severe infections such as HIV, chronic inflammatory diseases, diabetes mellitus, and cardiovascular disease are other conditions in which the CD4/CD8 ratio seems to be low (24). In this study, it has been observed that the CD4/CD8 ratio was low in 18.5% of the participants, and comorbid diseases other than infections were present in 9.7% of them. Despite the young age group, it is thought that the comorbidities might have been effective in the low rate.

In the literature, conflicting results have been reported regarding B lymphocyte subsets. Some previous studies have shown that the percentage and the number of cSMB cells decrease significantly with age. However, two different groups have reported an increase in the percentage of total memory B cells with age (25). A recent publication has determined that there were specific changes in B cell subpopulations and an overall age-related decrease in the percentage of CD19+ B cells. Also, a decrease in one or both of the absolute numbers and percentages of memory B cells has been observed in older individuals when compared to younger individuals (26). In this study, low CD19+ B lymphocyte levels were found in 10.9% of the participants, and comorbidity was observed in half of them. Although it has been reported that the number of B cells decreases with age, the data of our study, which consisted of a young population, were seen to be inconsistent with the current literature. In this case, comorbidities are thought to be effective at this low rate. In our study, only 28 of the cSMB cells were able to be examined. Miscarriage was found in 35.7% of those screened for cSM and comorbidity was observed in half of them. When the literature is reviewed, the data belonging to the changes in cSMB cell levels with age seem to be conflicting. However, the decrease in our study which consists of a young age group (between 21 and 28 years old) might be due to comorbidities.

Although NK cell deficiencies are rare, drugs, infections, and genetic factors can affect their number or function. A significant increase in the percentage of NK cells can be seen with age in healthy individuals. In contrast, individuals with chronic diseases have lower NK cytotoxicity (27). In this study, a low NK cell count was determined in 4.3% of the participants and comorbidity was observed in half of them.

In the literature, no significant change in the immune response specific for tetanus and pneumococcal antigens with age has been reported (28). In our study, tetanus-specific antibody deficiency was found in 3.3% of the participants and pneumococcal-specific antibody deficiency was found in 2.2% of them. However, it should be noted that a limitation of this study is that control antibodies could not be examined after vaccination. Although there are significant limitations in their usefulness, isohemagglutinins can be used as another way to assess polysaccharide response. In our study, isohemagglutinin titers were as low as 3.3% of the participants. Isohemagglutinins do not allow the assessment of IgG mediated immunity as they are both IgM and IgG antibodies. It can be recommended to be used in conditions with limited adult population resources (29).

It can be expressed that this study has several limitations. Firstly, it is a cross-sectional and retrospective study. Secondly, control measurements were not able to be made in individuals with low immunologic parameters, control responses could not be checked after vaccination in individuals with insufficient specific antibody responses, and long-term follow-up results could not be obtained. Lastly, a higher number of healthy patients might yield statistically stronger results.

In conclusion, it can be said that studies on the prevalence of immune parameters in young and healthy adults are limited. The low awareness of immunodeficiencies, especially rare diseases, was thought to be one of the obstacles to the detection of this prevalence. In our patient population with a high level of education and awareness, it has been observed that immune system parameters are lower than normal, especially selective IgA, selective IgM deficiency, and low IgG4 are observed to be more frequent. According to the data obtained from this study, more comprehensive and prospective studies are required to understand the reasons for low levels of these parameters better, how they affect the immune system in the long term, and what side effects they might cause in individuals.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the Necmettin Erbakan University of Medical Faculty Ethics Committee (Dated: 03.03.2023; Approval Number: 2023/4212).

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ORIGINAL ARTICLE

Molnupiravir detection by tandem mass spectrometry

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ABSTRACT

Background: After the COVID-19 epidemic 2019, studies on antiviral drugs accelerated. In clinical studies with both re-purposed and newly discovered drugs, the need for reliable methods that measure drug levels in the blood has increased. Molnupiravir is one of the drugs considered under the treatment of COVID-19 and is on the agenda with conflicting findings. However, limited validated methods report the measurement of molnupiravir levels. Therefore, our aim in this study was to develop a practical, robust, validated tandem mass spectrometric method that allows measuring molnupiravir levels.

Methods: Method development studies for the measurement of molnupiravir levels were performed with a liquid chromatography-tandem mass spectrometry (LC-MS / MS) device, and the method was validated according to CLSI (The Clinical & Laboratory Standards Institute) and FDA (Food and Drug Administration) protocols. Linearity, recovery, precision, stability, matrix effect, carry-over, and lower limit determination studies were performed.

Results: The method was linear with a correlation coefficient value of 0.993 in the 20 ng/mL-20 μg/mL range. The sensitivity of the method was 20 ng/mL. The CV% obtained from the intra- and inter-assay studies was below 6.2%, and the mean recovery was over 95%. The total analysis time was 5 minutes for each sample.

Conclusion: A simple, cost-effective, reliable tandem mass spectrometric method with high sensitivity and accuracy based on protein precipitation alone has been developed to measure molnupiravir levels.

Keywords: COVID-19, molnupiravir; pandemic; tandem mass spectrometry; drug monitoring.

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INTRODUCTION

COVID-19 disease, caused by the SARS-CoV-2 virus, has been associated with a significant increase in mortality and morbidity worldwide since 2019 and was declared a pandemic by the World Health Organization in 2020 [1]. The course of COVID-19 ranges from asymptomatic to life-threatening events. Early intervention is crucial, especially for asymptomatic and mild patients, while effective oral antiviral drugs are needed to reduce serious morbidities, hospitalizations, and mortality in severe clinical courses [2, 3]. During the COVID-19 pandemic, approved antivirals and candidates with broad-spectrum antiviral activity have been re-purposed, and studies for developing new molecules have accelerated [4]. Although some broad-spectrum antiviral drugs such as remdesivir, chloroquine, and favipiravir played an essential role at the beginning of the pandemic, studies on the development of small molecule, oral antiviral drugs targeting SARS-CoV-2 have accelerated with the understanding of COVID-19 [5]. Molnupiravir is a small-molecule oral antiviral prodrug effective against Sars-Cov-2 and other RNA viruses. Molnupiravir was developed by Merck and Ridgeback Biotherapeutics for the prevention and treatment of COVID-19 [6].

Molnupiravir is converted to the ribonucleoside analog N-hydroxycytidine (NHC) by host esterases in the plasma. NHC enters the systemic circulation and is intracellularly phosphorylated to NHC triphosphate. NHC triphosphate is incorporated into viral RNA by viral RNA polymerase and then misdirects viral polymerase to incorporate guanosine or adenosine during viral replication. Thus, it causes a series of lethal mutations in the viral genome that render the virus non-infectious. Results of the MOVE-OUT Phase 3 trial, published in 2021, reported a significant reduction of hospitalization and mortality in unvaccinated COVID-19 outpatients administered molnupiravir [7]. However, a recent Oxford PANORAMIC trial showed no decrease in hospitalization and mortality rates in vaccinated outpatients treated with molnupiravir [8]. Although it is stated that molnupiravir has mild side effects such as nausea, vomiting, and headache and is well tolerated by patients, safety concerns were expressed in an animal reproduction study due to its cytotoxic, mutagenic potential, and teratogenic effects. These concerns have been raised that molnupiravir may cause mutations similar to Sars-Cov-2, particularly in rapidly dividing human tissues. However, current health authorities, including the FDA, have approved the clinical use of molnupiravir, stating that it has low mutagenic and cytotoxic potential [9, 10].

Considering all these data, it is clear that further clinical trials on molnupiravir are needed. However, it has been observed that studies evaluating clinical findings of molnupiravir with the molnupiravir blood concentrations are lacking. Therefore, there is a need to develop reliable methods to measure molnupiravir blood levels and to interpret clinical findings together with blood levels in these clinical studies. Various High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV) [11], UV-spectroscopic [12], Highly Sensitive High-Performance Thin-Layer Chromatography (HP-TLC) [13], Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) [14, 15] methods have been developed for the measurement of molnupiravir levels until today. However, these methods had disadvantages such as laborious pre-treatment procedures, extended analysis time, and large sample volume requirements [11-17]. Our aim in this study is to develop a reliable and robust tandem mass spectrometric method for quantifying molnupiravir levels.

MATERIALS AND METHODS

Tandem mass spectrometric analysis

Chemicals and reagents

Molnupiravir capsule (200 mg) was obtained from Clinical Services. Acetonitrile (CAS Number: 75-05-8, HPLC grade, ≥99.9%), HPLC grade water (CAS Number: 7732-18-5, HPLC grade, ≥99.9%), formic acid (CAS Number: 64-18-6, reagent grade, ≥95%), methanol (CAS Number: 67-56-1, HPLC grade, ≥99.9%), carbamazepine (CAS Number 298-46-4, analytical standard, ≥99.9%), bovine serum albumin (CAS Number 9048-46-8, ≥98.5%), potassium chloride (CAS Number: 7447-40-7, ACS reagent, 99.0-100.5%), sodium chloride (CAS Number: 7647-14-5, ACS reagent, ≥99.0%), disodium hydrogen phosphate (CAS Number: 7558-79-4, ACS reagent, ≥99.0%), potassium dihydrogen phosphate (CAS Number: 7778-77-0, ACS reagent, ≥99.0%) were obtained from Sigma Aldrich (St. Louis, MO, USA).

To eliminate the problems associated with matrix effect, molnupiravir was dissolved in a surrogate matrix similar to the human matrix (serum or plasma). For this purpose, different matrices such as methanol, ethanol, acetonitrile, HPLC-grade water, phosphate-buffered saline (PBS)

solution (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4 at 25 °C) were evaluated. However, considering the factors such as protein content, ionic strength, and pH similar to plasma, it was seen that the most suitable surrogate matrix was PBS solution containing 1% BSA. 2 mg/mL stock solution was prepared by dissolving the molnupiravir capsule in PBS solution containing 1% BSA. Then, standard solutions were prepared in the concentration range of 20 ng/mL-20 μg/mL by serial dilution from the stock solution. 100 000 ng/mL stock carbamazepine solution was prepared by dissolving 100 mg carbamazepine standard in 1000 mL methanol. Then, the internal standard working solution of 100 ng/mL was prepared by diluting this stock solution in methanol at a rate of 1/1000. All working and standard solutions were freshly prepared and stored at +4 °C.

The ethical approval was obtained from the Selcuk University local Ethics Committee (Number: 2023/19, Date: 24/10/2023).

Equipment conditions

The analytes were detected using the API 3200 (Applied Biosystems/MDS Sciex) tandem mass spectrometer coupled with the Shimadzu HPLC system. Shimadzu HPLC system (Kyoto, Japan) consisted of a pump (LC-20 AD), an automatic sampler (SIL-20 AC HT), and a unit for online degasser (DGU-20A3). Mass spectrometric analyses were performed using an API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada) with an electrospray ion source (ESI) operating in positive mode. As the mobile phase, a mixture of mobile phase A (HPLC grade water containing 0.1% formic acid) and B (acetonitrile containing 0.1% formic acid) was applied by gradient elution. A Phenomenex Luna C18 reverse phase column (50 × 4.6 mm, 5 μm; part no: 00B-4041-E0) was used to separate the analytes. The column oven temperature was 35 ◦C, and the flow rate was 0.6 mL/min. The precursor/product ion transitions for molnupiravir and internal standard carbamazepine were 328.1/126.0 and 237.0/194.0, respectively. The method optimization parameters were as follows: ion spray voltage, 4500 V; ion source temperature, 500 ◦C; gas1, 60 psi; gas2, 60 psi; curtain gas, 30 psi; collision gas, 6 psi. Declustering potential (DP), collision cell exit potential (CXP), collision energy (CE), and entrance potential (EP) parameters were set to 50, 7, 30, 11 V and 30, 4, 48, 10 V for molnupiravir and carbamazepine, respectively.

