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FDA-Approved Molecular Tests Used to Define Human Papillomavirus (HPV) Infections which Cause Cervix Cancer

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Review Article

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ABSTRACT

Human papillomavirus (HPV) is a non-enveloped, commonly sexually transmitted virus with icosahedral symmetry and double-stranded circular DNA. Its genome, which is about 8 kb in size, encodes early genes (E1-8) and two late structural capsid genes (L1 and L2). Among the genes that play a role in viral pathogenesis, L1, E6, and E7 genes frequently exist. The E6 and E7 viral genes have a significant role in apoptosis inhibition, viral spread, development of squamous intraepithelial lesion (SIL), cell immortalization, neoplastic transformation, and invasive cancer.

Demonstrating the relationship between cervical cancer and HPV infections has led to increased interest in this subject and the classification of some HPV genotypes in the high-risk group (HR-HPV) for cervical cancer. Numerous commercial molecular tests have been developed to identify HPV genotypes involving different approaches. HPV molecular tests approved by the US Food and Drug Administration (FDA) include Hybrid Capture® 2 (HC2), Cervista™, cobas®, Aptima®, and BD Onclarity™. This article reviews five FDA-approved tests' methodologies, limitations, and commonalities. The HC2 and Cervista™ tests use non-PCR-based signal amplification methods, while the cobas® and BD Onclarity™ tests use PCR-based target amplification methods. On the other hand, the Aptima® test uses the mRNA transcriptional mediated amplification (TMA) method.

Each of these methods used in the diagnosis and follow-up of HPV has its strengths and weaknesses. These HPV molecular tests have high sensitivity and specificity. They are also more automated and repeatable than cytological methods. In addition to these advantages, there are also several limitations. Because of these limitations, molecular tests are no more perfect than cytological tests. This situation shows that these tests should not be used alone in the evaluation of HPV infections and cancer identification. On the contrary, HPV test results should be correlated with cytology or biopsy findings.

Keywords: Cervical cancer; Human papillomavirus; HPV; Molecular diagnosis

Rahim Ağzı Kanserine Neden Olan Human Papillomavirüs (HPV) Enfeksiyonlarının Tanımlanması için Kullanılan FDA Onaylı Moleküler Testler

Süreç

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ÖZ

İnsan papilloma virüsleri (HPV), zarfsız, genellikle cinsel yolla bulaşan, ikozahedral simetriye ve çift zincirli halkasal DNA'ya sahip bir virüstür. Yaklaşık 8 kb büyüklüğündeki genomu, erken genleri (E1-8) ve iki geç yapısal kapsid genini (L1 ve L2) kodlamaktadır. Viral patogenezde rol oynayan genler arasında sıklıkla L1, E6 ve E7 genleri bulunmaktadır. E6 ve E7 viral genleri, apoptoz inhibisyonu, viral yayılım, skuamöz intraepitelyal lezyon (SIL) gelişimi, hücre ölümsüzleşmesi, neoplastik transformasyon ve invaziv kanserde önemli bir role sahiptir.

Rahim ağzı kanseri ve HPV enfeksiyonları arasındaki ilişkinin gösterilmesi, bu konuya olan ilginin artmasına ve bazı HPV genotiplerinin rahim ağzı kanseri için yüksek riskli grup (HR-HPV) içerisinde sınıflandırılmasına neden olmuştur. HPV genotiplerinin tanımlanması için farklı yaklaşımları içeren çok sayıda ticari moleküler testler geliştirilmiştir. ABD Gıda ve İlaç Dairesi (FDA) tarafından onaylanmış HPV moleküler testleri arasında Hybrid Capture® 2 (HC2), Cervista™, cobas®, Aptima® ve BD Onclarity™ bulunmaktadır. Bu makalede FDA onaylı beş testin metodolojileri, sınırlamaları ve ortak özellikleri gözden geçirilmektedir. HC2 ve Cervista™ testleri PCR tabanlı olmayan sinyal amplifikasyon yöntemlerini kullanırken, cobas® ve BD Onclarity™ testleri PCR tabanlı hedef amplifikasyon yöntemlerini kullanır. Öte yandan Aptima® testi, mRNA transkripsiyonel aracılı amplifikasyon (TMA) yöntemini kullanır.

HPV tanı ve takibinde kullanılan bu yöntemlerin her birinin kendilerine özgü güçlü ve zayıf yönleri bulunmaktadır. Bu HPV moleküler testleri yüksek duyarlılık ve özgüllüğe sahiptirler. Ayrıca sitolojik yöntemlerden daha otomatik ve tekrarlanabilirlerdir. Bu avantajlara ek olarak, çeşitli sınırlamaları da vardır. Bu sınırlamalar nedeniyle moleküler testler sitolojik testlerden daha mükemmel değildir. Bu durum, HPV enfeksiyonlarının değerlendirilmesinde ve kanser teşhisinde bu testlerin tek başına kullanılmaması gerektiğini göstermektedir. Aksine, HPV test sonuçları sitoloji veya biyopsi bulguları ile ilişkilendirilmelidir.

Anahtar sözcükler: Rahim ağzı kanseri; İnsan papillomavirüs; HPV; Moleküler tanı

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Human papillomaviruses (HPV); are commonly sexually transmitted, non-enveloped, icosahedral symmetry, double-stranded circular DNA viruses [1]. The icosahedral structure consists of structural proteins, the major capsid protein (L1) and the minor capsid protein (L2) [2]. HPV has a genome of about 8 kb. This genome encodes early genes (E1-8) and two late structural capsid genes (L1 and L2). The genome also has a 0.9 kb non-coding region[3,4].

More than 100 HPV genotypes that differ in their oncogenic potential have been identified in [5]. Antigenically different genotype groups cause the formation of specific lesions. These groups are classified as "low risk (low risk, LR); probable high risk (PrHR); and high risk (high risk, HR) groups" according to their cancer responsibility. Members of the LR-HPV group may cause benign lesions (papilloma and condyloma), while members of the HR-HPV group may cause cancers such as cervical cancer [6]. Clinically, there are 14 HPV genotypes (HPV-16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, 56, 66, and 68, respectively) worldwide within the HR-HPV group [7].

HPV-16 and 18 are the two genotypes most associated with cervical cancer. The main functions of each gene of the HPV-16 virus are summarized in Figure 1. Among the genes that play a role in viral pathogenesis are frequently found in L1, E6, and E7 genes. E6 and E7 genes increase viral spread by inhibiting apoptosis. In addition, the expression of E6 and E7 oncogenes are associated with the development of squamous intraepithelial lesion (SIL), as well as having a prominent place in cell immortalization, neoplastic transformation, and invasive cancer development [7,8].

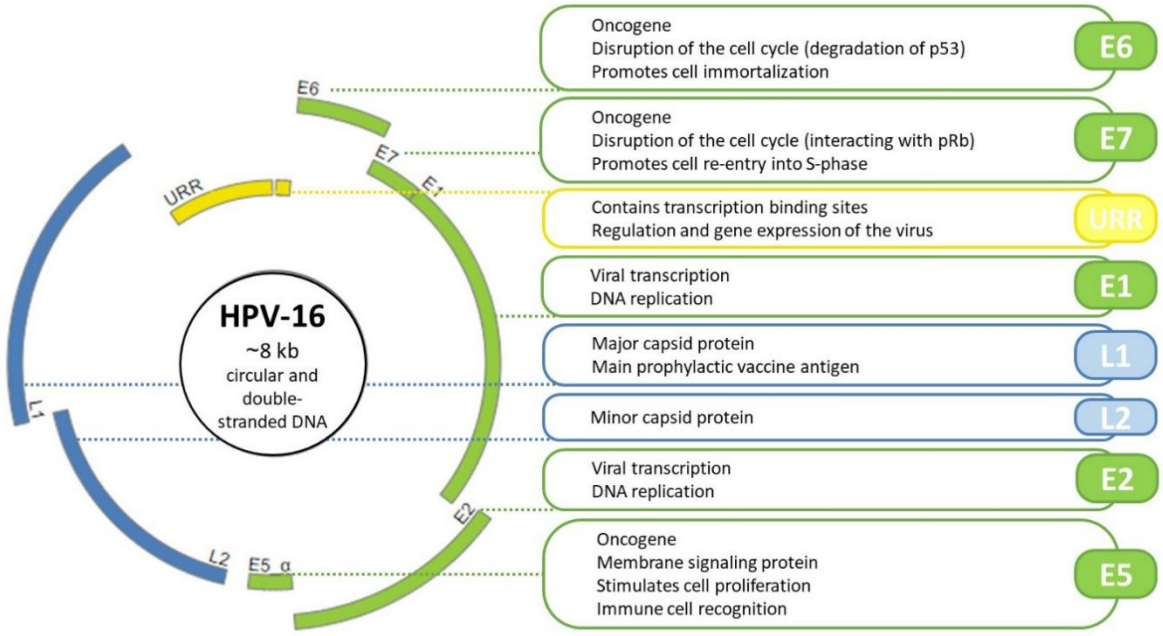

Figure 1. The genome of HPV-16 virus and functions of genes. The HPV genome consists of three main regions: the URR region containing the transcription binding region, the early gene (E1-8) regions, and the L1-2 late structural capsid gene regions [3,9]

HPV is the main cause of cervical cancer, one of the most common types of cancer in women worldwide [2,10]. Screening tests can identify this type of cancer. This situation has increased the importance of histopathological and molecular methods. Molecular tests are more sensitive than Papanicolaou (Pap) smear [2]. However, since most HPV infections are transient, it is not recommended to use molecular tests as direct screening tests. Because this case, patients may be exposed to unnecessary invasive procedures. Therefore, algorithms based on the combined use of Pap smear and molecular tests have been improved, which predict cervical cancer screening to be carried out within certain programs to avoid or minimize such situations. In addition, various methods are being developed to diagnose HPV by detecting biomarkers related to HPV[6]. In addition, the United States Food and Drug Administration (FDA) has approved that some commercial tests can be used as an adjunct screening test for HPV infections and for genotyping for certain genotypes [10]. This review aims to provide information about FDA-approved molecular tests that can be used to differentiate lesions at risk of progression into cervical cancer and discuss the limitations of these tests.

Molecular Tests Used in the Diagnosis of HPV

While HPV cannot be replicated in standard tissue culture, it can only be reproduced in special media such as xenograft or organotypic raft cultures. However, these methods are not used in routine virological diagnosis because their application is difficult and time-consuming. These methods are used only for vaccine development and research purposes [4]. For this reason, accurate identification is made by molecular biological techniques as well as cytological examinations [11]. There are three basic molecular methods used for HPV identification. These are nucleic acid-hybridization methods, signal amplification methods, and target amplification methods [6]. The main features of these methods are given in Table 1.

Table 1. Comparison of molecular methods used in the diagnosis of HPV

Method	Strengths	Weaknesses
Nucleic acid- hybridization	The Southern blotting method is the gold standard The presence of HPV is associated with morphology	Large amounts of purified DNA low sensitivity Time-consuming
Signal amplification	Commercial kits Quantitative method Lower false positive rate High sensitivity to genotyping	Certified licensed products Cocktail approach to genotyping
Target amplification	Flexible technology (viral load and genotype) Very high sensitivity Multiplex analysis	Contamination with previously amplified material can lead to false positives No standardization Low amplification signals for some HPV genotypes

Nucleic Acid-Hybridization Method

In nucleic acid-hybridization methods, radioactively labeled substances are used detection of HPV from cervical specimens [13,14]. These methods are Southern blotting, in situ hybridization, and dot-blot hybridization [13]. Among them, the Southern blotting method is the gold-standard method for HPV genomic analysis [15]. Although significant knowledge has been gained from these methods, the use of these methods is finite by low sensitivity, time-consuming procedures, and the need for relatively large amounts of purified DNA [13–15].

Signal Amplification Method

Signal amplification methods are an extension of direct probe techniques with increased sensitivity with innovations in detection methods [12]. In signal amplification systems, nucleic acids are first hybridized with specific probes. Then the degree of signals generated from these hybrid complexes is increased [4]. Hybrid Capture® 2 (HC2) method is the first signal amplification-based method approved by the FDA and used diagnosis of HPV. In addition, Cervista™ HPV HR and Cervista™ HPV 16/18 tests are commercial tests approved by the FDA and use the signal amplification method [11,17].

Target Amplification Method

The target amplification method is the most flexible and sensitive of all DNA analysis methods [17]. This method can be carried out for detection, viral load analysis, DNA sequencing, and mutation analysis [19,20]. Polymerase Chain Reaction (PCR) is one of these methods. PCR is a method that provides in vitro amplification and diagnosis of unique DNA regions in HPV infections, as in most viral infections. In addition, multiplex analyses, which allow simultaneous analysis of multiple target DNA sequences, can be performed to diagnose HPV infections [20].

Environmental contamination is very significant for PCR tests. Previously amplified materials may result in a false positive result for a negative sample [22]. There is an inherent risk of false-negative results when there is no competition between reagents, or there are multiple infections in low-copy samples.

Therefore, the detection of HPV genotypes may be adversely affected. In addition, recurrent infections constitute the disadvantage of this method [21].

The target amplification tests approved by the FDA are the cobas® HPV, APTIMA® HPV, and BD Onclarity™ HPV tests. Of these, the cobas® HPV and BD Onclarity™ HPV tests are based on DNA target amplification, while the APTIMA® HPV test is based on mRNA transcription-mediated amplification [8].

Some features of HPV molecular tests classified by signal amplification and target amplification methods and approved by the FDA are summarized in Table 2

Table 2. Comparison of FDA-approved HPV molecular tests[8]

Feature	Digene HC2 HR-HPV	Cervista™ HPV HR and HPV 16/18	cobas® HPV	APTIMA® HPV	BD Onclarity™ HPV
Manufacturer	Qiagen	Hologic	Roche	Hologic	Becton Dickinson
FDA approval date	2001	2009	2011	2011	2018
Method	Non-PCR-based DNA signal amplification	Non-PCR-based DNA signal amplification (Fluorescent resonance energy transfer FRET)	PCR-based DNA target amplification	mRNA transcription-mediated amplification (TMA)	PCR-based DNA target amplification
Target gene	Complete genome	L1, E6, and E7	L1	E6 and E7	E6 and E7
Detectable genotypes	13 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)	Cervista® HPV HR test; 14 HR- HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) Cervista® HPV 16/18 test detects HPV-16 and 18 genotypes	14 HR-HPV genotypes Channel 1: HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 Channel 2: HPV 16 Channel 3: HPV 18	APTIMA® HPV test; 14 HR-HPV genotypes (6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) APTIMA HPV 16 18/45 test; HPV-16, 18/45 genotypes	14 HR- HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68)
Internal control	No	Human HIST2H2BE	β - globin	An internal control transcript (HPV16 E6/7 transcript) is added to each reaction	β - globin
Collection medium	HC2 Specimen Collection Device (cervical brush and STM). PreservCyt Solution or BD SurePath	ThinPrep	PreservCyt	ThinPrep (2000 processor) Pap Test vials (containing PreservCyt Solution)	LBC media (BD SurePath ™, Hologic PreservCyt) BD Onclarity cervical brush
Test reading method	Automatic (chemiluminescence)	Fluorescence	Automatic (fluorescence)	Luminometer	Automatic (fluorescence)

FDA: US Food and Drug Administration, PCR: Polymerase Chain Reaction, HC2: Hybrid Capture 2, HPV: Human Papillomavirus, mRNA: messenger RNA, HR: High Risk.