Sample Preparation

200 μl working or standard solution, 100 μl internal standard (100 ng / mL carbamazepine), and 500 μl acetonitrile were added to eppendorf tubes and vortexed for 30 seconds. Afterward, the mixture was centrifugated at 3500 rpm for 10 minutes. Then, 25 μl supernatant was injected into the LC-MS/MS system.

Method Validation

The developed method was validated according to the CLSI (Clinical and Laboratory Standards Institute) [18] and FDA (Food and Drug Administration) [19] protocols. The validation process includes linearity, precision, matrix effect, recovery, carry-over, and stability studies.

Statistical analysis

The method validation performance was evaluated using the Ep-Evaluator Release 8.0 version (Data Innovations, South Burlington, VT) and Excel (2010) programs.

RESULTS

Linearity study

Linearity studies were performed according to the CLSI EP6-A protocol [18], and the linearity study findings were evaluated with the Ep Evaluator Release 8 program. The standard solutions were prepared in the concentration range of 20 ng/mL-20 μg/mL by serial dilution from the stock solution. Method linearity was evaluated by linear regression analysis, and the correlation coefficient was calculated as 0.993. The limit of detection (LOD) and limit of quantitation (LOQ) values were determined by signal/noise ratio according to CLSI EP17-A protocols [18]. A signal/noise ratio of approximately 3 was considered LOD, and a value of 10 was considered LOQ. Accordingly, the LOD value was determined as 5 ng/mL and the LOQ as 20 ng/mL. The The calibration curve was presented in Figure 1. Chromatograms of LOD (5 ng/mL) and LOQ (20 ng/mL) values were presented in Figure 2 and Figure 3, respectively.

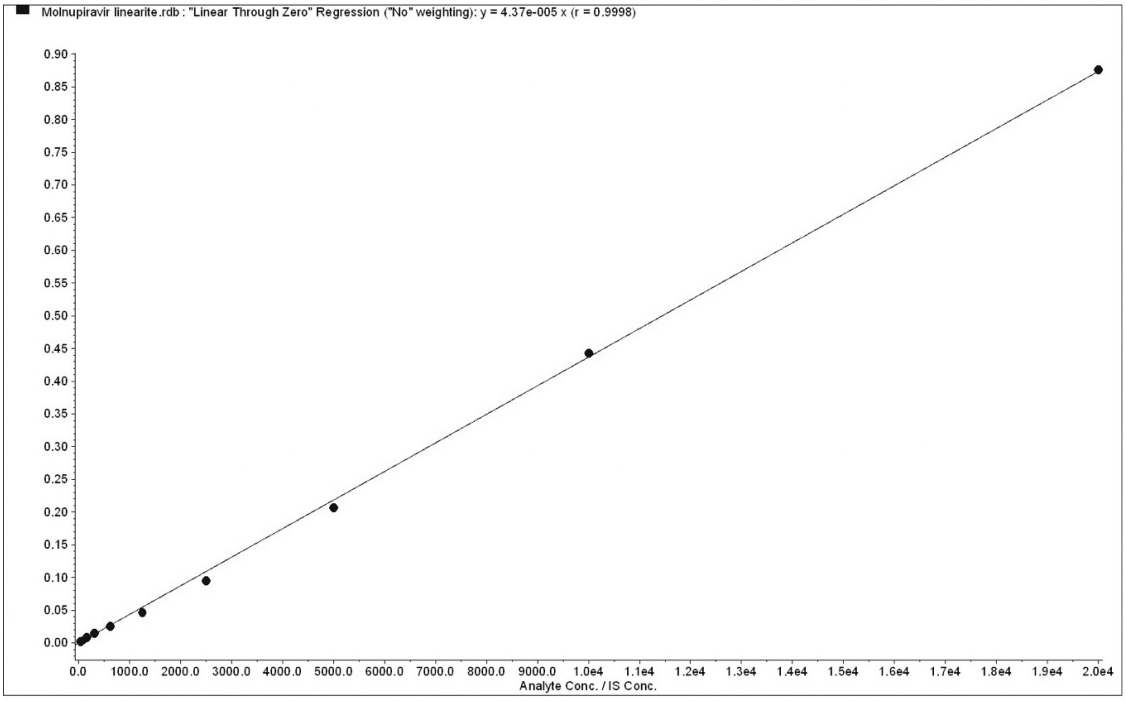

Figure 1. The calibration curve of molnupiravir.

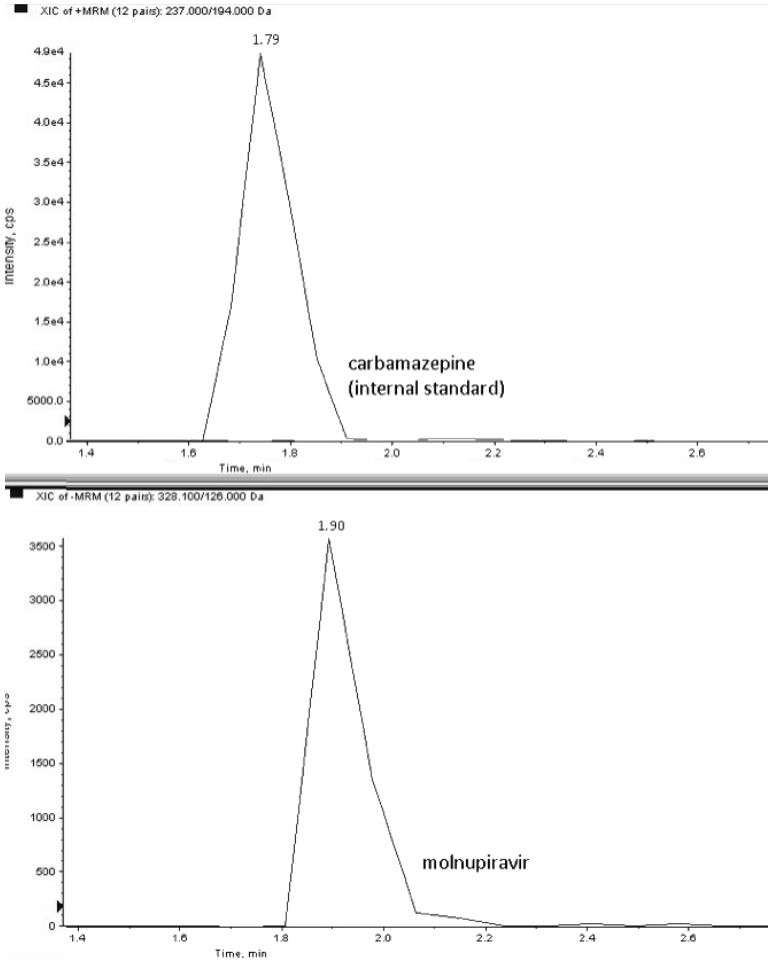

Figure 2. An example chromatogram of the molnupiravir standard at a concentration (LOD) of 5 ng/mL.

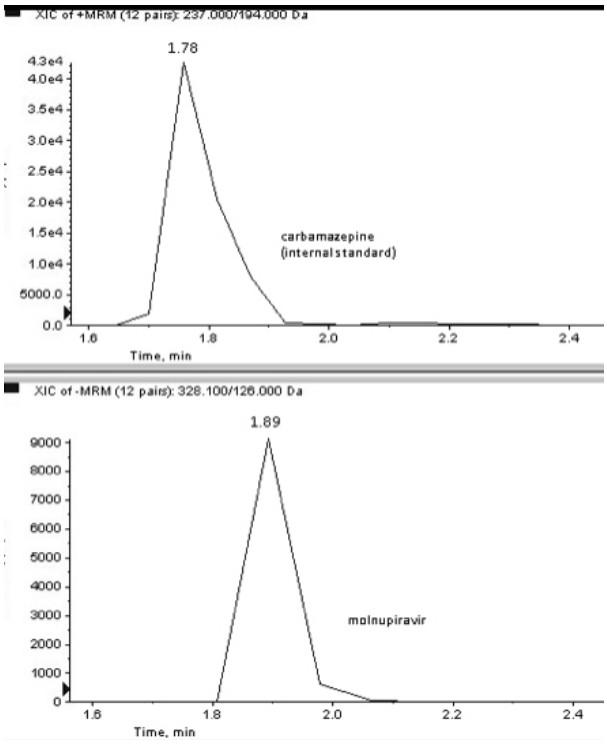

Figure 3. An example chromatogram of the molnupiravir standard at a concentration (LOQ) of 20 ng/mL.

Precision and accuracy study

The precision study was performed according to FDA protocols [19], including intra- and inter-assay precision. The precision study was performed with five different QC samples (LLOQ, low, medium 1, medium 2, and high QC). For the preparation of QC samples in the precision study, a 2 mg/mL stock solution of molnupiravir in PBS solution containing 1% BSA was used. During method validation, the QCs for accuracy and precision runs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within 3 times of the LLOQ (low QC), around 30% to 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC) [19]. Accordingly, five different QC samples (LLOQ, low, medium 1, medium 2, and high QC) were prepared using the solution from the stock. LLOQ level was 20 ng/mL. A low QC sample was prepared at a concentration of 3 times the LLOQ. Medium 1 level corresponds to the middle level of the linear range. Medium 2 level was 75% of the high QC sample. For inter-assay precision, 4 replicates of each level were run for 5 days. To calculate the intra-assay CV%, 40 replicates were run at each level, 20 in the morning and 20 in the afternoon. The method's intra- and inter-assay CV% values ranged between 4.3% and 6.2%. The precision (CV%) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, which should not exceed 20% according to FDA guidelines[19]. The results were expressed in Table 1.

Table 1. The results of molnupiravir precision study.

	Intra-assay				Inter-assay			
Concentration(ng/mL)	Mean	SD	CV%	Accuracy%	Mean	SD	CV%	Accuracy%
20	20.62	1.06	5.14	103.1	20.75	1.28	6.17	103.7
60	59.91	2.60	4.35	99.8	58.91	2.96	5.03	98.2
10000	10313	492	4.78	103.1	10221	613	6.01	102.2
15000	15184	688	4.53	101.2	15063	907	6.02	100.4
20000	20018	1017	5.08	100.1	19965	1112	5.57	100.1

An accuracy study was performed by analyzing 4 replicates per level at the LLOQ, low (LQC), medium 1 (MQC1), medium 2 (MQC2), and high-quality control (HQC) samples for five consecutive days. Accuracy was calculated as a percentage of the measured value to the expected value. The acceptability criteria for accuracy studies, according to the FDA guidelines, are that the bias value for LLOQ should be <20% and for other quality control (QC) values <15% [19]. The accuracy ranged between 98.2 and 103.7% for molnupiravir.

Recovery and matrix effect study

The recovery study was conducted with QC samples at 3 different concentration levels. The recovery study results were calculated as the average "measured value/expected value" ratio (%). The matrix effect study was performed according to the procedure specified by Chambers et al. [20]. The results were expressed in Table 2. In the matrix effect study, the response of the analyte in a neat solution was compared to the response of the spiked analyte in the pretreated surrogate matrix. Accordingly, 3 different levels of QC samples were prepared in the mobile phase mixture (water and acetonitrile, 1:1), and the response of the analyte in these QC samples was compared with the response of the analyte in the pretreated surrogate matrix. The matrix effect was calculated using the formula: (ME% = (mean post-extracted peak area / mean un-extracted peak area) × 100). The recovery value of the method was between 98% and 102%, and the matrix effect was below 7%.