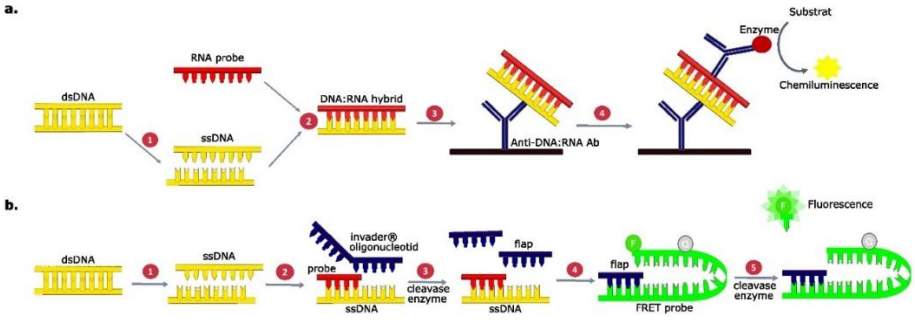

Figure 2. HPV signal amplification methods. A) Hybrid Capture® 2 method used in HC2 HR-HPV test. B) FRET probe method used in Cervista™ HPV HR and Cervista™ HPV 16/18 assays (inspired by resource 22).

HC2 HR-HPV Test

Hybrid Capture® 2 (Digene/Qiagen, Gaithersburg, MD, USA) is a signal amplification method used for the detection of HPV-DNA from endocervical and liquid cytological samples. It is used to identify 13 HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 LR-HPV genotypes. It is a reliable and reproducible method[23,24]. The patient sample collected in the test solution (PreservCyt) is prepared with the test kit (Digene). The test is a semi-quantitative molecular hybridization test that works by detecting radiation from a chemiluminescent substrate. That is, it is nonradioactive signal amplification based on the hybridization of target DNA to labeled RNA probes in solution. DNA-RNA hybrid formed in a consequence of the hybridization of HPV DNA and RNA probe generates a signal by enzyme-linked immune sorbent assay (ELISA) with alkaline phosphatase-conjugated monoclonal antibodies (Figure 2a). Results are appraised as the ratio of the positive control sample to relative light units (RLU). Luminous intensity is measured with a luminometer. The recommended 1 RLU/PC (positive cutoff value) of 1 pg/mL is equivalent to 5000 viral copies. Samples with an RLU/PC ratio ≥1.0 are considered positive [25]. While this test categorizes LR and HR viruses, it is not specific for HPV genotyping. That is, it cannot identify specific HPV types. If more than one genotype is positive, the distinction cannot be made. With this grouping, it can be determined that there is a precancerous lesion risk of 15% for HR- HPV -16/18 and below 3% for other HR types. HPV genotyping is important in determining the oncogenic types of HPV 6.

The HC2 test can only test for the presence of 13 HR-HPV genotypes [26]. It can cross-react with the HPV-66 genotype [27]. It is thought that the test results should be compared with the results of another method. Since the HC2 test can give false positive results (10%), HC2 negative samples should also be included in studies for analysis accuracy [26,27].

Cervista™ HPV HR Test

The Cervista™ HPV HR test (Hologic, Inc; Marlborough, MA) was approved by the FDA in 2009. It is a qualitative signal amplification assay that detects specific nucleic acid sequences (DNA) for 14 HR-HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). It uses the Invader® oligonucleotide (Hologic™, Inc) [27]. This method is based on the enzymatic degradation by the structure-specific 5' nuclease enzyme Cleavase® (Hologic™, Inc). Sequence-specific probes and Invader® oligonucleotides bind to the target DNA sequence to form a substrate for the enzyme. The probes in the target sequence open and close, and multiple 5' wings are formed in the target sequence. These multiple split wings bind to the universal hairpin fluorescent energy transfer (FRET) oligonucleotide, creating a different substrate for the enzyme. The enzyme breaks down FRET and creates fluorescent signals as the wings open and close (Figure 2b) [28]. The Cervista HPV HR test consists of two isothermal reactions, a primary reaction in the target DNA sequence and a secondary reaction that generates a fluorescent signal, and an internal DNA probe [27]. A mixture of 3 oligonucleotides is used as a reagent to test the 14 HR HPV types in total. The mix 1 (A5/16) is used for HPV types 51, 56, and 66; the mix 2 (A7) is used for HPV types 18, and the mix 3 (A9) is used for HPV types 16, 31, 33, 35, 39, 45, 52, 58, 59 and 68 [29]. The Cervista HPV HR is a method designed to reduce false-positive results caused by the cross-reactivity of low-risk HPV types. However, there is an internal control system that confirms that a sufficient amount of DNA has been tested [26]. It does not cross-activity with LR-HPV genotypes and non-oncogenic genotypes. It avoids most unnecessary colposcopy practices and can operate with a small amount of patient sample (2 mL). Thus, it increases the reliability of the test by reducing the number of insufficient DNA samples, and it prevents false-negative HPV results. The Cervista HPV HR test is among the most recommended molecular methods for HPV diagnosis. It reliably detects 14 HR HPV genotypes, including the HPV-66 genotype. The test has high specificity, sensitivity, and accuracy [27].

Cervista™ HPV 16/18 Test

The Cervista HPV 16/18 (Hologic, Inc; Marlborough, MA) test was approved by the FDA in 2011. It is a genotyping test used for the detection of HPV 16 and 18. The Invader® oligonucleotide (Hologic TM, Inc), which is the signal amplification method, is used to detect specific nucleic acid sequences [27]. It works in the same way as the Cervista HPV HR [28]. Besides the L1 gene region, it also targets other gene regions. There is a low rate of positivity among genotypes. The sensitivity of the test is 98% in cervical intraepithelial neoplasia (CIN 2) lesions and 100% in CIN 3 lesions. The Cervista is a qualitative signal amplification method using specific nucleic acid sequences and signal amplification method of 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). It is a test with high analytical

sensitivity and specificity against HPV-16/18 genotypes. However, this test cannot identify HPV genotype specifically [6]. For both the analytical and clinical performance of an HPV diagnostic test, the specificity of the test is so significant [27]. The Cervista HPV 16/18 test consists of a primary reaction at the target DNA sequence and a secondary reaction that generates a fluorescent signal [30]. The Cervista HPV 16/18 uses a mixture of 2 oligonucleotides, each containing a probe specific to the L1, E6, and E7 genomic regions. The Cervista 16 and 18 both contain two genes for the detection of cellular DNA in oligonucleotide mixtures targeting human histone protein. The "signal-to-noise" value for each test is evaluated above zero (FOZ). The FOZ threshold value for a positive result is considered 2.13 [29].

The Cervista 16/18 test has high specificity, sensitivity, accuracy, and interlaboratory repeatability. Because of the increased incidence of HPV-16 and 18 infections and cervical cancers, the data on the presence of these genotypes is significant for screening strategies for cervical cancers [31]. The Cervista tests are more useful for patients who do not have a sample of primary oropharyngeal carcinoma or for whom head-neck carcinoma is not identified. Because HPV-16 is the predominant, high-risk HPV genotype in oropharyngeal carcinoma, the Cervista 16/18 test provides an assessment of the health status of patients accurately with oropharyngeal carcinoma[32].

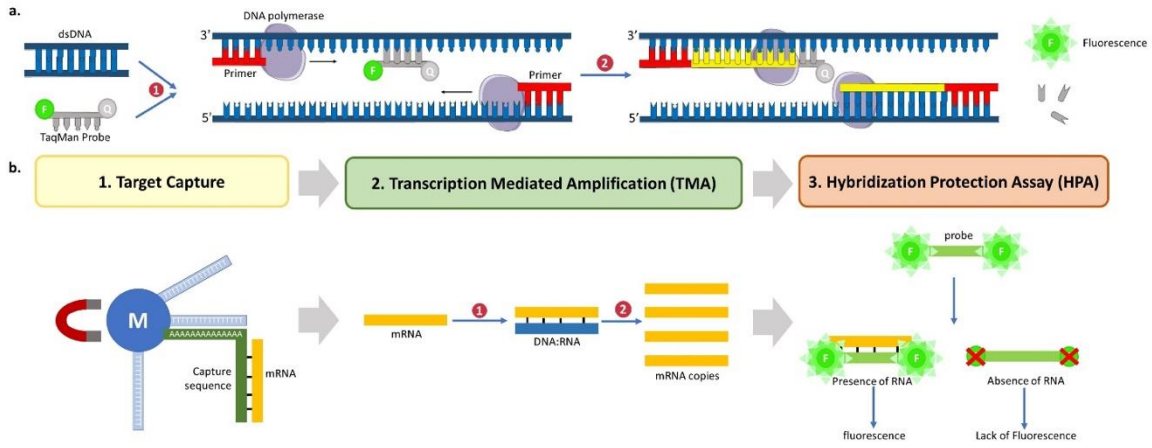

Figure 3. HPV target amplification methods. A) TaqMan probe method used in cobas® HPV and BD Onclarity™ HPV tests. B) Transcription-mediated amplification used in the APTIMA® HPV test (inspired by reference no: 22).

cobas® HPV Test

The cobas® HPV test, produced by Roche (Branchburg, NJ), was first approved for use in 2011 [33]. Liquid-based cervical swab sample is used as the test material [34]. This test makes automatic HPV identification using a real-time PCR technique. This test is a qualitative test that amplifies the DNA of the target gene L1 [35]. The reaction happens in a single PCR tube. There are four fluorescent probes for HPV-16, HPV-18, the other 12 genotypes, and each of the internal control (β-globin) [34]. Thus, the test can identify HPV-16 and HPV-18 types specifically [35].

The reaction occurs when the sample contains the L1 gene sequence of the HR-HPV genotypes. The specific primers bind to complementary sequences, and amplification occurs. One end of the TaqMan probes is labeled with a fluorophore and the other end with a quencher. The fluorescent emission does not occur due to the extinguisher. Once the probe is attached to an identifier sequence, it will degrade because of the continued qPCR reaction due to the 5'→3' exonuclease activity of the polymerase (Figure 3a). When the probe breaks down, fluorescent radiation occurs because the fluorophore is separated from the quencher. When the probe breaks down, fluorescent radiation occurs because the fluorophore is separated from

the quencher. The detection process is performed by detecting the fluorescent beam [36].

There are studies in the evaluation results of the cobas® HPV test that show that the test's repeatability is very consistent [37]. This fully automated test is easy to apply. It can provide reliable results in as little as 4 hours [38]. It has higher sensitivity compared to the HC2 HR-HPV test [39]. According to the study of Stoler et al. with 1578 cervical samples, the sensitivity of the HC2 HR-HPV test was 87.2%, while the sensitivity of the cobas® HPV test was 90% [40]. This test does not cross-react with other microorganisms. This test also does not interact with lubricants or antifungals [34]. This test can only differentiate between HPV-16 and HPV-18 genotypes. Because other HPV types have the same signal, their detection is not possible.

APTIMA® HPV Tests

The APTIMA® HPV test, produced by Hologic Gene-Probe (San Diego, CA) was approved for use by the FDA in 2011 [8]. As the second test in 2012, APTIMA® 16 18/45 was approved. ThinPrep cervical samples are used as test material [41]. All the tests approved so far detect the viral genome of DNA. However, the APTIMA® HPV test is the first FDA-approved test to detect mRNAs of the E6 and E7 gene regions. Detection of mRNAs of E6/E7 genes makes this test method more specific and sensitive than other DNA detection methods [42]. While the APTIMA® HPV test can identify 14 HR-HPV E6/E7 gene region mRNAs, only three genotypes (HPV-16, -18, and -45) can be detected with the APTIMA® 16 18/45 test [43].

The APTIMA® test consists of three steps, which take place in a single tube. These include target capture, transcription-mediated amplification, and detection of amplification products [41]. The samples are transferred to the sample transport medium, firstly. Thanks to this medium, cell lysis takes place, and mRNAs are released. Then, the target mRNAs bind to complementary oligonucleotides with a poly-deoxyadenosine (polyA) tail. Subsequently, these hybrids are linked by a poly-deoxythymidine (PoliT) tail attached to magnetic microparticles. This targeted mRNA is separated by a magnet. The complementary DNAs (cDNA) are built using reverse transcriptase and T7 polymerase enzymes (Figure 3B-1). The composed cDNAs allow the formation of new RNA multiplexes. The Hybridization Protection Test (HPA) is used to detect the resulting amplicons. With this method, the hybridization of the duplicated sequences with fluorescent-labeled oligonucleotide probes takes place. This results in fluorescent radiation. This emitted light is measured with a luminometer. If hybridization does not occur, the probe is degraded with the borate buffered solution (Figure 3b) [41].

The HPV E6/E7 mRNA marker is a better indicator of advanced disease than the commonly used HC2 method. According to the study conducted by Ratnam et al. (2011), the sensitivities of APTIMA® and HC2 HR-HPV tests were detected at 96.3% and 94.3%, respectively [44]. In addition, this test was determined to have a higher specificity compared to the cobas® HPV test. In a study of 1000 samples by Castle et al., they found that the APTIMA® HPV test was more specific than the cobas® HPV test [45]. It does not cross-react with LR-HPV strains and can be automatically processed. In addition to many advantages, it is stated as a disadvantage that it has a lower detection limit compared to other tests [43].

The APTIMA® HPV test, which emerged more recently than the HC2 method, is likely to have some limitations because it is less tested than the HC2 method. Preisler et al. detected cross-reactivity in 26, 61, 62, 67, 70, 82, and 83 strains for the APTIMA® test in their investigations with HC2 HR-HPV, cobas® HPV, and APTIMA® HPV tests [46]. In the studies conducted during the development of this test, it was determined that 26, 67, 70, and 82 strains were cross-reacted [41].

BD Onclarity™ HPV

The BD Onclarity™ HPV test, produced by Becton Dickinson (Sparks, MD), was FDA approved in 2018. After cervical samples are collected with swabs, they are collected in the BD SurePath™ and run from the solution. The DNA target amplification is performed using the Real-time PCR method, with this fully automated test. The E6/E7 oncogenes of 14 HR-HPV genotypes can be detected by this test[47]. The test consists of two stages. The first step consists of cell lysis and DNA isolation. The second stage is based on the TaqMan probe method, as is the method of the cobas® HPV test (Figure 3a) [47,48]. However, the reaction is performed in three separate tubes in this test, unlike the cobas® HPV test. A total of 15 probes and 4 fluorescent dyes are used, 14 for viral sequences and 1 for internal control. HPV-16, 18, and 45 genotypes are detected in the first of three PCR tubes, HPV-31, 33/58, and 56/59/66 are detected in the second PCR tube, and the HPV-51, 52, and 35/39/68 are detected in the third PCR tube. There is also a human β-globin gene region in each tube for internal control [47].

According to the results of studies conducted during the development of this test, the performance of the BD Onclarity™ HPV test to detect high-grade cervical disease was found to be higher compared to other FDA-approved HPV tests [47]. In addition, according to the study of Bottari et al., this test was found to be more specific and sensitive compared to the HC2 method [49]. Unlike other FDA-approved tests, this test has also been evaluated for people who have been vaccinated. Compared to unvaccinated women, this test has low sensitivity (80%) and high specificity (52.1%) in vaccinated women [47]. In addition, this test can distinguish between different genotypes[47,49]. It is easy to use because it is a fully automated system [47]. In addition to its advantages, the high probability of false negative results in the use of chemical drugs such as mucin, acyclovir, and clindamycin and the expensive test system is among the disadvantages of the test.

Conclusion

The molecular HPV tests, which are approved by the FDA, have some limitations. The cross-reactivity, false-positive, and false-negative results are among these limitations. It is also known that some drug use affects the tests. The studies were conducted to evaluate the analytical specificity of HPV molecular tests with the microorganisms (bacteria, fungi, viruses, etc.) that can be found in the female urogenital system microbiota and unclassified HPV types in an undetermined risk group. The studies were conducted to evaluate the analytical specificity of HPV molecular tests with the microorganisms (bacteria, fungi, viruses, etc.) that can be found in the female urogenital system microbiota and unclassified HPV types in an undetermined risk group. According to these studies, cross-reactivity was observed in the HC2 HR-HPV, Cervista™ HPV, Cervista™ HPV 16/18, and APTIMA® HPV tests, while no cross-reactivity was observed in the cobas® HPV and BD Onclarity™ HPV tests [34,41,47,50–52]. It was determined that there was cross-reactivity between the DNA test probe and the plasmid-pBR322, in the HC2 HR-HPV test. The presence of homologous sequences of this plasmid in genital samples has been reported. Therefore, false positive results may occur if there is a high level of bacterial plasmid in sample 50. The cross-reactivity was detected in the HPV-67 and 70 genotypes in the Cervista™ HPV HR test. The cross-reactivity was detected in the HPV-31 genotype in the Cervista™ HPV 16/18 test [51,52]. In the APTIMA® test, cross-reactivity was observed with low-risk HPV genotypes 26, 67, 70, and 8241. When the tests were examined in terms of cross-reactivities, it was determined that the reliability of cobas® HPV and BD Onclarity™ HPV tests were higher than the others. False positive results may occur in all molecular HPV tests due to cross-contamination. For this reason, it is necessary to use nuclease-free, disposable sterile materials to avoid cross-contamination. In the case of a low level of infection, there is a possibility that some tests will result in false negatives. These tests are the HC2 HR-HPV, Cervista™ HR HPV, and Cervista™ HPV 16/18 tests. When working with these tests, the possibility of HPV infection in negative results should not be ignored.

If the test samples are contaminated with certain drugs, creams, and/or gels, the possibility of false results increases. This affects the results of all tests negatively. In this context, attention should be paid to the use of antifungal cream and birth control gel in the HC2 HR-HPV test, the use of contraceptive jelly and/or antifungal cream in Cervista™ HR HPV and Cervista™ HPV 16/18 tests [51,52], the use of vaginal moisturizer in cobas® HPV test[34], use of antifungal medication in APTIMA® test [41], and the BD Onclarity™ HPV test, the use of vaginal creams containing mucin, acyclovir, and/or clindamycin [47]. People who will have these tests should stop using these drugs and/or creams for a certain period.

In the BD Onclarity™ and cobas® HPV tests, the presence of blood that causes the samples to discolor (red/brown samples) also affects the test's operation. The blood levels exceeding the 4% concentration for the BD Onclarity™ test and 2% for the cobas® HPV test increase the likelihood of false-negative results.

In conclusion, FDA-approved molecular tests have high sensitivity and specificity in the diagnosis of HPV. In addition, these tests are more automatic and reproducible than cytological methods. In addition to these advantages of the tests, the existence of limitations described in this review should not be overlooked. This indicates that molecular tests should not be used alone in the evaluation of HPV infections. Therefore, molecular HPV test results need to be correlated with cytological test results.

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Conflict Of Interest

There is no conflict of interest.

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Epidemic Diseases, Biological Weapons, and the Relationship of Religion with COVID-19

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ABSTRACT

As a result of the spread of the COVID-19 virus all over the world, starting from China in 2019, curiosity about the course of the epidemics that took place in history has increased. Many outbreaks have occurred in history, and many precautions have been brought against these diseases. Islam is a religion based on hygiene and cleanliness, both physically and spiritually. In the history of Islam, Hz. Prophet has many pieces of advice about the precautions to be taken against epidemics. In addition to outbreaks in the world, another factor that affects people both physiologically and psychologically and is released in a controlled manner is the microorganisms used as biological weapons. These weapons cause more chaos and terror than epidemics.

Moreover, even small amounts are enough to destroy millions of people. COVID-19 is the last pandemic in the world, and its effects are still ongoing. Although some claim that this virus is a biological weapon programmed in the laboratory, genetic sequencing has proven it to be natural.

Keywords: Epidemic, Pandemic, Disease, COVID-19, Bioweapon, Tibb al-Nabawi, Health

Salgın Hastalıklar, Biyolojik Silahlar ve COVID-19 ile Din İlişkisi

Süreç

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ÖZ

COVID-19 virüsünün 2019 yılında Çin'den başlayarak tüm dünyaya yayılmasıyla birlikte tarihte gerçekleşmiş salgın hastalıkların seyrine karşı merak da artmıştır. Tarihte pek çok salgın hastalık gerçekleşmiş ve bu hastalıklara karşı birçok farklı önlem alınmıştır. İslam hem bedenen hem de ruhen hijyeni ve temizliği esas alan bir dindir. İslam tarihinde Hz. Peygamber'in salgın hastalıklara karşı alınması gereken tedbirler konusunda birçok tavsiyesi bulunmaktadır. Dünyada salgın hastalıkların haricinde insanları hem fizyolojik hem de psikolojik olarak etkileyen ve kontrollü olarak salınan bir diğer etkenler biyolojik silah olarak kullanılan mikroorganizmalardır. Bunlar salgın hastalıklardan daha fazla kaos ve terör ortamı yaratırlar. Üstelik çok küçük miktarları dahi kitleleri yok etmeye yeterlidir. COVID-19 dünya üzerinde görülen son pandemidir ve etkileri halen devam etmektedir. Kimileri bu virüsün laboratuvarda programlanan bir biyolojik silah olduğunu iddia etse de doğal bir virüs olduğu genetik dizilimiyle kanıtlanmıştır.

Anahtar sözcükler: Salgın, Pandemi, Hastalık, COVID-19, Biyolojik Silah, Tıbbi Nebevi, Sağlık

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Introduction

Epidemic diseases are defined as diseases that infect a large part of humans, animals, and plants in a short time. Epidemics that have spread to a certain area are epidemics, and epidemics that have spread to the whole world are pandemics [1,2]. Pandemics are epidemic diseases that infect many countries simultaneously and spread over a wide area. Plague, Cholera, Spanish Flu, Typhoid, Swine Flu, and COVID-19 are among the epidemics and pandemics that have affected the world throughout history [3].

Perspective on Epidemic Diseases in Islam and the Tibb al-Nabawi

Hz. Prophet Mohammed said: "If you are aware of the presence of plague in a place, do not go to that area and stay where you are. If Plague disease starts in your place, do not leave your place." His words drew attention to the quarantine practice for the first time in the world. "Anyone with an epidemic disease should not go near a healthy person." and "Escape from a leper like a lion." He demanded that precautions be taken against infectious diseases [4,5]. While such precautions were taken against epidemics in the Islamic world, quarantine was applied in Europe for the first time after centuries in 1377 in Dubrovnik and Venice. The first quarantine application was made by the World Health Organization (WHO) in 1952 [6]. Hz. Prophet did not only restrict the quarantine practice to humans but also recommended that healthy animals and diseased animals be kept in separate areas. However, which states that a Muslim should wash at least once in seven days to maintain hygiene and that Allah has a right to that person, he draws attention to the importance Islam attaches to cleanliness in the hadith [7]. Along with these recommendations, he stated that diseases are a means of patience and gratitude for those who believe. He recommended that people who get sick seek healing, seek treatment and be patients [8]. As a matter of fact, from time to time, the Prophets also experienced difficult times related to diseases. Prophet Ayyub's patience, fortitude, and tawakkul attitude in the face of serious illnesses is a very important and meaningful example for Muslims [9,10]. When Hz. Prophet visited a patient and noticed that the patient's pain was increasing, he advised the relatives of the patient to bring a doctor for the patient. In response, the patient's relative asked Hz. Prophet surprisedly, "You say bring a doctor too, Rasulullah?" replied. Hz. Prophet said, "Yes, Allah has not given an incurable disease!" He recommended seeking a cure for diseases. Hz. Prophet stated that physical health began with maintaining oral hygiene and suggested that people should clean their mouths and teeth [8].

According to Islamic Historians, in a plague epidemic that took place in 639 years, thirty thousand people, including his companions such as Ebu Ubeyde bin Jarrah and Muaz bin Cabal, lost their lives due to the plague. When Hz. Omar went to Damascus with the Islamic Army; when he approached Damascus, he was informed that there was a plague epidemic at their destination, and the Companions disagreed about whether to go to the city or not. Hz. Omar consulted with the Companions, then stopped the army and sent it back. Thereupon, the army commander, Abu Ubeyde bin Jarrah, asked Hz. Omar: "Are you running away from the destiny of Allah, Omar?" When asked, Hz. Omar replied, "Yes, we are fleeing from one destiny of Allah to another destiny of Allah" [11]. Among the interesting events, the Egyptian King Mukavkis and the Greek Emperor Heraclius sent a doctor with many gifts to the Hz. Prophet. Even though days and months have passed since the gift was presented, the doctors said to Hz. Prophet, "O Mohammed! We have come here to serve You, but until today no patient has come to us. If you'll excuse us, we'd like to go now." they said. In response to this statement, Hz. Prophet said, "Know that even if you stay here for years, no one will come to you. Islam has shown the way not to be sick. My Companions also pay attention to cleanliness. They do not eat anything unless they are hungry and get up from the table before they are full." When the doctors replied, they returned to their country. However, it was stated that the European people were fighting diseases in these times. Madrasahs and hospitals opened in the cities of Cordoba, Baghdad, Ghazni, and Isfahan, which were the capitals of Islamic Civilization during the Middle Ages, became famous health centers [12].

Perspective on Epidemic Diseases in Christianity

The power of the priests, the right of the Church to speak, the punishment of scientists, and the nursing duty in hospitals by nuns were among the common practices in the Christian world. Under the influence of these practices, a famous mathematician like Pythagoras was burned to death along with his students as a result of the Church's fury. Galileo contraried with the Church in the field of science and died blind in 1642 while imprisoned at home. Copernicus hid his work so that he would not be excommunicated and tried in the inquisition. Columbus claimed that the world was round as a

result of his research, and these claims angered the Church. The Church tortured Columbus for this claim. Giordano Bruno, who claimed that the center of the universe is not the world, was burned to death at stake. Servetus was burned to death with his works because of the geography book he wrote[13]. Ignaz Philipp Semmelweis, an Austro-Hungarian scientist, and doctors was known as the "savior of mothers." He made sure that the postpartum fever disease was partially defeated with the advice of hand washing. In 1865, he faced the reaction of the Christian clergy in Europe and was imprisoned in a mental hospital. He died in a mental hospital from a wound on his hand [14].

The Development of Medicine as a Science and Searching for Solutions to Epidemics in the Islamic World

Ali bin Rabben et Tabari wrote the first Medicine book, Firdaws al-hikma, in the 850s. El-Razi mentioned Measles and Smallpox diseases in his work Al-Hawi in the 865s. Avicenna wrote the book El-Kanun Fit'tib, which was used as a coursebook in Europe from the 980s until the 1650s. Abul Kasim al-Zahravi became the unforgettable surgeon of Andalusia in the 1000s, and he wrote the medical book Al-Tasrif, which consists of 30 chapters [15-18].

Akşemsettin explained the existence of microorganisms in 1460, firstly. In 1150, a training and research hospital was built for the first time in Damascus, and the first private practice became operational. In the 1270s, patients were treated by the music in the Sifahiye Madrasah in Sivas, and the first eye surgery in the world was performed here[19,20]. While such positive developments were experienced in the Islamic World, a bad period was experienced in European countries due to the coercive and oppressive attitude of the Church and Priests against the people. According to them, diseases occur as a result of the disruption of the balance of four fluids in the body, which are blood, saliva, yellow bile, and black bile. In the 1850s, miasma (bad air) was considered the culprit of the cause of diseases in Europe. In Europe, street cats have been likened to the devil, and all street cats have been ordered by the Church to be killed. After the implementation of this request, there had almost no cats left on the streets of Europe. As a result, the rats overpopulated, and the plague became an inevitable disease in Europe [21,22].

Biological Weapons

Terrorist organizations or some states try to spread epidemics by using microorganisms as biological weapons. Because even the use of these agents in very small amounts affects large masses both physically and psychologically. The main purpose is mass panic, which is desired to be created in society rather than the extermination of the masses. Biological agents are the lowest-cost weapons of mass destruction that can be used to create a perception of panic in a possible biologic attack. For this reason, biological weapons are called "atomic bombs of poor countries". Incubation periods in biological weapons cause agitators to flee undetected. In today's conditions, agents that can be used as biological weapons can be easily produced in a laboratory for approximately 10 thousand dollars. Therefore, biological weapons can be produced at very low cost and in large quantities. Biological weapons are very difficult to detect by general security systems, and transportation of these weapons is also very easy. Biological weapons cause disease or death only in alive. Compared to other weapons of mass destruction, they do not have a destructive effect. Due to this feature, the detection of agents carrying biological weapons is very difficult. For this reason, states or terrorists prefer biological weapons [23,24].