Table 2. Recovery and matrix effect results of molnupiravir measurements.

Analyte	Concentration(ng/mL)	Recovery	Matrix effect
Molnupiravir	50	98.5%	-6.9
	5000	101.1%	4.5
	15000	97.4%	-4.9

Stability study

The stability study was performed according to the CLSI EP25-A protocol. For stability studies, 3 QC levels were prepared in the surrogate matrix, including the low, medium, and high QC samples. These samples were kept at -20 °C for freeze-thaw stability and then freeze-thawed 4 times with an interval of 5 days. For the long-term stability study, 4 aliquots were prepared for each QC level. The first replicate was run before freezing, and the other replicates were run on days 15, 30, and 45, respectively. The bias% values were calculated compared to the measured analyte levels on the collection day (expected value) via the following formula: Bias%=((measured value-expected value) / expected value) ×100

The results were expressed in Table 3

Table 3. The stability of molnupiravir at different temperatures (bias%).

Analyte	Concentration(ng/mL)	Frozen -(20C) for 45 day			Freeze-thaw stability		
		15. Day (%)	30. Day(%)	45. Day(%)	2.	3.	4.
Molnupiravir	50	3.66	5.12	7.53	4.15	6.63	8.65
	5000	2.98	4.97	5.88	3.94	5.36	7.39
	15000	4.15	5.56	6.52	2.63	4.58	6.36

The processed sample stability was also investigated by maintaining the QCs samples in an auto-sampler at 4 °C for 20 h, followed by analysis. The bias% values changed between 4.5% and 7.8%.

Carry-over study

This study has been performed according to CLSI EP10-A [18]. The high and low-level QC samples were analyzed using the order specified in the CLSI EP10-A protocol. The mean and standard deviations of the groups were calculated using the EP Evaluator Release 8 program. The carry-over study was conducted individually for each analyte. The orders of samples were expressed as follows: L1-L2-L3-H1-H2-L4-H3-H4-L5-L6-L7-L8-H5-H6-L9-H7-H8-L10-H9-H10-L11. The carry-over value was calculated as 1.22 ng / mL for molnupiravir. The EP Evaluator program determined the acceptability criteria based on CLSI protocol EP10-A3 guidelines, and according to EP Evaluator software, evaluation carry-over value was acceptable for molnupiravir. This study was approved by the clinical research ethics committee of the Selçuk University Faculty of Medicine (Date: 24.10.2023, Number: 2023/19).

DISCUSSION

With the declaration of COVID-19 as a pandemic, many therapeutic compounds have been re-purposed, and studies on developing new agents have accelerated. Molnupiravir is one of the antiviral drugs considered for the treatment of COVID-19. However, findings from clinical studies regarding the efficacy and safety of molnupiravir in the treatment of COVID-19 are contradictory. One of the most important limitations of these studies is the lack of co-evaluation of drug blood levels and clinical data. Therefore, there is a need for methods that allow reliable and practical measurement of molnupiravir levels [21].

Various HPLC methods have been reported for the measurement of molnupiravir levels. However, these methods were disadvantageous due to their low sensitivity, incomplete peak separation, long retention time, laborious pre-treatment procedures, and low recovery and precision [11,12,17, 22]. LC-MS/MS is accepted as the gold standard for drug-level measurement due to its high accuracy, sensitivity, precision, and low risk of interference [23].

For this reason, we developed a new method for quantitating molnupiravir levels in our study. However, limited studies report the development of a validated tandem mass spectrometric method for measuring molnupiravir levels. For example, Gouda et al. reported a tandem mass spectrometric method that allows measurement of molnupiravir levels in plasma. The method indicated was linear for molnupiravir in the 20 to 10000 ng/mL range. The CV% calculated from the precision study ranged from 0.7% to 9.4%. The extraction recovery % results ranged from 78.2 to 80.1%. The CV% calculated from the precision study was below 15%, and the recovery% ranged from 95% to 100%. However, the pre-treatment procedures consisted of a laborious procedure involving the concentration of samples under nitrogen [14]. Amara et al. reported a tandem mass spectrometric method based on measuring molnupiravir and its metabolite in plasma and saliva. The method's intra- and inter-assay CV% values ranged from 1.25% to 9.05%. The mean recovery was over 90% in plasma samples. In addition, the long and laborious pre-treatment steps based on the concentration of the samples under nitrogen were another disadvantage of the method [15]. Parsons et al. reported a validated tandem mass spectrometric method that allows the measurement of molnupiravir metabolites in plasma and peripheral blood mononuclear cell lysates. The CV% value of this method ranged from 1.42% to 11.8%. The average recovery was 74.2%. However, this method did not include validation of parent drug levels [16].

Considering the scarcity of reported methods for measuring molnupiravir levels, it is clear that new, practical, and reliable methods are needed. For this purpose, we developed a validated tandem mass spectrometric method for quantitating molnupiravir levels in our study. Our method was advantageous because it required a minimal sample volume, good sensitivity, expanded measurement range, high precision, low matrix effect, simple and economical pre-treatment procedures, and short analysis time.

Compared to other LC-MS/MS methods [14, 15], the method we developed relied only on a simple pre-treatment step involving protein precipitation followed by centrifugation of the samples. So, its simple, economical pre-treatment steps, short analysis time (3.5 min), and relatively low sample volume (200 μl) were significant advantages of our method.

The method we developed also had a wide measurement range (20 ng/mL-20 μg/mL). It had adequate sensitivity with LOD of 5 ng/mL and LOQ of 20 ng/mL. For example, Gouda et al., the measurement range of the tandem mass spectrometric method specified was 20-10000 ng/mL,

and the LOQ value was 20 ng/mL [14]. In various HPLC methods, the LOQ values for molnupiravir varied between 100 and 5000 ng/mL [24-26].

In the matrix effect study, molnupiravir was dissolved in a surrogate matrix similar to the human matrix (serum or plasma) to eliminate the problems associated with the matrix effect. Considering factors such as protein content, ionic strength, and pH similar to those of plasma, it was seen that the most suitable surrogate matrix was a PBS solution containing 1% BSA. As a result of our study, the matrix effect value was less than 7%. According to CLSI protocols, the matrix effect should be less than 15%. Therefore, the matrix effect value of the method was low and at an acceptable level [18].

The accuracy ranged between 98.2 and 103.7% for molnupiravir. The method's intra- and inter-assay CV% values ranged between 4.3% and 6.2%. According to the FDA guidelines, the acceptability criteria for accuracy studies are that the bias value for LLOQ should be <20% and for other quality control (QC) values <15%. The precision (CV%) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, which should not exceed 20% [19]. Therefore, our method had acceptable accuracy and precision.

In this study, a cost-effective, simple, robust, and reliable measurement method was developed to measure molnupiravir levels. However, our study has limitations regarding the lack of measurement of molnupiravir metabolite levels and drug and metabolite levels in real patient samples or biological matrices. The decrease in COVID-19 patients and the reduction in molnupiravir administration made working with the real patient population difficult. In this study, only the method for measuring parent drug levels was developed with molnupiravir commercial capsules. Further studies, including real patient samples, are needed. However, our study is important considering the limited studies in this area and the importance of measuring antiviral drug levels. There is a need for new studies that allow the measurement of molnupiravir and metabolite levels in the real patient population or various pharmacokinetic models.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the clinical research ethics committee of the Selçuk University Faculty of Medicine (Date: 24.10.2023, Number: 2023/19).The authors contributed equally to the study.

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ORIGINAL ARTICLE

Evaluation of telemedicine and health tourism awareness of healthcare professionals

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ABSTRACT

Background: Electronic infrastructures are important for improving the quality of health services. For this reason, our study wanted to evaluate the attitudes of healthcare professionals toward the use of electronic infrastructure and their awareness of telemedicine and health tourism.

Methods: This cross-sectional study, conducted among 108 healthcare professionals in a local hospital, analyzed the data obtained from the survey using the student t-test and the GraphPad Prism 9 statistical program.

Results: 42 male and 66 female volunteer healthcare workers participated in the study. When the participants' responses regarding the training they received regarding service provision to foreign patients were evaluated, the subject in which the training was most inadequate was foreign language (2.1%) ($p<0.05$). Physicians (50.1%), health license holders (69.8%), and non-healthcare workers (55.6%) thought that they could use telemedicine applications due to their profession ($p<0.05$). In addition, while healthcare professionals with less professional experience were more willing to use electronic infrastructure, their awareness levels were low ($p<0.05$).

Conclusion: More studies should be planned to eliminate deficiencies in health policies by effectively matching local needs with global expectations, especially health tourism and electronic health service provision.

Keywords: Healthcare Education, Medical Tourism, Telemedicine

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INTRODUCTION

Since ancient times, individuals have constantly migrated or traveled for centuries to regain health (1). The concept of health, which is very important, has been defined by the World Health Organization (WHO) as a state of complete mental, physical, and social well-being of individuals. This definition sets out the many goals of WHO, such as ensuring social welfare, preventing disease, and improving medical outcomes (2). With the beginning of seeing the individual's health as a concept above everything else, the health literacy of society has increased day by day to improve the state of physical or mental well-being. Parallel to this, "health tourism" emerged and promised people hope for complementary treatments in many areas, such as getting rid of diseases, fighting stress, and improving aesthetics (3). When health tourism is evaluated broadly, it has become a global industry (4).

As a reflection of digitalization in the healthcare sector, telemedicine provides benefits in supporting patient treatment and facilitating care services. These applications, which allow individuals to communicate with health institutions electronically without the distance barrier, offer various cost and quality advantages (5). As such, telemedicine is a technology that enables individuals to communicate with healthcare professionals by overcoming geographical barriers and bringing healthcare personnel together in the same environment (6). Telemedicine includes many applications, including information technologies, communication, and interactive video systems. While the first comprehensive examples of telemedicine were encountered in the 1960s, it showed significant development, especially in the 1990s (7). Since telemedicine is a vast field, various studies on field effectiveness, advantages, and costs continue. While facilitating access, increasing the quality of service, and reducing the cost of care are positive opinions, the deficiencies in the communication, organization, and quality structure between health professionals and patients, problems with health services, and data privacy protection are the aspects criticized (8).

While telemedicine applications were used in many health units, these applications had to be revised due to the significant expansion of the user universe during the global epidemic (9). Different opinions have been put forward regarding the use of telemedicine applications, especially in medical tourism; it is a common view that infrastructure investments such as call centers, support systems, and a qualified workforce are needed to integrate them into health tourism. Various studies show that integrating telemedicine and health tourism will affect tourists' travel behavior, and they can approach health tourism more broadly (10). Since telemedicine in health tourism will eliminate language-related, cultural, or geographical barriers, e-hospitals can serve more tourists (11).

Although electronic infrastructures have the potential to improve the quality of healthcare delivery, we do not have sufficient information about service providers' attitudes and awareness toward these technologies. For this reason, we planned our study to determine the knowledge levels and attitudes of health professionals who directly participate in providing health services. The research only includes the statistical evaluation of the results of the answers given to the survey questions and does not include the answers to all questions related to the field. The research is limited to August 2022 and was carried out at the local level. For this reason, it should be supported by more regional, national, or international studies.

MATERIALS AND METHODS

Study Design and Sampling

It is a cross-sectional survey study conducted among healthcare professionals in Bozyazı State Hospital of Mersin City in 2022. The universe size (N) is 150. The sample size was 108, considering the 95% confidence interval and margin of error α=0.05. There are 42 male and 66 female participants in the study population. Due to different demographic groups, the sample size (f: n/N) of the number of individuals to be included in the subsets of the universe was determined with a coefficient of 0.72 (Table 1).