Agents Used as Biological Weapons

The bacteria used as biological agents include *Vibrio cholerae* (Cholera), *Bacillus anthracis* (Anthrax), *Francisella tularensis* (Tularemia), *Coxiella burnetii* (Q Fever), and *Yersinia pestis* (Plague), and viruses as biological agents include Variola (Flower), Monkey Flower, Encephalitis (Western equine encephalitis virus, Eastern equine encephalitis virus, Venezuelan equine encephalitis virus), and Viral Hemorrhagic Fevers (Arenaviridae, Bunyaviridae, Filoviridae, Flavivirade) microorganism toxins and herbal toxins as biological agents include Ricin, Staphylococcal enterotoxin B (SEB), Botulinum toxins, and Mycotoxins (Trichotoxenes) [24].

One of the two biological weapons known to be effective in the world today is the Smallpox virus. There is no cure for the highly contagious virus. The mortality rate of this virus is 30% for the genus *Variola major* and 1% for the genus *Variola minor*. Another effective biological weapon is Anthrax. Although anthrax is originally a zoonosis, there is no human-to-human transmission. When it causes disease in humans, it causes different clinical pictures, such as lung and intestinal anthrax. Diseases do not last long, but death occurs in a short time [25].

Although biological weapons have almost invisible army-level power, they have a longer-lasting and more destructive effect compared to weapons of mass destruction or conventional weapons. While

the effects of chemical weapons are observed in the short term, the effects of biological weapons vary according to their incubation period. Parallel to the increase in the world population, the emergence of many different diseases is inevitable. There is a danger that every new disease or epidemic that emerges will turn into a biological weapon. The scope of biological weapons development programs is to create microorganisms that are fast-acting, have a short epidemic generation time, and cause a high rate of death [23,26].

Is COVID-19 Biological Weapons?

The first cases caused by the COVID-19 virus occurred on 17 November 2019 in Wuhan. The first death from Coronavirus occurred on 11 January 2020. The Coronavirus was isolated by WHO, and its genome sequence was shared on 12 January 2020. WHO declared a global emergency on 30 January 2020. WHO declared on 11 March 2020 that *"COVID-19 is a pandemic"* [27]. The COVID-19 virus is 88% similar in genome sequencing to the two types of Coronaviruses originating from bats. It is 79% similar to the SARS virus and 50% to the MERS virus. However, it is interesting that an anteater named Pangolin has 99% genome similarity to Coronaviruses in anteaters. Pangolins, an animal that is not well known in our country, is a species of scaly anteater. Most of the pangolins poaching in the world are sold to China. The possibility of an intermediate host between pangolins and humans is quite high [28-30].

Conclusion

It is thought that this virus may have been accidentally dispersed and transmitted from the Virology Laboratory in Wuhan. Because this laboratory was visited by a team from the USA in 2018 and was warned that no precautions were taken against risky studies and there was a security weakness. COVID-19 is a natural virus unless proven otherwise. When the DNA and genome structure of the virus are examined, the amino acid sequence of the S-protein (S1/S2) structure, which allows the virus to attach to the ACE-2 receptors in the lungs, seems completely natural. In the case of a laboratory intervention, this intervention can be clearly understood. The mortality rate for the SARS virus has been reported as 15% in those aged 45-64 and 50% in those aged 65 and over. The mortality rate for the MERS virus is 30% on average. The mortality rate of COVID-19 is 5.4% on average worldwide. SARS and MERS viruses have low infectivity but higher mortality. Therefore, its use as a biological weapon is very difficult. Viral diseases that will cause pandemics, including COVID-19, are seen as the nuclear bomb of poor countries. The smallpox virus is recognized as the strongest candidate to be used as a biological weapon in the future [28-30]. COVID-19 is a natural pandemic. But it is also a rehearsal for a future war that moves not tanks and submarines but the frontiers of scientific research.

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Conflict of Interest

The authors declare no conflicts of interest.

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Lithium Has Neuroprotective Effect On Neuroblastoma Cell Line In Low Dosages

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ABSTRACT

Lithium (Li) was presented as a protective agent in neuron degeneration, which is an important process in neurodegenerative diseases. This study aimed to determine the effect of specific amounts of Li on neuroblastoma cells by considering several specific genes which act on neuroprotection. Li solutions were prepared as 1 µM, 15 µM (low dosage), 30 µM and 45 µM (high dosage) concentrations and then applied to the neuroblastoma cell line. XTT and trypan blue assays were performed to determine cell proliferation and viability. mRNA expression levels of NES, BDNF, GRIN2A, LRRK2, PRKN, and SNCA were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Cell viability was detected as significantly increased in cells treated with low dosage Li. However, it was significantly decreased in high-dosage applied cells compared to untreated control. In addition, cell proliferation ratios were significantly decreased in high-dosage applied cells compared to the control. It was demonstrated that mRNA expression levels of several genes (NES, LRRK2, PRKN) were significantly upregulated.

Regarding BDNF, gene expression was significantly upregulated in the cells only treated with very low amount of Li. However, no significant data could be obtained for GRIN2A. Furthermore, the mRNA expression level of SNCA was determined as significantly downregulated compared to the control. Statistically significant expression of NES, LRRK2, PRKN, BDNF, and SNCA genes due to the variable Li concentrations applied to cells suggests that Li acts on transcriptional regulation of certain genes associated with neuronal survival. These findings support that dose-dependent Li treatment might have a protective effect on neurodegenerative diseases.

Keywords: Neurodegeneration, lithium, neuroprotection, gene expression

Lityumun Düşük Dozajlarda Nöroblastoma Hücre Hattı Üzerinde Nöroprotektif Etkisi

ÖZ

Lityum (Li), nörodejeneratif hastalıklarda önemli bir süreç olan nöron dejenerasyonunda koruyucu bir ajan olarak sunulmuştur. Bu çalışmanın amacı, belirli miktarlarda Li'nin nöroblastoma hücreleri üzerindeki etkisini, nöroproteksiyon üzerinde etki eden birkaç spesifik geni dikkate alarak belirlemekti. Li solüsyonları 1 µM, 15 µM (düşük doz), 30 µM ve 45 µM (yüksek doz) konsantrasyonlarında hazırlandı ve ardından nöroblastoma hücre hattına uygulandı. Hücre proliferasyonunu ve canlılığını belirlemek için sırasıyla XTT ve tripan mavisi deneyleri yapıldı. NES, BDNF, GRIN2A, LRRK2, PRKN ve SNCA'nın mRNA ekspresyon seviyeleri, kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qRT-PCR) ile tespit edildi. Hücre canlılığı, düşük dozda Li ile tedavi edilen hücrelerde önemli ölçüde arttığı tespit edildi, ancak yüksek doz uygulanan hücrelerde, tedavi edilmeyen kontrole kıyasla önemli ölçüde azaldı. Ayrıca yüksek doz uygulanan hücrelerde hücre çoğalma oranları kontrole göre önemli ölçüde azaldı. Birkaç genin (NES, LRRK2, PRKN) mRNA ekspresyon seviyelerinin önemli ölçüde yukarı regüle edildiği gösterildi. BDNF ile ilgili olarak, genin ekspresyonu, yalnızca çok düşük miktarda Li ile tedavi edilen hücrelerde önemli ölçüde yukarı doğru düzenlenmiştir. Ancak GRIN2A için anlamlı bir veri elde edilememiştir. Ayrıca, SNCA'nın mRNA ekspresyon seviyesinin kontrole kıyasla önemli ölçüde aşağı regüle edildiği belirlendi. Hücrelere uygulanan değişken Li konsantrasyonları nedeniyle NES, LRRK2, PRKN, BDNF ve SNCA genlerinin istatistiksel olarak anlamlı ifadesi, Li'nin nöronal sağkalım ile ilişkili bazı genlerin transkripsiyonel düzenlemesi üzerinde etkili olduğunu düşündürür. Bu bulgular, doza bağlı Li tedavisinin nörodejeneratif hastalıklar için koruyucu bir etkiye sahip olabileceğini desteklemektedir.

Anahtar sözcükler: Nörodejenerasyon, lityum, gen ekspresyonu

Süreç

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Introduction

Neurodegeneration is the main process of neurodegenerative disease. It is caused by the progressive loss of function and structure of a neuron. There are various neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's disease (PD), and Alzheimer's disease (AD). Today, there is no therapeutic application in reversing the progression of neuron degeneration[1, 2].

Li, which is used to treat mood disorders such as mania, hypomania, depression, and bipolar disorder, has a neuroprotective effect in the brain when it is daily used[3, 4, 5]. Li provides neuroprotection by inactivating N-methyl-D-aspartate (NMDA) receptor. It decreases gene expressions of pro-apoptotic proteins such as p53 and Bax while increasing the expression of cytoprotective proteins such as Bcl-2. In addition, Li activates Akt (Protein kinase B), known as 'cell survival kinase,' which plays an active role in cell survival[3, 6]. Moreover, it has been demonstrated that Li changes the expression of the *NES* gene, which is an intermediate filament and highly expressed in neural progenitor cells[4, 7, 8]. Despite these findings, the role of Li on neuron cells is still debated.

Neuroblastoma (NB) cell lines are derived from the neural crest and can be easily used for detecting the ability of neuronal differentiation with the application of various agents[9]. Many genes, predominantly expressed in neurons, play an active role in neuronal differentiation, survival, and regeneration. Of these, the Nestin protein, which NES encodes, is a biological marker for neuroblastoma cell lines. In addition, Brain-Derived Neurotrophic Factor (*BDNF*) encodes a nerve growth factor protein. The binding of this protein to its cognate receptor promotes neuronal survival in the adult brain. The expression of this gene is reduced in AD and PD patients[10]. Glutamate Ionotropic Receptor NMDA Type Subunit 2A (*GRIN2A*) encodes the NMDA receptor subunit, an ion channel protein. This structure plays crucial roles in certain kinds of memory and learning[11].

NES (OMIM 600915), *BDNF* (OMIM 113505), and *GRIN2A* (OMIM 138253) genes are all required for survival, regeneration, and proliferation of neural progenitor cells by mitogen stimulus[12-14]. Moreover, Leucine Rich Repeat Kinase (*LRRK2*; OMIM 609007), Parkin RBR E3 Ubiquitin Protein Ligase (*PRKN*; OMIM 602544) and alpha-synuclein (*SNCA*; OMIM: 163890) gene with a single-nucleotide polymorphism (rs356219) are also associated with the etiology of neurodegenerative diseases such as PD and AD[15].

Here, the effect of lithium concentrations on neuroblastoma cells was evaluated by considering neuron viability, proliferation, and transcriptional regulation of certain genes which play a role in neuronal survival. Interestingly, differential mRNA expression of several neuron-specific genes (*NES, LRRK2, PRKN*, *BDNF*, *GRIN2A,* and *SNCA*) in neuroblastoma cells treated with variable concentrations of Li suggests that certain amount of Li acts on neuronal survival via activating several pathways.

Material Method

This study was designed with the Gülhane Military Medical Academy, GATA Ethics Committee decision [23/10/2015- GATA/AR-2015-22/Ethics Committee Decision 2015- Session (240) 05].

Preparation Of Li Solutions: Li (499811-Sigma-Aldrich) as an element was used in our experiments. Li was diluted in isotonic saline solution as 1 µM, 15 µM, 30 µM, 45 µM, 60 µM, 75 µM, and 90 µM concentrations.

Cell Culture: Neuroblast cell line (NE-4C, ATCC, Cat. No: CRL-2925) was cultured and grown in 6 well-plates with RPMI-8226 1640 (Sigma-Aldrich-R8758) including 10 % (v/v) fetal bovine serum (Biochrom AG, Germany), 1% (v/v) l-glutamine and 1% (v/v) gentamicin (Biological Industries, Israel) at 37°C in 5% CO_2.

Application Of Lithium: LD 50 dosage was found as 75 µM Li concentration. For study groups, 1 ml of Li solution for each concentration [Low dosage: (1µM, 15µM)], High dosage: (30µM, 45µM)]) was used. Control group cells were grown in culture medium and not treated with Li solutions. The cells were examined immediately after the Li application and 24 hours later.

XTT Cell Proliferation Assay: The cytotoxic effects of Li solutions were analyzed by using the protocol of XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)- 5-[(phenylamino)carbonyl)]-2H-tetrazolium hydroxide) assay kit on neuroblastoma cells. In addition, the "XTT assay" kit (Trevigen XTT Cell proliferation assay kit-Cat No: 4891-025-K) was used to measure cell proliferation ratios in accordance with the manufacturer's instructions. In spectrophotometric viability tests such as XTT

assay, the viability of the untreated cells is accepted as 100%, and the viability of the treated cells is determined as a percentage (%) according to these cells[16]. In our experiments, neuroblastoma cells were seeded in a 96-well plate at a density of 10^3 - 10^5 cells/well in 100 µl of culture medium with or without lithium. Li was used in 1 µM, 15 µM, 30 µM, 45 µM, 60 µM, 75 µM, and 90 µM concentrations. We cultured the cells in a CO_2 incubator at 37°C for 24 hours. 10 µl XTT mixture was added to each well and incubated for 2 hours in a CO_2 incubator at 37°C. The results were obtained by using an "ELISA reader" with absorbance at 450 nm[17]. This study was repeated three times for each condition. LD 50 dosage was found as 75 µM Li concentration.

Cell Viability Assay: Trypan blue (Sigma Aldrich Co. 302643) was used as a stain to determine the cell viability. It was diluted at 0.8 mM in PBS and mixed with the cells in a 1:1 ratio. In total, a hundred cells were counted in each condition. Viable and non-viable cells were evaluated by using a hemocytometer (6+9)[18]. Three different studies were conducted for each condition.

RNA Isolation and Cdna Synthesis: All culture flasks were harvested by Trypsin-EDTA solution (Sigma Aldrich Cat No: T4049) 24 hours after Li applications. Total RNA was extracted via NucleoSpin RNA mini kit (NucleoSpin RNA, Mini kit for RNA purification/ Machenerey-Nagel) according to the manufacturer's protocol. cDNAs were synthesized by using RevertAid First Strand cDNA synthesis kit (ThermoFisher). The quality of cDNAs was checked with 2% agarose gel.

Qrt-PCR: The mRNA expression levels of *NES*, *BDNF*, *GRIN2A*, *LRRK2*, *PRKN*, and *SNCA* were analyzed. The primer sequences can be accessed in PrimerBank database (http://pga.mgh.harvard.edu/cgi-bin/primerbank). qRT-PCR conditions were 45 cycles of 95˚Cº 10'', 56˚C 15'', 72˚C 15'' (Roche Light Cycler 1.5). β-Actin [beta-actin housekeeping gene (5'-GTC CCT CAC CCT CCC AAA AG-3' (forward) and 5'-GCT GCC TCA ACA CCT CAA CCC-3' (reverse)] was used as an internal control. Each qRT-PCR reaction is performed in 20 µl. This study was repeated three times and results were analyzed by "Roche Light Cycler1.5 software" by $2^{-\Delta\Delta CT}$ method.

Statistical Analysis: Mean values and standard deviations were obtained for cell viability, proliferation assays as well as for qRT-PCR results. Student's *t* test was used to determine the statistical significance. $p<0.05$ was considered significant.

Results

NE-4C cells were treated with variable concentrations of Li solutions in low and high-dosage groups. It was detected that although cell viability was significantly increased in low-dosage Li-applied cells, it was significantly decreased in cells treated with high-dosage Li compared to untreated control neuroblastoma cells (Figure 1, Table 1). According to the XTT assay, all cell proliferation ratios were statistically insignificant in cells treated with low Li solutions. However, it was significantly decreased in the high amount of Li solution applied cells (Figure 2).

In this study, mRNA expression levels of *NES, BDNF, GRIN2A, LRRK2, PRKN*, and *SNCA* genes were determined by qRT-PCR both in the control and study group. Accordingly, it was demonstrated that mRNA expression of *NES* was significantly upregulated with the increased level of Li in the neuroblastoma cell line compared to untreated control (Figure 3A). the mRNA expression level of *LRRK2* was significantly upregulated by the treatment of 15 µM and 45 µM Li solutions to neuroblastoma cell line compared to control (Figure 3B). In addition, it was shown that *PRKN* gene expression was significantly increased only in 45 µM Li treated cells compared to untreated control (Figure 3C). No statistically significant data could be obtained for other applications. Furthermore, mRNA expression level of *BDNF* was significantly but slightly increased only in 1 µM Li treated cells compared to untreated control (Figure 3D). However, it was detected that mRNA expression of *SNCA* was significantly downregulated both in low and high dosage Li applied cells (Figure 3E). On the other hand, no significant data could be obtained for *GRIN2A* (Figure 3F). It should also be noted that there are some blank columns because of the clogged capillary tubes.

Table 1. Cell viability ratios of neuroblastoma cells in Li treated groups and untreated control.

		Low Dosage Group				High Dosage Group			
	Untreated	1µM	P value	15µM	P value	30µM	P value	45µM	P value
Neuroblastoma cells	93±4%	97±2%	p<0.05	95±4%	p<0.05	92±2%	p<0.05	90±3%	p<0.05

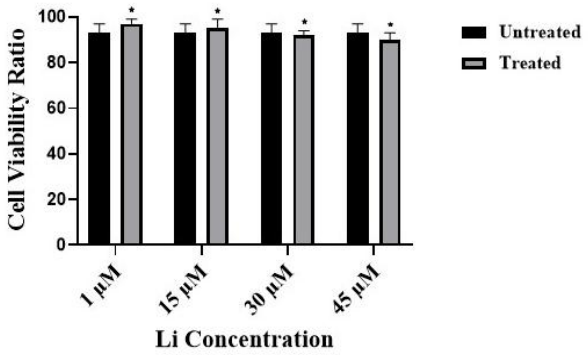

Figure 1. The effect of variable Li concentrations on viability of neuroblastoma cells.
X axis represents Li concentrations and y axis indicates cell viability ratios. P values represents the comparisons between untreated and treated cells. *: p<0.05

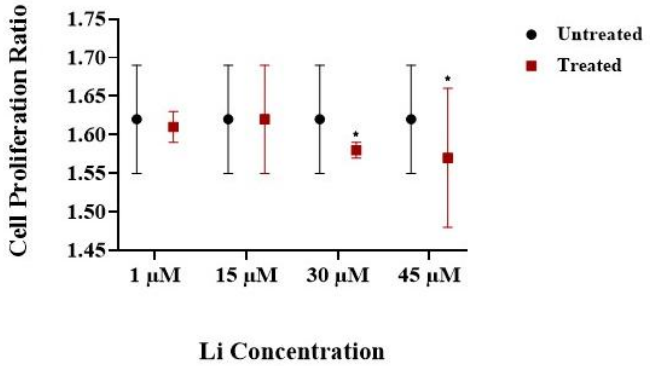

Figure 2. The result of XTT assay to estimate the proliferation ratio of neuroblastoma cells after Li treatments.
P values were calculated by the comparisons between untreated and treated cells. *: p<0.05
Proliferation ratios for 1 µM and 15 µM Li treated cells are not statistically significant (p≥0.05).

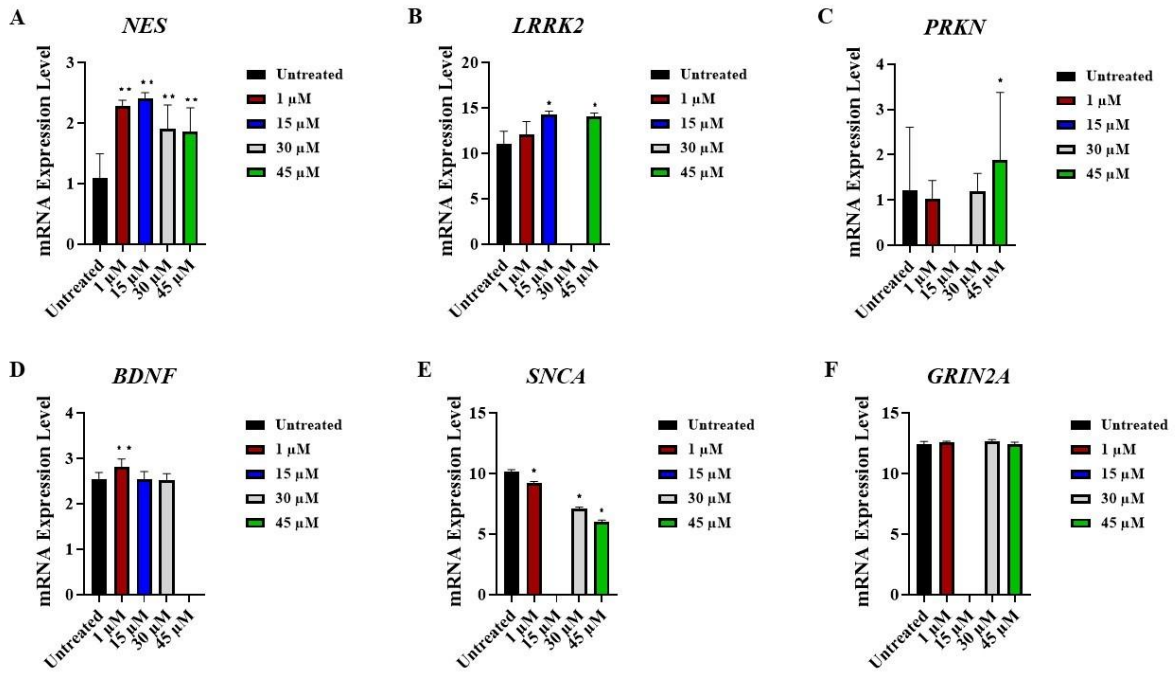

Figure 3. mRNA expression analysis of selected genes. Untreated represents control cells without Li application. *: p<0.05 **: p<0.005. P values were calculated by the comparisons between untreated and treated neuroblastoma cells. Blank columns represent not obtained data for related genes.

Discussion

The study results indicated that low-level Li increased neuron cell viability. However, cell viability and proliferation were decreased in high-level Li-applied neuron cells. Moreover, it was detected that Li was responsible for the transcriptional regulation of several neuron-specific genes.

Nerve cell degeneration can be identified as decreased functional activity and degeneration in nerve axons and their terminal branches. The destruction of the nerve cells occurs in the last step. These pathological conditions are pathognomonic in neurodegenerative diseases. The process of nerve degeneration is studied in the field of the neuroanatomy of the brain and neurophysiology of neural pathways[19]. Although specific protein accumulations and anatomic vulnerability typically define neurodegenerative diseases, they share many fundamental processes associated with progressive neuronal dysfunction and death caused by such as oxidative stress, programmed cell death, and neuroinflammation[20].

Li is an alkali metal which is naturally present in all vertebrate tissues and body fluids at low concentrations. It is used in manic-depressive illness as a drug. In addition, it was recommended to treat acute brain injury and chronic neurodegenerative diseases for its potentially beneficial effects[21, 22].

Interestingly, it was detected that neuron cell viability was decreased with the application of the increased amount of Li solution in this study. By the way, high dosage Li also decreased the cell proliferation. Li has been shown to increase cell proliferation in cerebellar granule and cerebral cortical cell cultures. Studies with bromodeoxy Uridine (BrdU) have demonstrated that this effect is in the S phase of replication[23]. This finding revealed that Li changed cell behavior (especially proliferation and apoptosis) by regulating gene expressions[23]. Furthermore, as high dosage Li decreased neuron cell proliferation in this study, it might be speculated that Li affected the mechanisms of necrosis or apoptosis. Reduced cell proliferation rate is correlated with decreased viability in cells treated with higher amount of Li. The results are consistent with the literature findings[24, 25]. This data also suggest that high level Li may be detrimental to neuron cells and dose adjustments should be done before use.

On the other hand, effects of Li were evaluated on the transcriptional regulation of several neuron specific genes which play an active role in neuron differentiation, survival, and regeneration. Accordingly, Li changed the mRNA expression level

of *NES* (Figure 3A). It is required for the survival, regeneration, and proliferation of neural progenitor cells[26]. Upregulation of *NES* suggests that it might stimulate neural progenitor cells and attempt to proliferate them upon application of Li. However, NES's mRNA expression level changed between low and high-dosage Li-applied cells. Although data are not statistically significant to make a comment on cell proliferation in cells treated with low dosage Li, it can be concluded that the proliferation ratio is decreased in high dosage Li applied neuron cells. This is also correlated with decreased mRNA expression of *NES* in neuroblastoma cells treated with high dosage Li. In addition, another neuron-specific gene, *LRRK2* encodes a kinase protein, and it is present in the mitochondrial outer membrane. Mutations (particularly G2019S) in this gene increase the kinase activity and have been associated with PD[27]. Several studies reported that LRRK2 protein involved in neurite outgrowth, autophagy, and immune cell functions[28,29]. Moreover, *PRKN* encodes parkin protein which functions as an E3 ubiquitin ligase and enhances cell survival by suppressing both mitochondria-dependent and -independent apoptosis[30]. Mutations in this gene are also known to cause PD [31]. Both *LRRK2* and *PRKN* genes were upregulated only in 15 µM and 45 µM Li applied cells, and data were not sufficient to make a precise comment about the effect of Li on these genes (Figure 3B-C). However, statistically significant upregulation of the gene m RNA levels in a certain amount of Li-treated neuroblastoma cells at least indicates the stimulation of neuroprotection-related pathways. In this situation, toxic effects of high amount of Li should be considered.

On the other hand, *BDNF* encodes a member of the nerve growth factor family proteins[32]. Expression of this gene is reduced in AD and PD patients. In addition, this gene plays a role in the regulation of the stress response and in the biology of mood disorders[33]. *BDNF* mRNA expression level was very slightly but significantly upregulated in neuroblastoma cells treated with a very low amount of Li, demonstrating the neuroprotection effect of low dose Li in neurons (Figure 3D). However, *GRIN2A* gene is associated with *NMDA* receptors, memory, and learning[33]. mRNA expression of *GRIN2A* was either not obtained or not statistically significant. For this reason, no precise comment could be declared in terms of the effect of Li on *GRIN2A* expression. Furthermore, alpha-synuclein is a neuronal protein that regulates synaptic vesicle trafficking and subsequent neurotransmitter release. It is abundant in the brain and is located mainly in the axon terminal of the presynaptic neurons. The release of neurotransmitters relay signals between neurons and are critical for normal brain function. *SNCA* has also been implicated in the pathogenesis of PD and SNCA peptides are a major component of amyloid plaques in the brains of patients with AD[34, 35].

Moreover, it is accumulated after spinal cord injury and causes damaged nerve fibers and neurons[36]. It was reported that downregulated alpha-synuclein triggered functional recovery, reduced neuroinflammation, and microglial activation in rats with spinal cord injury[36]. In addition, Zhao et al. reported that Li was involved in epigenetic regulation by decreasing DNA methylation, which eventually reduced the expression of alpha-synuclein[37]. Our study also showed that mRNA expression of *SNCA* was downregulated depending on the application of the increased amount of Li. It can be concluded that Li regulates the expression of *SNCA* via novel pathways or mechanisms to protect nerve cells.

To sum up, our data suggest that dosage-dependent Li treatment of neurodegenerative diseases such as AD and PD may reduce symptoms and disease progression via regulating the expression of several gene mRNA levels. This implies Li is a protective agent for neurodegenerative diseases. However, further clinical and molecular biology-based experimental studies should be done to understand how Li affects the expression levels of mRNAs of these genes and to reveal the exact mechanism of neuroprotection in more detail.

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Conflict Of Interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Effect of Thiamine on Morphine Analgesia and Tolerance in Rats

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Research Article

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ABSTRACT

The latest research has demonstrated that inflammation, oxidative stress, and apoptosis play a major role in morphine analgesia and tolerance development. This search goal is to examine the possible role of thiamine use on oxidative stress, inflammation, and apoptosis in the development of morphine analgesia and morphine tolerance in rats.

Methods: Thirty-six male Wistar rats were used in this study. The rats were severed into six groups: saline, 100 mg/kg thiamine, 5 mg/kg morphine, thiamine + morphine, morphine tolerance, and thiamine + morphine tolerance. The resulting analgesic effect was measured by hot plate and tail movement analgesia tests. TAS and TOS, inflammation parameters, and apoptosis protein levels of the dorsal root ganglion tissues sample were measured using an ELISA kit.

Results: When thiamine was given alone, it did not show an anti-nociceptive effect ($p>0.05$). In addition, thiamine enhanced the analgesic effect of morphine ($p < 0.05$) and also significantly reduced tolerance to morphine ($p < 0.05$). However, it reduced TOS when administered with a single dose of morphine and tolerance induction ($p < 0.05$). In addition, thiamine reduced apoptosis protein levels after tolerance development ($p < 0.05$).

Conclusion: Consequently, these results may attain by reducing TOS, inflammation, and apoptosis.

Keywords: Thiamine, Morphine Analgesia, Morphine Tolerance, Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Apoptosis, İnflammation

Tiaminin Ratlarda Morfin Analjezisi ve Toleransı Üzerine Etkisi

Süreç

Geliş: 03/08/2022
Kabul: 26/03/2023

ÖZ

Özet: Son araştırmalar inflamasyon, oksidatif stres ve apoptozun morfin analjezisi ve tolerans gelişiminde önemli bir rol oynadığını göstermiştir. Bu araştırmanın amacı, sıçanlarda morfin analjezisi ve morfin toleransının gelişiminde oksidatif stres, inflamasyon ve apoptoz üzerinde tiamin kullanımının olası rolünü incelemektir.

Yöntemler: Bu çalışmada 36 adet erkek Wistar rat kullanıldı. Sıçanlar salin, 100 mg/kg tiamin, 5 mg/kg morfin, tiamin+morfin, morfin toleransı ve tiamin+morfin toleransı olmak üzere altı gruba ayrıldı. Ortaya çıkan analjezik etki, hot plate ve tail flick analjezi testleri ile ölçüldü. Dorsal kök ganglion doku örneğinin TAS ve TOS, inflamasyon parametreleri ve apoptoz protein seviyeleri bir ELISA kiti kullanılarak ölçüldü.