Table 1. Determination of sample size and stratified sampling

$n = \frac{N\,t^2\,p}{d^2(N-1)+t^2\,p\,q}$	$n = \frac{150 \times (1.96)^2 \times 0.50 \times 0.50}{(0.05)^2 \times (150-1) + (1.96)^2 \times 0.50 \times 0.50} = \frac{144}{1.33} = \underline{\mathbf{108}}$
(f: n/N): 108/150: 0.72	
Profession	**n**
Medical Doctor	16x0.72=11.52
Graduate	2x0.72=1.44
Undergraduate	87x0.72=62.64
Associate degree	4x0.72=2.88
Elementary school, high school	3x0.72=2.16
Non-health worker	38x0.72=27.36

Demographic strata were selected using a simple table of random numbers, in line with the principle of equal representation. After the informed consent form was obtained from the volunteers participating in the research, they were informed about the research by the researcher (S.C), and it was stated that they could withdraw from the research at any stage of the study if they wished.

Analysis of data

The survey questions were prepared by considering the academic data presented before and scientific studies in the literature on health tourism and telemedicine. The data obtained in the survey were analyzed using the GraphPad Prism 9 statistical program, and the difference between the groups was examined using the student-t test. The difference between the groups was considered significant when $p<0.05$. This study was approved by Alaaddin Keykubat University Scientific Research and Publication Ethics Committee (Date: 25.05.2022, Number:12) and Mersin Provincial Directorate of Health (Date: 28.07.2022 Number:55)

RESULTS

The answers of the health professionals who volunteered in our study to the survey questions about their awareness of health tourism and their views on the integration of telemedicine applications into health tourism were evaluated statistically.

42 male (38.9%) and 66 female (61.1%) female volunteer health workers participated in the study. 11.1% of the participants are physicians, 63.9% are health personnel, and 25% are administrative personnel. The numerical distribution of the participants is related to the number of personnel working in the hospital and the power of representation. When the distribution by age group is evaluated, 44.5% of the participants are in the 20-40 age range, and 55.5% are over 40. While 17.6% of the participants had 0-5 years of working life experience, it was seen that 54.6% had more than 16 years of professional experience (Table 2).

When the participants' answers were evaluated regarding the training received for providing services to foreign patients, it was revealed that 35.4% had yet to receive training on this subject. The subject in which the most inadequate

Table 2: Demographic information of participants

		n	%
Gender	Male	42	38.9
	Female	66	61.1
	Total	108	100.0
Profession	Medical Doctor	12	11.1
	Graduate	1	0.9
	Undergraduate	63	58.3
	Associate Degree	3	2.8
	Elementary school, high school	2	1.9
	Non-health worker	27	25
	Total	108	100.0
Age	20-29	21	19.5
	30-39	27	25
	40-49	51	47.2
	+50	9	8.3
	Total	108	100.0
Professional Experience	0-5	19	17.6
	6-10	10	9.3
	11-15	20	18.5
	+16	59	54.6
	Total	108	100.0

training was reported was foreign language (2.1%). It was observed that examination services (71.3%) could benefit more through electronic infrastructures compared to consultation (24.1%), nutritional health services (17.5%), and rehabilitation services (p<0.05). The preference for using electronic infrastructures for health tourism (71.3%) was found to be significantly positive (p<0.05) (Table 3).

Table 3: Evaluation of the training received by participants for service delivery and their preferences in using telemedicine applications

		n	%	Sum	p##
◊ Distribution of training received to serve foreign patients	Health services to be provided to the patient (a)	33	22.9	a-c a-d a-e a-f b-c b-d b-e b-f c-d c-e d-e d-f	<0.05
	Patient rights (b)	30	20.8		
	Services to be provided to the patient's relatives (c)	15	10.4		
	Foreign Language (d)	3	2.1		
	None (e)	51	35.4		
	Other (f)	12	8.4		
	Total	144	100.0		
Regarding the use of applications and electronic infrastructures such as telephone/video calling in various healthcare services	In Pre-Inspection Services (a)	73	44	a-b a-c a-d a-e b-d b-e c-d c-e	<0.05
	Tele Consultation (b)	40	24.1		
	Nutrition and Healthy Living Services (c)	29	17.5		
	Rehabilitation Services (d)	16	9.6		
	Other (e)	8	4.8		
	Total	166	100.0		
Inspective controls can be evaluated using electronic infrastructures	Strongly Disagree (a)	1	0.9	a-c	<0.05
	Disagree (a)	19	17.6		
	Neither agree nor disagree (b)	11	10.2		
	Agree (c)	53	49.1		
	Strongly Agree (c)	24	22.2		
	Total	108	100.0		
Telemedicine integration is required in health tourism	Strongly Disagree (a)	1	0.9	a-c	<0.05
	Disagree (a)	3	2.8		
	Neither agree nor disagree (b)	27	25		
	Agree (c)	46	42.6		
	Strongly Agree (c)	31	28.7		
	Total	108	100.0		

Student t-Test

◊ Participants were able to tick more than one option.

a, b,c: Responses were combined according to the strength of certainty statements and compared with apparent approval, disapproval, or indecision.

Male participants (54.7%) prefer the pre-inspection more than female participants (48.5%), while female participants (72.7%) prefer the post-inspection more than male participants (69%) (p<0.05)

Table 4: Participants' evaluations of telemedicine use in gender variable telemedicine applications

		n	%	Sum	p##
I prefer the use of electronic infrastructure for my pre-inspective examination (woman)	Strongly Disagree(a)	4	6.1		
	Disagree(a)	19	28.7		
	Neither agree nor disagree (b)	11	16.7	a-c a-b b-c	<0.05 <0.05 <0.05
	Agree(c)	25	37.9		
	Strongly Agree(c)	7	10.6		
	Total	66	100.0		
I prefer the use of electronic infrastructure for my pre-inspective examination (men)	Strongly Disagree(a)	2	4.8		
	Disagree(a)	9	21.5		
	Neither agree nor disagree (b)	8	19	a-c a-b b-c	<0.05 <0.05 <0.05
	Agree(c)	19	45.2		
	Strongly Agree(c)	4	9.5		
	Total	42	100.0		
I prefer the use of electronic infrastructure for my post-inspective examination (woman)	Strongly Disagree(a)	0	0		
	Disagree(a)	16	24.3		
	Neither agree nor disagree (b)	2	3	a-c a-b b-c	<0.05 <0.05 <0.05
	Agree(c)	32	48.4		
	Strongly Agree(c)	16	24.3		
	Total	66	100.0		
I prefer the use of electronic infrastructure for my post-inspective examination (men)	Strongly Disagree(a)	1	2.4		
	Disagree(a)	3	7.2		
	Neither agree nor disagree (b)	9	21.4	a-c a-b b-c	<0.05 <0.05 <0.05
	Agree(c)	21	50		
	Strongly Agree(c)	8	19		
	Total	42	100.0		

Student t-Test

a, b,c: Responses were combined according to the strength of certainty statements and compared with apparent approval, disapproval, or indecision.

Sum: Comparison between groups

Physicians (50.1%), healthcare licensees (69.8%), and non-health workers (55.6%) assumed that they could use telemedicine practices in terms of their profession (p<0.05). 100% of the physicians, 82.6% of the health licensees, and 70.4% of the administrative staff expressed positive opinions about the benefits of integrating these practices into health tourism (p<0.05)

Table 5: Evaluation of telemedicine applications in health tourism in occupational variable

		n	%	Sum	p##
Considering professionally, i can use telemedicine applications in my profession (physician)	Strongly disagree(a)	1	8.3	a-c	<0.05
	Disagree(a)	1	8.3		
	Neither agree nor disagree (b)	4	33.3		
	Agree(c)	4	33.3		
	Strongly agree(c)	2	16.8		
	Total	12	100.0		
Considering professionally, i can use telemedicine applications in my profession (Undergraduate)	Strongly disagree(a)	1	1.6	a-c	<0.05
	Disagree(a)	8	12.7		
	Neither agree nor disagree (b)	10	15.9		
	Agree(c)	33	52.4		
	Strongly agree(c)	11	17.4		
	Total	63	100.0		
Considering professionally, i can use telemedicine applications in my profession (non-health worker)	Strongly disagree(a)	0	0	a-c	<0.05
	Disagree(a)	4	14.8		
	Neither agree nor disagree (b)	8	29.6		
	Agree(c)	13	48.2		
	Strongly agree(c)	2	7.4		
	Total	27	100.0		
In the future, telemedicine applications should be integrated into health tourism (physician)	Strongly disagree(a)	0	0	a-c	<0.05
	Disagree(a)	0	0		
	Neither agree nor disagree (b)	0	0		
	Agree(c)	9	75		
	Strongly agree(c)	3	25		
	Total	12	100.0		
In the future, telemedicine applications should be integrated into health tourism (Undergraduate)	Strongly disagree(a)	3	4.8	a-c	<0.05
	Disagree(a)	4	6.3		
	Neither agree nor disagree (b)	4	6.3		
	Agree(c)	28	44.5		
	Strongly agree(c)	24	38.1		
	Total	63	100.0		
In the future, telemedicine applications should be integrated into health tourism (non-health workers)	Strongly disagree(a)	0	0	a-c	<0.05
	Disagree(a)	0	0		
	Neither agree nor disagree (b)	8	29.6		
	Agree(c)	13	48.2		
	Strongly agree(c)	6	22.2		
	Total	27	100.0		

Student t-Test

WW◊ Participants were able to tick more than one option.

a,b,c: Responses were combined according to the strength of certainty statements and compared with apparent approval, disapproval, or indecision.

In addition, considering the age of the participants, it was observed that healthcare professionals with less professional experience were more willing to use electronic infrastructure, but their awareness level was low (p<0.05)

Table 6: Definition of telemedicine in age variable and use of technological infrastructure

		n	%	Sum	p##
The state of knowing the definition of tele-medicine (20-29)	I know exactly (a)	6	28.6	a-c	<0.05
	Neither agree nor disagree (b)	6	28.6		
	I don't have enough information(c)	9	42.8		
	Total	21	100.0		
The state of knowing the definition of tele-medicine (30-39)	I know exactly (a)	14	51.9	a-c	<0.05
	Neither agree nor disagree (b)	6	22.2		
	I don't have enough information(c)	7	25.9		
	Total	27	100.0		
The state of knowing the definition of tele-medicine (40-49)	I know exactly (a)	26	51	a-c	<0.05
	Neither agree nor disagree (b)	7	13.7		
	I don't have enough information(c)	18	35.3		
	Total	51	100.0		
The state of knowing the definition of tele-medicine (50+)	I know exactly (a)	4	44.4	a-c	>0,05
	Neither agree nor disagree (b)	1	11.2		
	I don't have enough information(c)	4	44.4		
	Total	9	100.0		
I prefer to use technological devices and applications that contain my health information (20-29)	Strongly Disagree(a)	0	0	a-c	<0.05
	Disagree(a)	1	4.7		
	Neither agree nor disagree (b)	0	0		
	Agree(c)	13	62		
	Strongly Agree(c)	7	33.3		
	Total	21	100.0		
I prefer to use technological devices and applications that contain my health information (30-39)	Strongly Disagree(a)	0	0	a-c	<0.05
	Disagree(a)	0	0		
	Neither agree nor disagree (b)	1	3.7		
	Agree(c)	16	59.3		
	Strongly Agree(c)	10	37		
	Total	27	100.0		
I prefer to use technological devices and applications that contain my health information (40-49)	Strongly Disagree(a)	1	2	a-c	<0.05
	Disagree(a)	4	7.8		
	Neither agree nor disagree (b)	3	5.9		
	Agree(c)	31	60.8		
	Strongly Agree(c)	12	23.5		
	Total	51	100.0		
I prefer to use technological devices and applications that contain my health information (50^{+})	Strongly Disagree(a)	1	11.1	a-c	<0.05
	Disagree(a)	0	0		
	Neither agree nor disagree (b)	1	11.1		
	Agree(c)	4	44.5		
	Strongly Agree(c)	3	33.3		
	Total	9	100.0		

Student t-Test

Sum: Comparison between groups

DISCUSSION

E-applications developed today have become an integral part of health systems. On the other hand, telemedicine includes integrating some of these applications into health service delivery and modifications specific to this field. In addition to aiming to reveal a local result in this field by evaluating the awareness levels of health tourism and telemedicine at a cross-sectional level in terms of health workers, our study also aims to determine the target groups we will focus on in terms of providing better quality service and our shortcomings in this regard, based on the results obtained.