Bulgular: Tiamin tek başına verildiğinde antinosiseptif etki göstermedi ($p>0,05$). Ek olarak, tiamin morfinin analjezik etkisini arttırdı ($p < 0.05$) ve ayrıca morfine toleransı önemli ölçüde azalttı ($p < 0.05$). Ancak, tek doz morfin ve tolerans indüksiyonu ile uygulandığında TOS'u azalttı ($p < 0.05$). Ek olarak, tiamin, tolerans gelişiminden sonra apoptosis protein seviyelerini azaltmıştır ($p < 0.05$).

Sonuç: Sonuç olarak, bu sonuçlara TOS, inflamasyon ve apoptozu azaltarak ulaşılabilir.

Anahtar Kelimeler: Tiamin, Morfin Analjezisi, Morfin Toleransı, Toplam Antioksidan Durumu (TAS), Toplam Oksidan Durumu (TOS), Apoptoz, İnflamasyon

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Introduction

Morphine is an analgesic, which is often applied the relieve violent and chronic pain today. If the antinociceptive properties of morphine develop tolerance, the duration of analgesic action decreases. However, despite many studies, the mechanism for developing opioid tolerance is unclear[1,2].

Research on morphine tolerance has generally focused on the effect of neurons in this development. However, it was recently reported that glial cell activation also plays a role in morphine tolerance[3–5]. As a result of the activation of glial cells, many proinflammatory cytokines are secreted[6]. Prolonged use of morphine induces the activation of microglia, which are glial cells in the central nervous system[7,8]. Microglial cells cause pain by releasing many proinflammatory cytokines, such as interleukin-1 (IL-1β). In this case, morphine binds to toll-like receptor 4 (TLR4) and releases proinflammatory cytokines[9]. In addition, IL-1β, which is the most critical factor in inflammation processes, is produced by a nod-like receptor protein 3 (NLRP3) as pro-inflammatory cytokine interleukin-1β (Pro-IL-1β) and then secreted as IL-1β[10]. These cytokines are involved in increasing the hyperactivity of dorsal root ganglia (DRG), which causes morphine to reduce analgesic efficacy and sensitivity[11]. In addition, long-term morphine treatment leads to oxidative stress (OS) in various cells[12,13]. Oxidative stress on some cellular mechanisms resulted in neuronal apoptosis in DRG[12,14].

Thiamine (TIA) is an essential water-soluble vitamin critical for carbohydrates, amino acid catabolism, and gluconeogenesis. It also acts as a cofactor of enzymes Cut-off time in almost the entire organism[15,16]. The fact that thiamine is involved in both energy pathways and defense mechanisms developed against oxidative stress suggests that it will help diagnose and treat many diseases in the field of health[17]. In the absence of thiamine, apoptosis, and neurodegeneration occur in cells. The reduction in thiamine phosphate and thiamine-dependent enzymes multiplies oxidative stress and leads to neurodegeneration. But the effects of thiamine on the development of morphine analgesia and morphine tolerance are still unknown.

This search aims to examine the possible role of thiamine use in rats on apoptosis inflammation pathways, and oxidative stress in the development of morphine analgesia and morphine tolerance.

Methods

Animals

Wistar Albino (230-250 grams for each group; n=6) rats were used in the study. The rats were provided by the Animal Center Laboratory of Sivas Cumhuriyet University. At a steady temperature (22 ± 3°C), the ad libitum was kept in standard conditions with a standard diet and water in a 12 h light-dark period. The experiment took place between 09:00 and 17:00. The study protocol was approved by Sivas Cumhuriyet University's Animal Ethics Committee. (Approval No: 65202830-050.04.04-448), experimental processes were initiated.

Drugs

Thiamine (B1) (Solgar) and morphine sulfate (Sivas Cumhuriyet University Hospital, Turkey) were dissolved in a saline solution. Thiamine and morphine were freshly prepared and injected into the animals during the experimental days. Before the analgesia tests, morphine (5 mg/kg) and thiamine (100 mg/kg) was given subcutaneously (s.c.) and intraperitoneally (i.p.), respectively.

Experimental protocol

Analgesic effects of thiamine and morphine were utilized at 30-minute intervals (30, 60, and 90 minutes) using tailf-lick and hot-plate antinociception tests. The animals were divided into six groups: Saline, thiamine (TIA), 5 mg/kg of morphine (M), TIA+ M, morphine tolerance (MT), and TIA+MT. Thiamine and saline were given to animals as intraperitoneal and morphine subcutane in the specified doses (volume of administration, 1 ml/kg). After analgesic tests were carried out, the animals were sacrificed by decapitation. Dorsal root ganglion (DRG) (T12-L5 levels) were collected from animals evaluated (Figure 1).

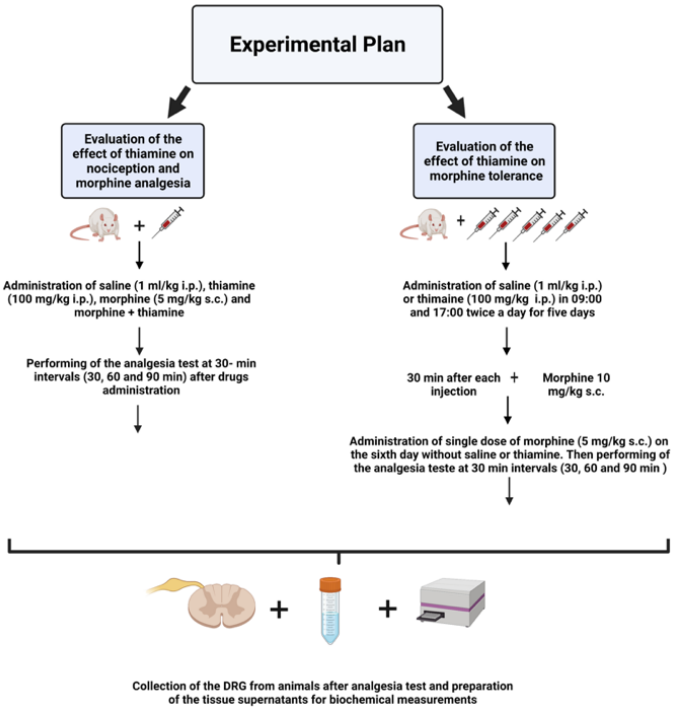

Figure 1. The study's experimental design.

Antinociception tests

Tail-Flick test

Thermal nociception was measured using a common tail-flick device (May TF 0703 Tail-flick Unit, Commat, Turkey). After administering saline or drugs, the radiant heat source was focused at a distance of 3 cm on the perpendicular portion of the tail. Following the administration of saline or drugs, tail-flick latencies (TFL) were measured. Cut-off times were established 15 seconds to prevent tissue injury[1,2].

Hot Plate test

It is thought that the antinociceptive reaction in hot plates occurs under the influence of central and peripheral mechanisms. The rats were placed individually on a hot plate (May AHP 0603 Analgesic Hot-plate, Commat, Turkey) at 54 ± 3 ° C. The delay up to the first claw licking or splash reaction to avoid heat was recorded as a pain threshold indicator. Cut-off time is set to 30 seconds to prevent damage to the paws[1,2].

Morphine tolerance induction

The procedure used to induce morphine tolerance was defined in earlier investigations 18. To create morphine tolerance, animals were randomly chosen and administered 10 mg/kg of morphine s.c. twice daily (at 9:00 and 17:00) for five days18-20. In addition, morphine (10 mg/kg) was given 30 minutes after every thiamine administration to determine the effects of thiamine (100 mg/kg) on morphine tolerance. Analgesic morphine dosage recommendation (5 mg/kg). It was administered on day six without saline or thiamine, and tail-flick and hot-plate tests were measured at 30-minute intervals (30, 60, and 90 minutes) to assess tolerance.

DRG tissue homogenate preparation

DRG tissue samples in the cold phosphate buffer saline solution of the animals were homogenized using a mechanical homogenizer (Analytic Jena Speed Mill Plus, Jena, Germany) and centrifuged for 10 minutes at a temperature of 4 ºC at 4000 rpm. Then supernatants were obtained and stored at -80 °C until biochemical analysis. A Bradford protein test kit (Merck, Germany) was used to determine the total protein levels in the samples[21].

Total antioxidant status (TAS) measurement

TAS concentrations at tissue level were determined by an automated test method previously developed by Erel based on monitoring the reaction rate of free radicals by measuring the absorption of colored dianidil radicals during free radical reactions that begin with hydroxyl radical production in a Fenton reaction. Antioxidants in tissue samples should suppress coloring in proportion to their concentration[22]. The results were expressed as micromolar Trollox equivalents per gram tissue protein (µmol Trollox Eq /g protein).

Total oxidant status (TOS) measurement

Tissue TOS concentrations were measured by Erel's automated test method [23]. Since iron ion is oxidized to iron ion when sufficient amounts of oxidant are present in the environment, the technique allows measuring TOS levels by measuring the tissue level of iron ions using xylenol oranges. Hydrogen peroxide was used for calibration of the analysis 23. The test results were expressed as the equivalent of micromolar hydrogen peroxide per gram tissue protein (µmol H2O2 Eq /g protein).

Measurement of NLRP-3, pro-IL-1β, IL-1β, Caspase-1, Caspase-3 and Caspase-9

DRG supernatants NLRP-3, pro-IL-1β, IL-1β, Caspase-1, Caspase-3, and Capase-9, were measured using rat ELISA commercial kits (Shanghai Sunred Biological Technology, Shanghai, China).

The operational protocols were by the manufacturer's instructions. In short, standard and tissue samples were added to a plate and incubated for 60 minutes at 37 ° C. After washing, dyeing solutions were added and incubated for 15 minutes at 37 ° C. The stop solution was added and read at 450 nm. Standard curves were used to calculate all kits. The coefficients of variation in and between the plates were less than 10%.

Analgesic tests data analysis

To calculate the maximum percentage of antinociceptive action (MPE), tail movements and hot plate delays (in seconds) were converted into an antinociceptive activity percentage with the following equation: % MPE = [(post-drug delay - primary delay) / (cutting value - Basic delay)] × 100 [18].

Statistical analysis

The results are given as SEM (standard error of average) ± average. Antinociceptive effect was measured, and average MPE was calculated. Variance analysis (One-Way Anova) and posthoc Tukey test were used in the analysis of the data. The significance value was regarded as $p<0.05$.

Results

Effect of Thiamine on Nociception, Morphine Analgesia, and Morphine Tolerance

Tail flick and hot plate tests were used for 90 minutes with a 30-minute interval to assess the analgesic effect of thiamine. Although there was an increase in antinociceptive tests in the 30th, 60th, and 90th minutes compared to the thiamine saline group, this increase was not significant (Figure 2A, 2B, $p>0.05$). This data showed that thiamine alone does not have a significant analgesic effect. However, in tail-flick and hot-plate tests, the administration of thiamine together with morphine boosted the antinociceptive impact of morphine ($p<0.05$; Figure 2A, 2B). In addition, the maximum increased effect of thiamine on morphine was demonstrated in the tail-flick test in the 60th minute and the hot-plate test in the 30th minute.

Morphine analgesia tests showed their peak in the 60th minute. However, in both tests, the MPE was statistically higher in the morphine-given group than in the morphine tolerance group. In both the tail-flick and hot-plate tests, the administration of thiamine to morphine-tolerant rats dramatically reduced their morphine tolerance compared to morphine-tolerant rats. ($p<0.05$) (Figure 2A, 2B).

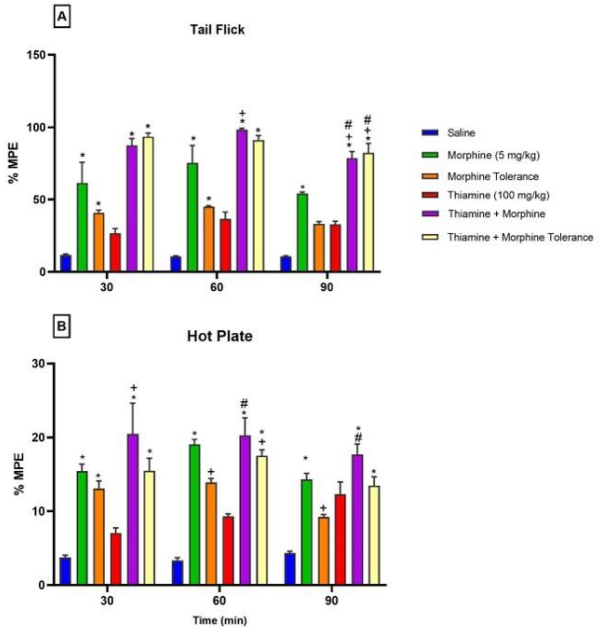

Figure 2. Thiamine's effect on nociception, morphine analgesia, and tolerance to morphine. In the tail flick test (A); In the hot plate test (B. The percent MPE (n = 6) values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on TAS and TOS parameters in morphine analgesia and tolerance in DRG

TAS and TOS levels in dorsal root ganglia are shown in Figure 3A and Figure 3B. Compared to the saline group, the morphine tolerance group's TAS levels drastically decreased ($p< 0.05$) (Figure 3A). However, the application and combination of thiamine did not influence the development of

morphine and morphine tolerance in DRG (p>0.05) (Figure 3A).

Morphine alone enhanced TOS levels in DRG compared to saline. However, the morphine tolerance group enhanced TOS levels compared to saline and morphine groups. However, the application of thiamine with morphine reduced TOS levels compared to both the morphine and morphine tolerance group (p<0.05) (Figure 3B).

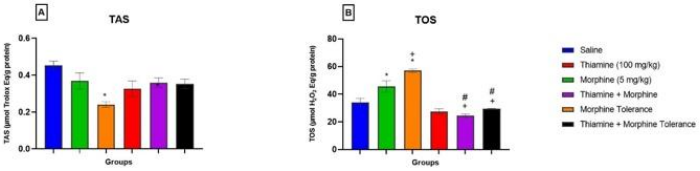

Figure 3. In morphine analgesia and tolerance in DRG, the effect of thiamine on total antioxidant status (TAS) (A) and total oxidant status (TOS) (B) levels was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on apoptosis in morphine analgesia and tolerance in DRG

The levels of caspase-9 and caspase-3 in the dorsal root ganglia are shown in Figure 4A and Figure 4B. The morphine tolerance group increased caspase-9 and caspase-3 compared to saline. In addition, the combination of thiamine with morphine did not alter caspase-9 and caspase-3 levels compared with both the morphine and morphine tolerance group. However, the thiamine + morphine tolerance combination reduced caspase-9 and caspase-3 levels, compared to the morphine tolerance group (p<0.05) (Figure 4A, 4B).