While evaluating the place of telemedicine in health services, Zywietz's study stated that innovative applications should be included in health and tourism. He noted that improving devices and infrastructure services would benefit service quality (12). In the study of Martinez et al., an alternative e-application design in health tourism was presented. The application considered to be developed in this study offers solutions for the problems that patients may encounter during travel and health care (13). When our study results are evaluated, our results suggest that telemedicine and health tourism integration will support positive results in this field.

Telemedicine is a clinical practice that connects a patient to specialist care counselors via electronic platforms, potentially improving patient self-management and allowing the care of patients with limited access to healthcare (14). Artificial intelligence-supported telemedicine applications are used in many fields, from ophthalmology (15) to migraine treatment (16), from respiratory diseases (14) to addiction treatment (17). Shih et al. conducted a study on tele-organ transplants using a face-to-face interview with 50 people in 3 different institutions. 80% of the participants stated that, in this case, a violation of the medical law might be in question, 74% said that tele-systems could be integrated into this field, and 36% stated that there might be cost fluctuations (18). Moving some medical interventions from a central location to the patient's home can reduce healthcare costs (14). When patients are disadvantaged in receiving specialist access or advanced care, telemedicine models can offer an opportunity to equalize care at different levels. Telemedicine services are expanding, but empirical research on best practices and consequent problems for telemedicine models and subsets still needs to be done (19). Research highlights deficiencies in the accessibility of telemedicine among older people, particularly in West African countries (20).

Our study shows that physicians and health licensees similarly support integrating telemedicine applications in the professional field. When the study results are evaluated at the local level, it shows that the target of education planning should be young health workers, especially those at the beginning of their careers, and improvements should be made for all health professionals working in this field. The deficiency is mainly in knowing a foreign language and serving foreign patients.

All doctors working at an online e-hospital that opened in 2015 were bilingual doctors who provided services from teleconsultation to international transfer and treatment to the United States. Such telemedicine platforms for medical tourism save patients the trouble of identifying and connecting with an appropriate healthcare provider and minimize language and cultural barriers (11). However, Sorensen reported that traveling to another country carries some risks. This situation has been associated with the absence of information about the patient's health history in the hands of healthcare professionals (21). The greatest challenge to the combined use of telemedicine and health tourism applications is the inability to reliably assess healthcare quality across borders. The increasing complexity of healthcare delivery and the rapidly rising cost of medical care require transparency in pricing and standardization of quality assessment. There is a particular need for a reliable cross-border comparable assessment system for international patients. As Shaw argues, we need to standardize healthcare delivery, and more effort is required to improve regulation, institutional licensing, accreditation, and transparency (22).

On the other hand, although the studies on patient satisfaction are very new, in a study evaluating the telemedicine service offered to epileptic patients during the COVID-19 pandemic, 39.1% of the patients stated that they were unaware of this service. In addition, 95% of the service recipients indicated satisfaction with this application (23).

All these data show that with the increase in age and professional experience of healthcare professionals, awareness of telemedicine applications increases, and healthcare personnel generally have a favorable opinion on this issue. However, when the training received is considered, it has been observed that the health personnel received training in a foreign language at a lower level than other training. It is seen that this education on approaching foreign patients and service delivery requirements needs to catch up with the education in different fields. In general, it is seen that integrating telemedicine services into health

tourism, especially in pre-examination service, is welcomed more positively, followed by services related to consultation and nutrition counseling. Female participants are more optimistic about performing post-inspective controls using electronic infrastructures.

In conclusion, our study reveals regional health professionals' views, expectations, and attitudes regarding providing electronic infrastructure services, especially in health tourism. Providing a quality service seems closely related to language education, cultural situations, and technical infrastructure support. However, since cultural structure is a crucial component in service delivery, differences between countries may cause differences in the attitudes and views of health professionals. When the literature data is evaluated, it is seen that more research is needed in this area.

Declarations:

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This study was approved by Alaaddin Keykubat University Scientific Research and Publication Ethics Committee (Date: 25.05.2022, Number:12) and Mersin Provincial Directorate of Health (Date: 28.07.2022 Number:55)

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ORIGINAL ARTICLE

Turkish validity and reliability study of the falsified hand sanitizer identification scale

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ABSTRACT

Background: The objective of this study was to assess the validity and reliability of the Falsified Hand Sanitizer Identification Scale in Turkish culture and language, which was developed to help consumers identify safe and effective hand disinfectants.

Methods: Between February and April 2021, this methodological study used an online questionnaire to survey state university staff members. 355 people were reached. The scale's Turkish validity was assessed using language (translation-back translation), construct (confirmatory factor analysis (CFA)), and known group validity. Cronbach's alpha internal consistency coefficient was used to assess reliability, as well as item-total correlation analysis and the test-retest method (Spearman correlation analysis).

Results: The majority of the participants (62.18%, n = 217) were male. The mean age of all participants was 41.14 ± 9.80 years. In DFA, $x^2/sd = 3.67$, with CFI = 0.98, GFI = 0.92, NFI = 0.97, and RMSEA = 0.08. As the frequency of daily use of sanitizer increased, the score obtained from the scale increased ($p < 0.001$). When all of the items were removed from the scale, its Cronbach's alpha coefficient decreased. The Cronbach's alpha coefficient for the scale was 0.934, with 0.892 for factor 1, 0.891 for factor 2, and 0.818 for factor 3. The corrected item-total correlation coefficients for all items ranged between 0.584 and 0.758. The test-retest correlation coefficient was 0.859 ($p < 0.001$).

Conclusion: The Turkish Falsified Hand Sanitizer Identification Scale is a valid and reliable 5-point Likert scale consisting of 12 items and three sub-dimensions.

Keywords: Disinfectant, COVID-19, Validity, Reliability.

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INTRODUCTION

Hand hygiene is one of the most effective measures that can be taken to reduce the spread of pathogens and prevent infections, including the COVID-19 virus (1). In cases where hand washing is not possible, it is recommended to use alcohol-based hand sanitizer if the hands are not visibly dirty (2). A meta-analysis of 18 randomized controlled studies indicated that alcohol-based hand sanitizers were more effective in reducing the incidence of acute respiratory infections than soap and water (3). The reason for this is that alcohol-based hand sanitizers are easier to use, take less time, and produce less irritation to the skin (4).

Global hand sanitizer sales have risen dramatically in response to COVID-19 prevention (5). Because of the huge demand for alcohol-based hand sanitizers, many manufacturers from various sectors have started to produce hand sanitizers. However, many may not have adequate facilities or a quality management system to ensure that the raw materials used are up to the required standards to manufacture alcohol-based hand sanitizers (6). In this process, an increase was observed in methanol-containing hand sanitizers, although the label listed ethanol as an active ingredient (7). As a result, the number of people suffering from methanol poisoning has increased (8).

There are two basic types of falsified hand sanitizers: sanitizers containing methanol, not typically listed as an ingredient, and sanitizers with an alcohol percentage less than sufficient (9). Methanol has toxic effects when inhaled, swallowed, or absorbed via the skin. Methanol poisoning is characterized by metabolic acidosis, ocular toxicity, and nervous system toxicity, and in severe cases, it can result in blindness and death (10). Given the widespread popularity of hand sanitizers and their importance in preventing the spread of COVID-19, falsified hand sanitizers pose an important public health problem (9).

Hand sanitizer labels must include the product's commercial name, any descriptive descriptions, the name of the active substance, the purpose of use, the instructions for use, the possible side effects, the first aid instructions, the batch number, and the expiration date (11).

The Falsified Hand Sanitizer Identification Scale (12) was developed by Jairoun et al. to help consumers identify safe and effective hand sanitizers. There is no similar scale developed in the Turkish language. As a result, our study aimed to evaluate the validity and reliability of this scale in Turkish culture and language.

MATERIALS AND METHODS

Study design, Setting, and Participants

This research is methodological type. The research was carried out between February and April 2021 by applying a 2-stage online survey to the staff of Fırat University (academic, administrative, contracted personnel, and permanent workers). Inclusion criteria for the study: Being a staff member of Fırat University and consenting to participate in the study. Exclusion criteria: Never used or purchased a hand sanitizer in their life. In the first stage, an official letter containing the necessary explanations, the permission of the ethics committee, and the questionnaire link was sent to all personnel via the Electronic Information System at Fırat University. Individuals who consented to participate in the study filled out an online questionnaire. The second-stage questionnaire was sent to the people who filled out the questionnaire in the first phase for a test-retest two weeks after they filled out the questionnaire. The first stage reached 355 people, while the second stage reached 66 people. A sample size of 300 people, or ten times the number of items, is considered sufficient for the validity and reliability analysis to develop and/or adapt the measurement tool (13). Therefore, the present study provided an adequate sample size (355 people).

Data Collection Tools

In the first stage of the research, the questionnaire consisted of two parts. The sociodemographic information form was used in the first part, and the Falsified Hand Sanitizer Identification Scale was used in the second part. In the second stage, the questionnaire consisted of a single part and included only scale questions. Additionally, the first and second-stage questionnaires also included a question asking for people's e-mail addresses. Participants in the first and second stages of the questionnaire were matched based on their e-mail addresses.

Falsified Hand Sanitizer Identification Scale: It is an English-language self-rating scale created by Jairoun (2020) et al. (12). The 12-item scale is of the 5-point Likert type (never, rarely, sometimes, often, always). The lowest score that can be obtained from the scale is 12, and the highest score is 60. The scale consists of three sub-dimensions: Safety Measures (5 questions), Identity Measures (4 questions), and Efficacy Measures (3 questions).

Evaluation of Data

Translation

The scale was translated from English to Turkish by two unrelated language translators who know both cultures and languages. The Turkish scale questions were compared by the responsible researcher and a third-language translator and turned into a single form. The expressions were then checked for compliance with Turkish by two Turkish language experts. Corrections were made based on their suggestions. The scale, which was translated from English to Turkish, was translated back from Turkish to English by separate fourth- and fifth-language translators. The researchers compared the original and translated English scales, evaluated the contradictory points, and completed the translation. Because hand sanitizers in Turkey contain 70% alcohol, the alcohol percentage of at least 60% specified in item 11 on the original scale has been revised to 70%.

Validity

Construct validity

Confirmatory Factor Analysis (CFA) was used to determine construct validity. The CFA examined the x^2/sd (standard deviation) coefficient, the Comparative Fit Index (CFI), the Goodness of Fit Index (GFI), the Normed Fit Index (NFI), and the Root Mean Square Error of Approximation (RMSEA). "Acceptable fit" criteria included $x^2/sd < 5$, CFI ≥ 0.95, GFI ≥ 0.85, NFI ≥ 0.90, and RMSEA ≤ 0.08. "Good fit" was defined as having $x^2/sd < 3$, CFI ≥ 0.97, GFI ≥ 0.90, NFI ≥ 0.95, and RMSEA ≤ 0.05 (14).

Known group validity

The hypothesis that those who use hand sanitizer more frequently daily would score higher on the scale for identifying falsified hand sanitizer was tested.

Reliability

Reliability was evaluated with Cronbach's alpha internal consistency coefficient, item analysis based on item-total correlation, and the test-retest method. The Cronbach's alpha internal consistency coefficient indicated "sufficient" between 0.60-0.70, "high" between 0.70-0.90, and a "very high" reliability level above 0.90 (15). A corrected item-total correlation coefficient below 0.20 indicated that the relevant item should be removed from the scale (14). As a result of the Pearson correlation analysis applied for the test-retest analysis, it was expected to be $0.80 \leq r \leq 0.90$ (15).

Statistical Analysis

SPSS 21.0 (IBM Corp., Armonk, NY, USA) and LISREL (SSI Inc., Michigan, USA) programs were used for statistical analysis. Descriptive statistics were frequency (n) and percentage (%) for categorical variables and mean ± standard deviation (mean ± sd) and/or median (1st quarter–3rd quarter) for continuous variables. The Shapiro-Wilk test was used to test compliance with the normal distribution. Since the data did not comply with a normal distribution, the Kruskal-Wallis H test was used to test known group validity, and Spearman correlation analysis was used for test-retest analysis. Statistical significance was evaluated at the $p < 0.05$ level.

Permissions

Ammar Abdulrahman Jairoun, the responsible author of the scale's article, was contacted via email, and permission to adapt the scale to Turkish was granted on November 27, 2020. The Fırat University Non-Interventional Research Ethics Committee approved the research (letter dated January 7, 2021, numbered 1899). The institutional approval was granted by the Rectorate of Fırat University via a letter numbered 8160 dated January 26, 2021.

RESULTS

Characteristics of study participants

The majority of the participants (62.18%, n = 217) were male, and the mean age of all participants was 41.14 ± 9.80 years. The characteristics of the study participants are presented in Table 1. 72.60% (n = 257) stated their socioeconomic level as middle, and 65.35% (n = 232) stated their education level as university. 47.61% (n = 169) of the participants are healthcare workers. The most common answer to the question questioning the frequency of daily use of hand sanitizer was "often" (40.56%, n = 144).

Table 1. Characteristics of participants

	First stage, n (%)	Second stage, n (%)
Gender (n = 349)*		
Male	217 (62.18)	32 (48.5)
Female	132 (37.82)	34 (51.5)
Socioeconomic level (n = 354)*		
Low	42 (11.86)	2 (3.0)
Middle	257 (72.60)	46 (69.7)
High	55 (15.54)	18 (27.3)
Education (n = 355)		
Elementary school, middle school, and high school	80 (22.54)	1 (1.5)
University	232 (65.35)	52 (78.8)
Postgraduate	43 (12.11)	13 (19.7)
Health employees (n = 355)		
Yes	169 (47.61)	38 (57.6)
No	186 (52.39)	28 (42.4)
Frequency of daily use of hand sanitizer (n = 355)		
Never	4 (1.13)	1 (1.5)
Rarely	78 (21.97)	16 (24.2)
Sometimes	97 (27.32)	25 (37.9)
Often	144 (40.56)	19 (28.8)
Always	32 (9.02)	5 (7.6)

** There was missing data.*

Validity

Construct validity

The CFA results were as follows: $x^2/sd = 3.67$ ($p < 0.001$), CFI = 0.98, GFI = 0.92, NFI = 0.97, and RMSEA = 0.08 (Table 2).

Table 2. Confirmatory factor analysis

	Turkish Falsified Hand Sanitizer Identification Scale	Acceptable fit	Good fit
x^2/sd	3.67 ($p < 0.001$)	≤ 5	≤ 3
CFI	0.98	≥ 0.95	≥ 0.97
GFI	0.92	≥ 0.85	≥ 0.90
NFI	0.97	≥ 0.90	≥ 0.95
RMSEA	0.08	≤ 0.08	≤ 0.05

CFI: Comparative Fit Index; GFI: Goodness of Fit Index; NFI: Normed Fit Index; RMSEA: Root Mean Square Error of Approximation

Known group validity

The results of the analysis performed to test the known group validity are given in Table 3. Those who "always" or "frequently" use hand sanitizer daily scored significantly higher on the scale than those who "sometimes" use it ($p < 0.001$).

Table 3. Known group validity analysis

	Turkish Falsified Hand Sanitizer Identification Scale Median (1st Quarter–3rd Quarter)	p
Frequency of daily use of hand sanitizer		< 0.001*
Never	26.50 (22.50–31.00)	
Rarely	30.50 (24.00–41.00)	
Sometimes	38.00 (26.00–46.00) [a, b]	
Often	39.50 (33.00–48.00) [b]	
Always	41.00 (34.00–54.00) [a]	

Note: a, b A statistically significant difference was found between the values with the same letters. * Kruskal-Wallis H test

Reliability

Reliability analysis results are shown in Table 4. When each item was removed from the scale, the Cronbach's alpha coefficient of the scale decreased. The Cronbach's alpha coefficient of the full scale was found to be 0.934; the Cronbach's alpha coefficient of factor 1 was 0.892; factor

2 was 0.891; and factor 3 was 0.818. The corrected item-total correlation coefficients of all items ranged between 0.584 and 0.758. The test-retest correlation coefficient was 0.853 ($p < 0.001$).

Table 4. Reliability analysis

	Cronbach's alpha if the item deleted	Corrected item-total correlation	Cronbach's alfa
Factor 1			0.892
Item 1	0.929	0.685	
Item 2	0.927	0.739	
Item 3	0.928	0.705	
Item 4	0.926	0.758	
Item 5	0.930	0.653	
Factor 2			0.891
Item 6	0.926	0.752	
Item 7	0.927	0.727	
Item 8	0.927	0.750	
Item 9	0.927	0.737	
Factor 3			0.818
Item 10	0.928	0.710	
Item 11	0.933	0.584	
Item 12	0.929	0.695	
Scale			0.934

The final version of the Turkish Falsified Hand Sanitizer Identification Scale is presented in Table 5.

Table 5. Turkish Falsified Hand Sanitizer Identification Scale

El dezenfektanın etiketinde bulunan aşağıdaki bilgileri ne sıklıkla kontrol edersiniz?	Hiç	Nadiren	Bazen	Sıklıkla	Her zaman
Faktör 1: Güvenlik Önlemleri					
Madde 1: Kullanım talimatının etikette açıkça belirtilmiş olduğunu kontrol ederim. Örnek: Avucunuzun içine gerekli miktarda el dezenfektanı dağıtın ve kuruyana kadar ellerinizi hızlıca ovalayın.					
Madde 2: Uyarı/ikazların ürün etiketinde açıkça belirtilmiş olduğunu kontrol ederim. Örnek: Yalnızca harici kullanım içindir. Çocukların erişemeyeceği yerlerde saklayın; gözlerle ve mukoz membranlarla doğrudan temastan kaçının, yanıcıdır.					
Madde 3: İlk yardım önleminin etikette açıkça belirtilmiş olduğunu kontrol ederim. Örnek: Göz teması veya cilt tahrişi durumunda lütfen bir doktora danışın. Yutulursa Zehir Danışma Merkeziyle iletişime geçin.					
Madde 4: Saklama koşullarının etiket üzerinde açıkça belirtilmiş olduğunu kontrol ederim. Örnek: Oda sıcaklığında saklayın, ateş veya alevden uzak tutun.					
Madde 5: Son kullanma tarihi/üretim tarihinin etiket üzerinde açıkça belirtilmiş olduğunu kontrol ederim.					
Faktör 2: Kimlik Ölçütleri					
Madde 6: Barkodun silinmez bir şekilde ürün etiketine basılmış veya yazdırılmış olduğunu kontrol ederim.					
Madde 7: Parti numarasının silinmez bir şekilde ürün etiketi üzerine basılmış veya yazdırılmış olduğunu kontrol ederim.					
Madde 8: Üreticinin adı ve logosunun okunaklı ve doğru olduğunu kontrol ederim.					
Madde 9: Menşei ülkenin, ürün etiketinde açıkça belirtilmiş olduğunu kontrol ederim.					
Faktör 3: Etkililik Ölçütleri					
Madde 10: Biyosidal etki (zararlı organizma üzerinde kontrol edici etki) ile etiketlenmiş olduğunu kontrol ederim. Örnek: Antiseptik/dezenfektan					
Madde 11: En az %70 alkol içeriği ile etiketlenmiş ürün olduğunu kontrol ederim.					
Madde 12: Aktif madde adının (bilimsel ad) doğru yazılmış olduğunu kontrol ederim.					

DISCUSSION

This study assessed the validity and reliability of Jairoun et al.'s (12) Falsified Hand Sanitizer Identification Scale in Turkish culture and language. The current study concluded that the Turkish Falsified Hand Sanitizer Identification Scale is a valid and reliable measurement tool.

According to a study, it was found that the frequency of hand sanitizer use in adults increased during the pandemic compared to before the pandemic. Additionally, adults participating in this study stated that their frequency of hand sanitizer use would remain the same after the pandemic as during the pandemic period. Therefore, it is important to research the use of hand sanitizer in the post-pandemic period (16).

A CFA is used to validate a predetermined model or structure. The researcher, who makes the adaptation study of a measurement tool developed in a different language, should evaluate the adaptation of this structure to her or his own culture and language instead of re-determining the structure of the scale. Therefore, instead of doing exploratory factor analysis (EFA) during the measurement tool adaptation process, the model fit should be examined by performing CFA after language validity (13). In the current study, 12 items and three sub-dimensions were examined with CFA. In the model of the current study, $x^2/sd \leq 5$ and RMSEA ≤ 0.08 indicated acceptable fit, while CFI ≥ 0.95, GFI ≥ 0.85, and NFI ≥ 0.90 indicated good fit (Table 2).

For known group validity, it was evaluated whether the scale could distinguish between groups. As the frequency of daily use of hand sanitizer increased, the score obtained from the scale increased (Table 3, $p < 0.001$). The hypothesis of known group validity was provided. The relationship between the level of knowledge about hand hygiene and hand hygiene practices among university students in India was examined, and it was determined that students with good knowledge about hand hygiene washed their hands more frequently (17).

Cronbach's alpha coefficient evaluates the overall reliability of the scale. In addition, it evaluates whether the items on the scale form a whole to question or explain a homogeneous structure (15). Cronbach's alpha coefficient of the full scale, which was found to be 0.934 in the current study, was 0.90 and above, indicating "very high" reliability (Table 4). Cronbach's alpha coefficients of 0.892 for factor 1, 0.891 for factor 2, and 0.818 for factor 3 indicated a "high" level of reliability, as they range from 0.70 to 0.90 (Table 4). In the study of the original scale, Cronbach's alpha coefficient was 0.867 for the full scale, 0.848 for Factor 1, 0.821 for Factor 2, and 0.736 for Factor 3 (12), and it is seen that the original scale also has a high level of reliability.

The relationship between each item and the overall scale is determined using item-total correlation analysis. If these associations are high, the scale is thought to have a high degree of internal consistency (14). The current study has determined that the scale has internal consistency because the corrected item-total correlation coefficient for all items was more than 0.20 (Table 4).

In the test-retest method, the scale is applied to the same group a second time after a certain time, and the correlation coefficient between the results of the two applications is calculated. The high value of this coefficient indicates that the scale's measurement results do not change over time, are stable, and therefore have high reliability (14). In the current study, this coefficient was found to be 0.859, indicating that the scale has high stability and reliability ($p < 0.001$). In the article on the original scale, the test-retest correlation coefficient was stated as 0.770 ($p < 0.01$) (12).