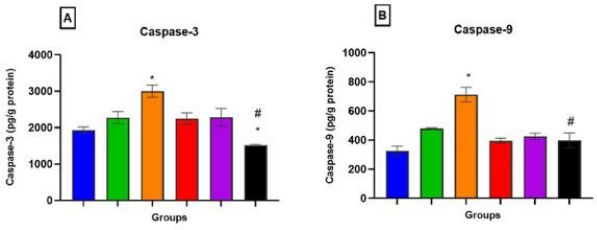

Figure 4. In morphine analgesia and tolerance in DRG, the effect of thiamine on apoptosis caspase-3 (A), caspase-9 (B) was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on inflammation in morphine analgesia and tolerance in DRG

To better understand the effect of inflammation in the development of morphine analgesia and morphine tolerance of thiamine, we determined caspase-1, IL-1β, NLRP-3, and pro-IL-1β levels in dorsal root ganglia with ELİSA kits (Figure 5A, Figure 5B, Figure 5C, and Figure 5D). The application of a single dose of morphine increased caspase-1 levels in DRG compared to the saline group. (Figure 5A). Single-dose morphine administration and morphine tolerance enhanced pro IL-1β levels in DRG compared to saline, as shown in Figure 5C (p<0.05). In addition, the morphine tolerance group was found to statistically increase the level of pro-IL-1β compared to the morphine group (p<0.05). The combination of thiamine+morphine and thiamine+morphine tolerance reduced pro IL-1β levels statistically (p<0.05) (Figure 5B). Il-1β levels increased statistically significantly compared to the saline group of the morphine group alone (p<0.05). Furthermore, morphine tolerance increased IL-1β levels (p<0.05) (Figure 5C). As shown in Figure 5C, compared to the saline, the combination of thiamine +morphine and thiamin+morphine tolerance reduced IL-1β levels in DRG (p<0.05). As shown in Figure 5D, single-dose morphine and morphine tolerance increased NLRP-3 levels compared to saline (p<0.05). In addition, the morphine tolerance group increased NLRP-3 levels in DRG compared to morphine (p<0.05) (Figure 5D).

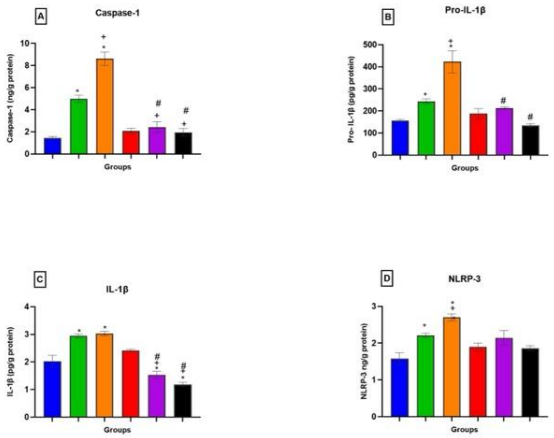

Figure 5. In morphine analgesia and tolerance in DRG, the effect of thiamine on inflammation parameters caspase-1 (A), Pro- IL 1 β (B), IL-1 β (C) and NLRP-3 (D) was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the

morphine group and #p<0.05, compared to the morphine tolerance group.

Discussion

Morphine is an opioid frequently used to treat severe and chronic pain[23]. Prolonged use of opioids causes analgesic effects and side effects such as respiratory depression, euphoria, sedation, and nausea[25,26]. Different analgesic approaches, such as low-dose morphine or a mix of auxiliary medications, are utilized to lessen these unfavorable effects of morphine [27]. In this study, thiamine in analgesia tests, also known as B1vitaminin, increased morphine analgesia and reduced analgesic tolerance when combined with morphine. According to our data, Thiamine's effects on morphine analgesia and tolerance building may be brought about by its ability to inhibit inflammatory and apoptotic pathways. However, our results showed that thiamine alone does not have an analgesic effect.

Numerous studies have demonstrated that both acute and ongoing morphine treatment can result in a considerable reduction in TAS levels in the mouse liver, as well as the rodent and human brain[28-30]. It has also been shown that chronic morphine therapy affects superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx), that is, enzymes involved in endogenous antioxidant defenses [31,32]. In line with previous studies, our study has also found that morphine and morphine tolerance alone reduce DRG's antioxidant status (TAS). However, thiamine did not change tas levels combined with a single dose of morphine or in DRG. It may be suggested that morphine can suppress the antioxidant system, causing the development of tolerance. In addition, single doses of morphine and chronic morphine application have been shown to cause oxidative stress (TOS) in DRG. In addition, TOS levels in DRG increased more than the application of chronic morphine compared to a single dose and showed similar results from previous studies. This might be connected to the development of tolerance. However, thiamine reduced these morphine effects.

Vitamin B types effectively control inflammatory or neuropathic pain in different animal models and humans[33]. In some research in experimental animals, the combination of thiamine/pyridoxine/cyanocobalamin has shown an antinociceptive effect[34]. These vitamins have also been shown to increase the antinociceptive effect of nonsteroidal anti-inflammatory drugs [35]. Recently, riboflavin, another B complex vitamin, has been found to show anti-inflammatory effects. Riboflavin has been shown to reduce the synthesis of tumor necrosis factor (TNF) α, interleukin (IL)-1, and IL-6 from inflammatory cytokines caused by lipopolysaxkaritis (LPS)[36]. Thiamine (B1 vitamin), another vitamin B, which we also use in our study, is considered to have painkiller properties through antinociceptive, anti-inflammatory, and anti-neuropathic mechanisms. Our study found that the treatment of thiamine alone did not show antinociceptive, anti-inflammatory, or antiapoptotic effects. This may be due to the different doses of thiamine, the way it was given, and the experimental model.

Braga et al. thiamine has been shown to significantly reduce TNF-α concentrations in the DRG of animals treated with practicality. proinflammatory sickins (TNF- α, Il1-β, Il6) are excised in healthy spinal cord cells at low levels. Still, there is an increase in expression levels after peripheral nerve damage and inflammation [34]. The main source of this increase in the central nervous system (MSS) is activated glia. However, glial activation has been shown to inhibit inhibitors in developing hyperalgesia and allodin due to nerve damage and inflammation[37]. With acute and chronic administration of morphine, glial activation occurs. During this treatment process, the degree of glial activation increases. The administration of morphine and increased glial activation causes the release of more proinflammatory cytokines[38]. Increased proinflammatory cytokines then reduce the analgesic effect of morphine, causing the development of morphine tolerance[39]. In our study, morphine and morphine tolerance alone increased the levels of IL1-β, and pro-IL1-β. However, morphine tolerance increased inflammatory parameters in DRG (IL1-β, pro-IL1-β) more than a single dose of morphine. These results are in line with those of earlier research. In addition, the combination of thiamine, morphine, and morphine tolerance has alleviated these parameters. These findings showed us that thiamine reduces inflammation and exhibits antinociceptive activity with combined therapy.

Liu et al. have demonstrated that exposure to chronic morphine increases NLRP3, caspase-1, pro-IL1-β, and IL1-β, and these levels decrease with melatonin treatment. Chronic morphine exposure leads to excessive cellular ROS production and inflammatory activation of NLRP3 in microglia. Increased NLRP3 activation causes caspase-1 oscillation, and caspase-1 activation increases pro-IL1-β cytokine, causing an increase in IL1-β level. And this increase causes morphine analgesic tolerance and hyperalgesia. To reduce these side effects due to the use of morphine, thiamine can reduce ROS and, as a result, inhibit the activation of NLRP3 inflammation, suppressing the overactive IL-1β signal, which ultimately weakens the development of morphine analgesic tolerance[40]. Proinflammatory cytokines such as IL-1β, IL-6, and TNF-α released from glia in the central nervous system may be responsible for the development of central sensitization[41]. Increased spinal proinflammatory cytokines and glia activation not only induce central sensitization, but also affect the antinociceptive properties of morphine [42] . Raghavendra ve diğerleri, 2002). Suppress glial activation or inhibition of spinal proinflammatory cytokines reduces morphine tolerance and enhances the acute antinociceptive effect of morphine in neuropathic rats [43]. Moreover, beige-J mice, a species of mutant mouse with immunological deficiencies, have previously been shown to be resistant to morphine analgesia and have high IL-1 levels[44].

Various cellular mechanisms such as oxidative stress and inflammation in the initiation and spread of apoptosis directly trigger apoptosis, increasing the production of ROS. However, antioxidants and anti-inflammatories have been shown to reduce ROS production and increase its anti-apoptocytic effect[45]. Previous research has shown that prolonged exposure to morphine causes apoptosis cell death by activating mechanisms such as oxidative stress, inflammatory and endoplasmic reticulum (ER) stress in the dorsal horn regions of the spinal cord, which are critical to opioid analgesia [46,47]. Our findings found that morphine tolerance in DRG increased caspase-1, caspase-3, and caspase-9 levels. It was in line with earlier research in the literature[18,48,49]. Even though a single dose of morphine raised levels of inflammation and TOS, it did not change the caspase-3 and caspase-9 but caused an increase in caspase-1 levels, which are pyroptosis markers. This result may be that a single dose of morphine has a threshold value in the apoptosis process. On the other hand, using thiamine in combination with morphine tolerance reduced caspase-1, caspase-3, and caspase-9 levels in DRG. In the clinical management of pain, it can be concluded that thiamine has a therapeutic effect by maintaining a significant delay in the development of tolerance to morphine analgesia, suppression of inflammation, and apoptosis.

Conclusion

As a result, the data presented in this study suggest that thiamine is a useful adjuvant in the treatment of long-term opioids. For this medication to be utilized in conjunction with opioid drugs, more research is required.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Individual Impact of COVID-19 Pandemic in a State of Uncertainty on Trust Basis

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ABSTRACT

The COVID-19 pandemic is a rapidly spreading virus outbreak affecting people's lives globally in terms of health, economy, social and psychological aspects. Governments have taken various measures to protect the functioning of societal life and individual health. To prevent the spread of the virus in society, measures such as lockdowns, travel restrictions, a ban on gatherings in enclosed spaces, closure of certain businesses, school closures, and transition to remote learning were taken. In addition, on an individual level, measures such as wearing masks, practicing personal hygiene, and maintaining physical distance were taken. The uncertainty of the situation has weakened people's sense of security. Health concerns, job losses, social isolation, financial turmoil, and low morale have increased people's worries about the future. The long-term effects of the pandemic are still not fully understood, but it is known that the sense of security plays a significant role in both the quality and level of life of society and individuals. The sense of security of individuals is important in every aspect of life, and the loss of this feeling can negatively impact their economic, physical, emotional, and social health. This study, conducted using a literature review method, aimed to identify the psycho-social and economic problems arising during the pandemic process. The results of the study indicated that the pandemic experienced globally caused serious problems for both individuals and society and that these problems are likely to have impacts on the "new normal" life after the pandemic.

Bilinmezlik Durumu Olarak COVID-19 Pandemisinin Güven Temelinde Birey Yaşamına Etkisi

Süreç

Geliş: 07/01/2023
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ÖZ

COVID-19 pandemisi, tüm dünyada hızla yayılan bir virüs salgını olup insanların hayatını sağlık, ekonomik, sosyal ve psikolojik yönden etkiledi. Devletler, toplumsal yaşamın işleyişini ve birey sağlığını korumak amacıyla çeşitli önlemler aldılar. Virüsün hayatın her alanına yayılmasını önlemek için toplumsal planda sokağa çıkma yasağı, seyahat kısıtlamaları, kapalı alanlarda toplanma yasağı, bazı sektörlerde işyerlerinin kapatılması, okulların tatil edilmesi ve uzaktan eğitime geçiş gibi tedbirler alındı. Bunun yanı sıra bireysel planda, maske takma, kişisel bakım-hijyene özen gösterme ve fiziksel mesafe koyma gibi önlemler alındı. Bir bilinmezlik durumu olarak yaşanan süreç, insanların güven duygularını zayıflatmıştır. Sağlık endişeleri, iş kayıpları, sosyal izolasyon, finansal bozulmalar ve düşük moral gibi faktörler bireylerin gelecek kaygılarını artırmıştır. Pandemi sürecinin uzun dönem etkileri henüz tam olarak anlaşılmamakla birlikte, güven duygusunun hem toplumun hem de insanların yaşam kalitesi ve düzeyinde önemli bir rol oynadığı bilinmektedir. Bireylerin güven duyguları, yaşamının her alanında önemlidir ve bu duygu kaybı, insanların ekonomik, fiziksel, duygusal ve sosyal sağlığını negatif yönde etkileyebilir. Literatür taraması yöntemi ile yapılan bu çalışmada pandemi sürecinde ortaya çıkan psiko-sosyal ve ekonomik sorunların belirlenmesi amaçlanmıştır. Çalışma sonucunda küresel düzeyde yaşanan pandeminin hem birey hem de toplum yaşamında ciddi sorunlar oluşturduğu belirlenmiş ve bu sorunların "yeni normal" olarak isimlendirilen pandemi sonrası yaşamda da muhtemel yansımaları olacağı kanaatine varılmıştır.

Anahtar sözcükler: COVID-19, Belirsizlik, Güven, Kişilerarası İlişkiler, Ruh sağlığı

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Introduction

Outbreaks have occurred in some periods of human history and have caused the deaths of millions of people, leading to multi-faceted changes in individual and societal life. Outbreaks have caused negative impacts on individuals' cognitive, emotional, and behavioral structure and socioeconomic life. At the same time, they have contributed to the disruption of the administrative, cultural, and economic order of societal life [1].

The COVID-19 pandemic can be evaluated as both uncertainty and a trust crisis. During this process, significant trust losses are said to have been experienced among countries, companies, and individuals [2]. The restrictive nature of the measures taken to protect health during the pandemic has caused differentiations and changes in individuals' attitudes and behaviors, beliefs and values, philosophical thoughts and world views, and perceptions of freedom and security. These changes are shaping a way of life referred to as the "New Normal". The "New Normal" will be a period surrounded by an eroded sense of trust due to the pandemic, affecting the individual's outlook on themselves, other people they are in relationships and communication with, and the future.

The "New Normal" is defined as individual and societal life shaped by insecurity and uncertainty. How will these changes manifest in the "New Normal"? How will the erosion of trust and uncertainty be resolved in the "New Normal" period? To what extent will individuals be able to rebuild trust in their personal relationships, working lives, and social relationships? Can the limitations and disconnections caused by the insecurity and uncertainty experienced in international social, political, and economic relationships during the pandemic be overcome? How will "physical distance" be implemented in human relationships in the "New Normal"? What direction will the understanding and practices of hygiene evolve? To what extent will the risk perception of individuals and societies be elevated? What will be the role and importance of internet technology, which is the most widely used tool for communication and information sources, education and economic activities during the pandemic, in the "New Normal" era?