This study has some limitations. First, the results of the study cannot be generalized to the population, as the present study was conducted among staff of a university. Secondly, the results of this study were only compared with the original scale, as the scale did not have a validity and reliability study in different languages. Finally, the limitation of the study is that no comparison was made using another scale with proven validity and reliability in the known group validity analysis.

It has been found that the Turkish Falsified Hand Sanitizer Identification Scale is a valid and reliable measurement tool with a 5-point Likert scale consisting of 12 items and three sub-dimensions. The scale measures consumers' awareness of hand sanitizers. Training can be provided to increase consumers' awareness about hand sanitizers. Thus, the appropriate use of hand sanitizers can be encouraged, and possible dangers can be prevented (18). This finally helps prevent the transmission of pathogenic microorganisms. In future studies, it is recommended to use the Turkish Falsified Hand Sanitizer Identification Scale and to adapt the Falsified Hand Sanitizer Identification Scale to different languages and cultures.

Declarations

The authors received no financial support for the research and/or authorship of this article. There are no conflicts of interest.

This study was approved by The Fırat University Non-Interventional Research Ethics Committee (Date: 07.01.2021, Number: 1899) and Rectorate of Fırat University (Date: 26.01.2021, Number:8160)

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ORIGINAL ARTICLE

Inadequate informative capacity of arthroscopic lateral epicondylitis treatment-related youtube videos

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ABSTRACT

Background: In the literature, the quality of YouTube videos about lateral epicondylitis has been investigated by researchers so far. However, the quality of YouTube videos related to arthroscopic treatment has not been evaluated yet. So, this study aims to evaluate the informative capacity of YouTube videos related to arthroscopic lateral epicondylitis treatment.

Methods: For the study, a standard YouTube search has been conducted by using the terms 'tennis elbow arthroscopic treatment' and 'lateral epicondylitis arthroscopic treatment. For each search query, the 50 most "relevant" videos, as determined by YouTube's algorithm, have been taken into consideration (a total of 100 videos). After the exclusion of several videos, a total of 58 videos are included in the analysis. The informative quality and capacity of the videos have been evaluated by using the Journal of the American Medical Association (JAMA), Global Quality Score (GQS), DISCERN, and Lateral Epicondylitis Specific Score (LESS) scoring systems.

Results: According to DISCERN, 18.97% of the videos are of poor-to-very poor quality. The rate is 34.49% for LESS. According to the GQS and JAMA scores, the rate of low-quality videos is 36.21% and 44.83%, respectively. In addition, it is also seen that the mean DISCERN, LESS, GQS, and JAMA scores are significantly higher in videos uploaded by non-physicians than in those uploaded by physicians ($p<0.05$).

Conclusion: It can be concluded that YouTube videos related to arthroscopic lateral epicondylitis treatment have a poor informative capacity. This issue has to be paid attention to by orthopaedic surgeons and they should lead the patients to safer sources. Patients should be advised to consider searching for better quality and more informative resources when they want to seek information about in the arthroscopic treatment of lateral epicondylitis.

Keywords: Lateral epicondylitis, arthroscopy, YouTube, video, information, quality.

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INTRODUCTION

It is known that YouTube is the second most popular search engine for general Internet queries after Google. Moreover, it has been increasingly utilized by patients to access healthcare information easily (1). However, this trend among patients raises concerns about the quality and the accuracy of medical informative YouTube videos, which are not peer-reviewed (2). Patients who obtain treatment-related information from YouTube videos have the potential to develop misconceptions even if the clinicians assert otherwise (3).

Clinicians have been investigating the quality of orthopaedic disease-related and treatment-related YouTube videos for several years. Because a patient is always able to access inaccurate information through this platform. In addition, the results of these studies have demonstrated that the quality of YouTube videos related to numerous orthopaedic diseases is inadequate (3-9). Besides many other orthopaedic diseases, the quality of YouTube videos on lateral epicondylitis has also been being investigated (10-13).

The quality and the accuracy of online information on lateral epicondylitis varies substantially based on the search term, the author of the website, and the ranking of search results (14). The most common treatment for recalcitrant lateral epicondylitis is arthroscopic treatment, and it is one of the increasingly popular surgical procedures (15). Despite this, it can be seen that studies investigating YouTube videos on lateral epicondylitis have not included search terms related to arthroscopy. This study aims to evaluate the quality of YouTube videos on lateral epicondylitis and arthroscopic surgery.

MATERIALS AND METHODS

For the internet search, the history was deleted on Google Chrome (version 92.0.4515.159-64 bit) and it was used with cookies on May 8th, 2021. The standard www.YouTube.com website was accessed through the browser and YouTube was searched by entering the search terms 'tennis elbow arthroscopic treatment' and 'lateral epicondylitis arthroscopic treatment.' Then the first 50 videos for each search term were evaluated. It is known that it has been a previously used method (9). There are some inclusion criteria as the primary content relevance to lateral epicondylitis, English language, and acceptable audio-visual quality. Furthermore, repetitive videos, videos consisting only of audio or video, non-English videos, and videos irrelevant to lateral epicondylitis or arthroscopy have been excluded. After the exclusion, a total of 58 videos are included in this study. As only publicly available data have been used in the study, patient consent or ethics committee approval is waived.

For each YouTube video included in the study, several video characteristics are extracted as the title, the duration, the number of views, the source/uploader, the type of content, the number of days since upload, the rate of views (views/per day), and the number of likes. Also, video sources and uploaders are categorized into seven groups; academicians (related to authors or uploaders affiliated with research groups, universities, or colleges), physicians (independent physician or physician group with no research, university, or college affiliation), non-physician (healthcare professionals other than licensed medical doctors), educator, medical resource (content or animations from healthcare websites), patient, and commercial (1). Moreover, the content of the videos is categorized as surgical technique or approach, non-surgical management, and postoperative rehabilitation (9).

OrthoInfo is a website created by the American Academy of Orthopaedic Surgeons for Patient Education (16) and the Lateral Epicondylitis Scoring System (LESS) was designed based on the OrthoInfo website for lateral epicondylitis (https://orthoinfo.aaos.org/en/treatment/elbow-arthroscopy/). This method has been previously used to evaluate YouTube videos (6,17). The LESS consists of 24 items with five subheadings: patient presentation, information about lateral epicondylitis, diagnosis and evaluation, conservative treatment, surgical treatment, postoperative care, and complications. The maximum score that can be obtained from the LESS is 25. According to the scores obtained from the LESS, videos are categorized as very poor (scores between 0 and 5), poor (scores between 6 and 10), fair (scores between 11 and 15), good (scores between 16 and 20), and very good (scores between 21 and 25).

The Global Quality Score (GQS) and DISCERN scoring systems are used to assess the quality of videos included in the study (18, 19). The GQS provides a nonspecific assessment of the training quality. It consists of five items, and each item is worth 1 point. DISCERN was developed in Oxford, United Kingdom to assess written health information. The original DISCERN consists of 16 questions. Each question is scored between 1 and 5. Therefore, the minimum score that could be obtained from DISCERN is 16, and the maximum score is 80. According to the score ranges obtained from DISCERN, videos are categorized as

very poor (points between 16 and 28), poor (points between 29 and 41), fair (points between 42 and 54), good (points between 55 and 67), and excellent (points between 68 and 80) (6,9).

The JAMA (Journal of the American Medical Association) scoring system is used to evaluate the accuracy and reliability of the videos (11). JAMA provides a non-specific assessment of source reliability. JAMA consists of four items and 1 point is given for each item. A maximum score of 4 indicates the reliability of the source and a score of 0 represents poor reliability of the source (6,9).

The video links included in the study were presented as tables to the two observers. They blindly evaluated the videos and scored them according to the scoring system. Then, they discussed the scores in a consensus meeting until there was a full agreement for each video. Ethical Committee approval is not required for this research because of the research was conducted with publical available data.

Statistical Analysis

For the statistical analysis, categorical variables are presented as relative frequencies with percentages, and continuous data are reported as means and standard deviations and as medians with range values. The Shapiro-Wilk test is used to evaluate the distribution of the data and the Mann-Whitney U test is used to compare different groups. Also, Spearman's rho correlation coefficient is used to analyse the relationship between the usefulness scores generated for each video and their corresponding technical characteristics. The value of the Spearman rho coefficient correlation is interpreted as weak between 0 and 0.39, moderate between 0.40 and 0.59, strong between 0.60 and 0.79, and very strong between 0.80 and 1.0. A value of P< .05 is considered to indicate a statistical significance. The data are analysed via R Studio version 2023.09.0+463.

RESULTS

The duration, the number of views, the number of days since upload, the rate of views, the number of likes and dislikes, the liking rate, and the VPI values of the 58 videos included in the study are summarized in Table 1.

Table 1. Descriptive data on the characteristics of the videos included in the study.

	Mean ± SD	Median (Range)
Video Duration (sec)	422.13 ± 504.16	262 (41-2614)
No of views	49642.34 ± 151207.13	1827.5 (28-892760)
No of days after upload	1963.48 ± 1293.69	1871 (120-5806
View rate	19.73 ± 59.93	1.915 (0.02-322.64)
Like	453.84 ± 2089.19	10 (0-1300)
Dislike	3.81 ± 23.76	0 (0-180)
Like rate	88.63 ± 30.59	100 (0-100)
Video power index	19.48 ± 59.48	1.915 (0-322.64)

The mean DISCERN value is 39.36 ± 20.86. According to DISCERN, 9 of the videos are rated as very poor (15.52%), 2 of them are rated as poor (3.45%), 11 of them are rated as fair (18.97%), 10 of them are rated as good (17.24%), and 26 of them are rated as excellent (44.83%). According to the LESS, the average value of the videos is 6.31 ± 5.36. When all the videos are evaluated, it is seen that 9 of the videos are rated as very poor (15.52%), 11 of them are rated as poor (18.97%), 5 of them are rated as middling (8.62%), 7 of them are rated as good (12.07%), and 26 of them are rated as very good (44.83%). According to the JAMA criteria, 26 (44.83 %) of the videos are awarded as 2 or less. According to the GQS, 21 (36.21 %) of the videos received a score of 2 or less. When the uploaders were analysed, it was seen that there were 22 academicians, 27 physicians, 3 non-physicians, 1 educator, 3 medical resources, 1 patient, and 1 commercial resource. The comparison of videos uploaded by physicians and non-physicians is summarized in Table 2.

Table 2. Comparison of videos of non-physician and physician uploaders according to scores.

	Non-physician (n=21)		Physician (n=37)		p
	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)	
JAMA	3.125 ± 1.115	3 (0-4)	1.823 ± 1.266	1 (0-4)	0.0004
GQS	3.333 ± 1.340	4 (1-5)	2.205 ± 1.200	2 (1-5)	0.0020
DISCERN	47.083 ± 21.932	48 (16-80)	35.676 ± 16.223	32 (10-80)	0.0423
LESS	11.166 ± 7.833	8 (1-25)	6.794 ± 4.952	6 (1-22)	0.0372

According to the correlation analysis, it is seen that there is a very strong correlation between JAMA and GQS (rho = 0.863), DISCERN (ϱ = 0.842), and DISCERN and GQS (0.876) ($p<0.05$). Also, there is a moderate positive correlation between LESS and JAMA (ϱ = 0.491), GQS (ϱ = 0.573), and DISCERN (ϱ = 0.591) ($p<0.05$). Besides, there is a moderate positive correlation between the number of likes and JAMA (rho = 0.535), GQS (rho = 0.512), DISCERN (rho = 0.426), and LESS (rho = 0.467) scores. The results of the correlation analysis are shown in Table 3.

Table 3. Correlation analysis results.

		Number of views	Like	Like rate	VPI	JAMA	GQS	DISCERN	LESS
Number of views	*Rho*	-							
	p								
Like	*Rho*	0.735	-						
	p	<0.001							
Like rate	*Rho*	0.018	0.264	-					
	p	0.891	0.040						
VPI	*Rho*	0.778	0.859	0.261	-				
	p	<0.001	<0.001	0.046					
JAMA	*Rho*	0.116	0.535	0.168	0.281	-			
	p	0.380	0.003	0.204	0.031				
GQS	*Rho*	0.220	0.512	0.295	0.344	0.863	-		
	p	0.094	0.008	0.023	0.008	<0.001			
DISCERN	*Rho*	0.143	0.426	0.293	0.238	0.842	0.876	-	
	p	0.278	0.035	0.025	0.069	<0.001	<0.001		
LESS	*Rho*	0.220	0.467	0.253	0.291	0.491	0.573	0.591	-
	p	0.093	0.018	0.053	0.025	<0.001	<0.001	<0.001	

DISCUSSION

An important result that has been concluded from this study is that 18.97% of the videos are of poor to very poor quality according to the DISCERN. Also, according to the LESS, 34.49% of the videos are rated as poor or very poor. When the GQS scores are taken into consideration, it is seen that 36.21% of the videos are also of low quality. Also, according to the JAMA criteria, 44.83% of the videos scored 2. The video quality of non-physicians is higher than that of physicians. These findings suggest that the quality of YouTube videos on lateral epicondylitis and arthroscopic surgery is low and the source is unreliable.

The Internet is considered to be a limitless source of information. However, as almost none of the sources are peer-reviewed, the accuracy of the information provided should always be questioned. YouTube is one of the most prominent online social media platforms with videos on almost every topic, including diseases and treatment methods (2). However, the quality of the information found on online platforms is uncertain and uneven, which might mislead the patients and destabilize the relationship between the clinician and the patient (2). Studies evaluating YouTube videos on lateral epicondylitis have shown that the quality of these videos is inadequate (10-12). The results of our study are also similar. It can be cited as evidence that if a patient who is recommended arthroscopy for the treatment of lateral epicondylitis uses YouTube, there is a high probability of accessing incorrect or inadequate information. It might cause confusion and misconception among patients. A possible solution seems to be to lead the patients to the platforms where they can access accurate information and/or to produce Internet resources that provide accurate, detailed, and reliable information by physicians.

In a study evaluating the quality of YouTube videos on carpal tunnel syndrome, the overall reliability and educational quality of YouTube videos were rated as low. However, the quality of videos from academician and physician uploaders or the quality of videos related to surgical techniques and disease-specific information was significantly higher than the other video sources and content (8). In addition, in another study evaluating YouTube videos on lateral epicondylitis, it was determined that the quality of videos from uploaders who were physicians was significantly higher (20). However, in our study, it is seen that the quality of the videos of non-physician uploaders was higher than that of physicians. One of the striking features of this study is the result that the majority of videos included surgical

techniques or surgery-related videos. Arthroscopic surgical videos showed the arthroscopic surgery process and how it was performed. Although it can be thought that these videos might provide useful information regarding surgical techniques, they were of poor quality in terms of patient information related to the nontechnical aspects of surgical treatment. So, it can be stated that videos that provide information about the course of the disease at the very beginning, the indications, the postoperative management process, and complications are required even if the surgical technique is explained. Designing videos by professionals and leading the patients to these videos would be a solution to this issue.

The high number of likes and views on YouTube videos can create a misperception among patients about the quality of the video and it might end in misinformation for the patient (2). A study by Kuru et al. found a negative correlation between the quality of the video and the number of likes, which might indicate that high-quality videos are not as popular as low-quality videos (5). However, there is evidence that low-quality videos are preferred in some studies (21,22). However, the findings of this study seem to be contradictory. There was a positive correlation between the number of likes of the videos and scoring systems. As a result, it can be concluded that patients undergoing arthroscopy for the treatment of lateral epicondylitis are aware of the quality issue. Findings in this study show that these patients were not able to access the quality information they wanted. Thus, it is clear that there is a need for resources that provide quality information for these patients.

This study has some limitations. Firstly, it is known that YouTube might suggest different video rankings specific to a person and location and it is a platform that is constantly updated by newly uploaded videos (5). In this case, it might result in different videos and rankings for each search. In this study, the videos that were searched in a single period have been evaluated. A second limitation might be that only the first 50 videos were evaluated for each search term. However, it is known that it is a previously used method (6). Thirdly, YouTube is mostly known as an entertainment platform and normally it does not require a peer review. The videos uploaded on the platform can be of poor quality because they contain a part of patient education. However, this study has aimed to determine the quality of patient information. Additionally, videos that include a single treatment method and do not provide information about alternatives might also be confusing. Finally, the LESS is a scoring system that has not been used before, and its reliability has not been proven. On the other hand, similar scoring systems have been used in previous studies (6,9). It also showed a positive correlation with other scoring systems.

In conclusion, it is determined that YouTube videos on lateral epicondylitis and arthroscopy are of poor quality. Orthopaedic surgeons are advised to be careful about this problem and should lead the patients to safer sources. Additionally, associations and/or physicians can prepare high-quality educational videos on the subject. So, patients would be able to be directed to these resources.

Declarations

The authors have no conflicts of interest to declare. The authors declared that this study has received no financial support.

Ethical Committee approval is not required for this research because of the research was conducted with publical available data.

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CASE REPORT

Repair of recurrent umbilical hernia with Duramesh™, a suturable mesh: Our first application experience

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ABSTRACT

Suture tension due to the structure of the sutures used during closure of laparotomies and the technique applied, or the cutting of the tissue while the stitches are being pulled, predisposes to incisional hernia. It is known that repairs made with the use of mesh provide more successful results in incisional hernia surgery compared to primary closure of the defect with sutures. For this reason, a multifilament suturable mesh was developed to prevent the suture from cutting the tissue due to tension at the stitch and tissue interface. In this case, the approach and early results of a recurrent umbilical hernia case operated on with suture-shaped mesh (Duramesh™), a new product developed for use in incisional hernias and abdominal closure, are presented.

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INTRODUCTION

Despite advances in surgical techniques and suture technology, incisional hernia (IH) remains a common complication after abdominal surgery. It is known that IH develops in approximately 15% of patients in the general population after midline laparotomies (1). Complication rates after IH repair are high and recurrence rates vary between 23 and 50% (2). There are many factors that contribute to the risk of developing IH, including obesity, age, diabetes mellitus, smoking and wound infections. The incision site, the suture material used and the closure technique are among other etiological reasons (3).

It is thought that the sharp suture filament used to close the laparotomy incision acts like a sharp wire, cutting the abdominal wall tissues and causing IH formation over time. Therefore, to limit shrinkage while approximating tissues, a suturable mesh of individual polypropylene filaments caged together in a macroporous cylindrical configuration was developed (Duramesh™, Mesh Suture Inc., Chicago, IL) (4).

We present our observations and early results of our case of recurrent umbilical hernia, which we operated on with the suture-shaped Duramesh™, a new product developed for use in IH and abdominal closure.

CASE REPORT

A 51-year-old male patient, who had no additional disease in his history and underwent mesh-free repair of a 2 cm umbilical hernia a year ago, presented with complaints of recurrent umbilical hernia. A defect area of approximately 4 cm in the umbilicus was detected in the physical examination and ultrasonography. Preoperative preparations and informed patient consent were completed. The surgical incision was performed with a smile-shaped incision below the navel. After the hernia defect in the fascia was isolated, the 4 cm defect was closed with Duramesh™ (Msİ, Chicago) number "0" using one by one suturing technique (Figure 1).

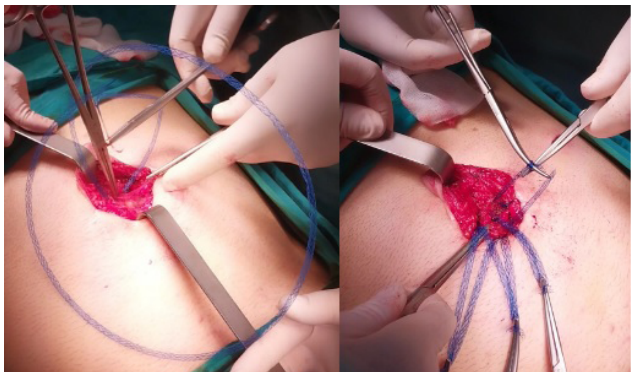

Figure1: Suturation image with suture-shaped mesh (Duramesh™)

No additional mesh was needed (Figure 2).

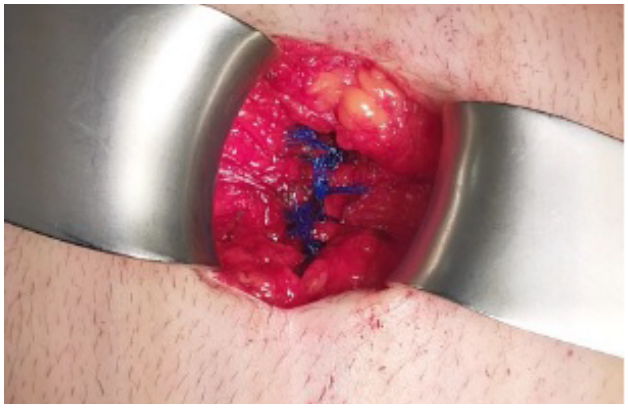

Figure 2: Final image after intraoperative suturing

The patient was discharged on the first postoperative day without any problems. There was no problem at the postoperative 10th day, first month and third month follow-ups, and the wound healed without any problems. The follow-up of the patient, who has no recurrence, continues.

DISCUSSION

Many methods are being tried to prevent the development of incisional hernia, such as the "small bite" suture technique, closing the incisions with prophylactic mesh, releasing the anterior and posterior components, botulinum toxin injection into the lateral abdominal muscles, and preferring minimally invasive techniques instead of laparotomies (1,3). After midline laparotomy, it has been shown that closing the fascia using the small bite technique with a 4:1 suture length/wound length ratio reduces the possibility of incisional hernia development (5). However, the primary culprit in the development of IH, which occurs in 24% of laparotomy closures, is shown to be cutting the tissue due to pulling of the sutures (6). Therefore, Duramesh™,

defined as a suturable mesh, increases the implant surface area in contact with the tissues compared to a standard suture. In an animal study, monofilament was rated as "non-irritating" to tissues compared to polypropylene (4). Results comparable to reported standard materials in the tendon repair model made with suturable mesh and the sternum closure model show that the usage area of this material can be expanded (7,8).

Yurtkap et al. (9) reported in their animal study that Duramesh™ had similar results to Polydioxanone (PDS), a traditional laparotomy closure material. They also think that it may help prevent IH by enhancing wound healing thanks to its three-dimensional, macroporous structure (9).

In the case we presented, there was no difficulty in using the product during primary closure of the defect with Duramesh™ in umbilical hernia repair. Thanks to its structure that can easily pass through tissues and its ability to be tied like a suture, the defect could be closed primarily. We describe it as encouraging for the use of the product that no complications or recurrences such as wound infection or seroma were observed in the early patient follow-ups. However, its results in clinical practice are not clear because it is not widely used yet. As the use of Duramesh™ becomes widespread, it will be possible to compare its advantages and disadvantages through controlled studies.

Informed Consent

Informed consent was obtained from the patient.

Declarations

This study was accepted as a poster presentation at the "23rd National Surgery Congress" organized by the Turkish Surgical Association on 24-28 April 2024 / Antalya. The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest. Ethical committe approval is not required because of this article is a case report.

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