The ongoing COVID-19 pandemic, which has an unpredictable outcome and continues with changing variants, raises many questions. It is still being searched for answers to these questions as humanity has lived in fear and insecurity due to extraordinary circumstances for a long time. How will the pandemic affect the concept of freedom and the beliefs of humanity who live in insecurity due to security reasons? The problems caused by the pandemic have raised many questions that require answers.

Methods

In this study, a literature review was conducted using various academic databases and online resources to investigate the impact of the COVID-19 pandemic on individual life. The studies related to COVID-19 were evaluated in terms of their effects on individuals' bio-psychological and socioeconomic life. The majority of the studies on the subject in the literature were conducted during the pandemic period, and efforts were made to identify the problems that the pandemic has created in the lives of individuals and society. During the pandemic, which has continued for almost two years, the measures taken have had both positive and negative effects, leading to the emergence of a new way of life, referred to as "new normal" in the literature.

COVID-19 Pandemic

Pandemics are global outbreaks of infectious diseases that have a major impact on various aspects of life. Throughout history, pandemics such as the bubonic plague, cholera, typhus, HIV, and SARS have posed a dangerous threat to humanity. These outbreaks are global health issues that can alter how people live and lead to changes in societal structures and governance. Because of their rapid spread, unknown causes, lack of protection, treatments, and ability to cause direct death, pandemic diseases create a shock effect on people. When people face this danger, their first reaction is often to escape their environment and sever connections with others [3].

Pandemic diseases have always caused mass panic and anxiety for humanity. The first bubonic plague outbreak that started in China in 161-162 AD resulted in the death of one-third of those infected in the Mediterranean region. In the 20th century, about 300 million people died from the disease. The bubonic plague originating from China and Central Asia caused the death of approximately one-third of Europe's population in 1347. The Black Death that occurred in Italy in the 1300s resulted in the death of 75-125 million people. Cholera spread in Japan in 1817, in Moscow in 1826, and in Berlin, Paris, and London in 1831. The Spanish Flu infected approximately half a billion people between 1918 and 1920 and caused the death of 17-50 million people. HIV appeared in 1960 and caused 940,000 deaths and 1.8 million new HIV infections by 2017. SARS in 2003, Influenza A H5N1 (bird flu) in 2006, Influenza A H1N1 (swine flu) in 2009, MERS in 2012, Influenza A H7N9 in 2013, Ebola in 2014, and Zika in 2015 caused numerous deaths [1].

The COVID-19 pandemic, which is a rapidly spreading infection disease, has affected all societies and caused chaos, as have previous outbreaks.

China reported to the World Health Organization (WHO) on December 31, 2019, about an unknown respiratory illness in Wuhan. The WHO announced on January 7, 2020, that the cause of the disease was a new coronavirus and named it 2019-nCoV. Scientists in China identified the virus on January 9, 2020 [4].

The WHO declared the situation as a pandemic on March 11, 2020 [5]. As the outbreak quickly spread around the world, the WHO provided preventive measures and recommendations. Individual measures can be summarized as wearing masks, following hygiene rules, and maintaining a physical distance. Different types of vaccines were also produced to prevent their spread. The COVID-19 outbreak also created a global "crisis" and was referred to as a "health crisis" due to its dangerous results for human health and its causes of global fear and panic [6].

A crisis is defined as an event that emerges quickly and unexpectedly, causing fear and panic and is difficult, risky, and dangerous [7]. The spread of a virus across the world has created an environment that is referred to as the "new normal." To protect against the virus, measures and regulations have been taken, and the environment has changed, so people continue to lead their daily lives while also having to comply with decisions made by public authorities. At the individual level, the most serious issue was the fear and uncertainty caused by the unknown. As a result, people evaluated the outbreak based on their beliefs, perceptions, and understandings and displayed their own attitudes and behaviors as a result of these evaluations.

The public authorities in each country took measures based on their own resources to prevent the spread of the virus that had spread worldwide and caused the death of many people. The positive aspects of the measures taken for the protection of health during the pandemic period have also had negative results. Agamben[8] evaluates the resulting situation as the sacrifice of beliefs, normal life, social relationships, work, emotional closeness, and religious and political convictions to the risk of illness.

Before the pandemic, some countries did not take measures due to the belief that herd immunity would develop, but when it became clear that this approach was not effective, the necessity of taking some measures emerged. The measures taken had both positive and negative impacts on individual and social life. Individual measures include wearing masks, maintaining physical distance, and paying attention to hygiene. Social measures cover a wide range of activities, from education, travel, and work-life to entertainment life. The main social measures are curfew, travel restrictions and reduction of face-to-face education to remote learning, suspension or restriction of activities in businesses such as shopping centers, restaurants, and barber shops, limited use of public transportation, restrictions on intercity and international travel, closure of certain workplaces in certain sectors, remote work, certain workers with certain age or chronic illnesses being granted leave, closure of places of worship, ban on mass events, a ban on artistic, sports and cultural activities, etc. [1,9].

The procedures of each country were seen to have similar characteristics during the process. Although these measures were gradually relaxed, the pandemic changed people's regular lifestyles, thoughts, communication styles, and practices in a sudden and profound way and caused them to reorganize their perception of time and space. When the COVID-19 pandemic emerged, health protection measures became a priority. Problems arose in terms of sources that people could trust in an uncertain and unknown environment. During the COVID-19 outbreak, social media usage increased, and manipulative and false information and conspiracy theories on these platforms caused information pollution and increased people's anxiety. It can be said that incorrect information provided by social media during crisis periods can lead to emotional reactions such as stress, depression, anxiety, hopelessness, insomnia, anger, and others [10].

During the process, false information about COVID-19 led to some people not following necessary measures or not getting vaccinated. A series of negative opinions was expressed about the vaccines developed to prevent the spread, which is still being discussed. A study by Petersen et al. [11] found that providing accurate and transparent information about the negative effects of vaccines increased confidence in health authorities and decreased the likelihood of getting vaccinated, while unclear and suspicious communication increased vaccine acceptance and reduced confidence. A relationship has been found between mistrust of official information and guidance provided by institutions and the preference for alternative medical treatments [12]. Generally, belief in conspiracy theories and individuals having false information has also triggered belief in conspiracy theories during the COVID-19 process.

The accuracy of information provided by institutions can sometimes be questionable, and this can increase distrust. It can be said that distrust of institutions can affect relationships among individuals and groups and lead to negative outcomes [13]. Research has shown that trust issues related to institutions can lead to the acceptance of false information, and individuals perceive themselves to be at risk in terms of economics and healt[14]. As uncertainty in information increases, people tend to accept information and narratives from sources they trust as being true.

Transparency is required for society to receive reliable information during crisis periods. Institutions must

provide clear information about preventive and supportive measures taken to prevent harmful behavior. A positive correlation has been found between the reliability of information and trust in institutions and measures taken [14]. The measures should aim to reduce uncertainty and decrease the effects of socioeconomic shocks [15].

During the Covid-19 pandemic, each country's government informed the public through various communication tools, and also regulated social interactions through various organizations [13]. The Ministry of Health in Turkey established a Scientific Board to overcome uncertainty, panic and anxiety, manage the crisis and ensure confidence. This board implemented numerous health measures, such as emergency measures to control and prevent the spread of the epidemic, and treatment of infected individuals. The ministry also informed the public about the implementation of Covid-19 screening tests and ways to protect from the virus, among other activities. The Ministry transparently communicated daily numbers of cases, deaths, and recoveries, vaccination efforts, and hospital occupancy rates to the public and gained public trust. The Ministry also played a coordinating role during the crisis and assisted institutions and organizations with their necessary work.

Initially, the media was a platform where information was spread uncontrollably and caused confusion, but over time, it became an environment where real data released by official institutions was followed. During this period where individual and social life was sustained with a number of restrictions, institutions focused on the bio-physiological aspect of the outbreak and carried out their precautions and information accordingly. However, it can be said that there was not enough information and guidance for the psycho-social aspect of the problem, which was equally important, and that adequate measures were not taken to protect health.

Trust

Like many concepts, there is no agreement on the definition of trust. Trust is an important emotion that forms the basis of human relationships and communication and enables individuals to protect and sustain their existence. As humans are social beings, they have a requirement for constant communication with others. At this point, the level of trust between individuals is a determining factor in social life that directly affects it, and is widely studied as an important variable in social sciences. The level of trust felt or experienced between individuals provides direction for evaluating and maintaining situations to be shared in a secure and manageable manner. Trust is not just an emotional and cognitive situation [16], it is a structure that interacts with the perception and belief that an individual has about the expected situation in human relationships [17].

Trust has a dynamic structure and differentiating features based on socioeconomic relationships and interactions between people [18]. Trust, which forms the foundation of relationships and cooperation between individuals and groups, ensures the continuation of living and cooperation together [19]. Communication and interaction between people changes, causing the trust to change as well, so constant trust cannot be referred to. Trust has an important role in the regular maintenance and determination of social life. Individuals are part of structures with different characteristics such as family, neighborhood, workplace, cultural, artistic, political and economic groups, and trust determines these relationships.

The formation and sustainability of social life require the emergence and institutionalization of various organizations. Individuals have relationships not only with other individuals, but also with institutions and structures. Trust plays a crucial role in the functioning, development, and relationships between institutions and structures and between these entities and individuals.

An individual's sense of trust is influenced by their familiarity, closeness, and sense of belonging with the group they are in. This takes the form of partial trust for family and acquaintances, and social trust for strangers. Partial trust refers to a high level of trust between individuals, while social trust is characterized by a cautious approach to strangers and is considered a low level of trust [20]. Social trust refers to mutual trust among people living and interacting in a society, even if they do not know each other [21].

Social trust serves not only as a source for protecting and maintaining institutions and processes within society, but also facilitates socialization of individuals [22]. Trust can be classified into three categories: trust in institutions and structures, trust in close family members and friends, and generalized trust towards strangers. Some approaches that include trust in social capital studies view it as a result of social capital, while others see it as a cause. When associating trust with social capital, it highlights the importance of trust towards strangers rather than trust in family and friends [23].

Social capital, which is considered one of the key variables affecting a society's socioeconomic and political success and is considered more important and prioritized than human and physical capital, covers trust. Trust has a positive relationship with social development and reflects indicators such as fair income distribution, human development, cooperation, and educational success. Trust increases or decreases cooperation among individuals. Trust encourages

cooperation and partnership in solving collective problems in social life and makes it easier for the individual to participate in social and political life [24]. Trust forms the foundation of relationships and communication within society and supports the formation and socialization of the individual's social identity. As trust increases, transaction costs decrease, cooperation and social integration become stronger [25] according to Bozkurt.

Studies have shown a negative correlation between trust and crime rates and corruption, and an increase in distrust leads to an increase in social risk [26]. Putnam [27] describes distrust as a pathology and says it affects every aspect of individual and social life. In societies with increased distrust, there is a rise in isolation, alienation, and illegal organization among individuals. Trust forms the foundation of almost every implementation in social life. The gaps and deficiencies that arise from the fact that the entire daily operations cannot be regulated by legal regulations and institutional practices are resolved through trust-based relationships. At this point, it can be said that trust both shapes the legal structure and has a positive impact on the complex operations of daily life [28].

Fukuyama [26] categorizes societies as having high and low trust and emphasizes the impact of trust between individuals on the economic development of society. Lack of trust is considered a cost-increasing factor that negatively affects a society's economic activities, while secure societies do not incur this cost. Therefore, trust has a dynamic feature that guides a society's socioeconomic development, social integration, stability, and growth [29]. High-trust societies prefer tolerance, compromise, and finding common solutions in resolving conflicts and are considered to be societies with developed capacities [30]. As a result, cooperation and solidarity between individuals in these societies are realized at a higher level.

Trust is evaluated as desired expectations regarding the actions and intentions of others [31] and is a factor that determines an individual's attitude towards risk and is established through mutual dependency [32]. Individuals make decisions based on the social trust of authorities managing the risks when they are lacking information about the risks of danger and determine their perception of risk and benefit [33]. Trust is a crucial component in decision-making based on information [34]. Trust is a necessary component in social relationships that potentially require taking risks [35]. In studies on risk perception and acceptance of danger, trust is considered an explanatory variable [36]. The relationship between trust and risk perception is studied by risk management researchers and it has been found that individuals' risk perceptions show differences based on cultural context and risk factors and there is a strong connection between perceived risk probability and trust [37,38].

Natural disasters and pandemics threaten the basic desires of people to protect and continue their existence, causing disruption of the sense of trust. The disrupted sense of trust affects an individual's cognitive strategies, decision-making and behaviors [39–41]. Additionally, all outbreaks have generally had negative impacts on health, national security, the economy, and consumption in [42]. The COVID-19 outbreak has created an atmosphere of uncertainty and insecurity. The measures taken to manage the "health crisis" caused by the outbreak turned into a crisis that disrupted the social and economic order of society. The pandemic and the measures taken have caused fear and panic in all countries, as well as imposed changes in leisure activities, threatened habits, disrupted plans, changed lifestyles, and fundamentally changed established relationships and communication [43].

Due to its multi-dimensional uncertainty, the fear of getting sick and unintentionally spreading the disease to others has eroded mutual trust among individuals and paved the way for new endeavors. The most frequently used resource during the process was communication technology. Communication technology facilitates communication between people but can also contribute to an increase in insecurity due to its virtual and complex nature [16]. The widespread use of internet technology during the pandemic also revealed the reality that digital society is both a risk society and a surveillance society [44].

The report of the Police Academy highlights many aspects of the new normal that will emerge after the COVID-19 pandemic. One of them is the changing of individual behaviors. The report indicates that long-term staying at home creates tension in social relationships, causing harm to people's mental and emotional abilities and socioeconomic lives. At the same time, it draws attention to the fact that limited communication through the internet and phone due to physical isolation, spending countless hours aimlessly on social media, and the possibility of people believing in conspiracy theories, can increase various addictions and interpersonal violence, potentially leading to the deterioration of social relationships in the long term [45].

Intolerance Of Uncertainty

It can be said that intolerance of uncertainty, one of the concepts that have been widely studied in social sciences, like trust, lacks agreement in its definition, as many concepts in the field. Intolerance of uncertainty refers to the state of discomfort and unease experienced by individuals in uncertain situations. It is defined as a tendency to believe in a negative outcome of an event and an inability to accept the event without considering the probability of it occurring [46,47].

Individuals with low tolerance of uncertainty perceive uncertain situations in their environment as dangerous [48] and tend to interpret these situations as threats [49,50]. Carleton [51]states that the source of intolerance of uncertainty and fear of the unknown is negative belief and lack of information, resulting from an individual's susceptibility.

The relationship between uncertainty and negative beliefs has also been expressed by Dugas et al. [52]. The relationship between uncertainty tolerance and conditions such as anxiety and general anxiety disorder has been studied [53], and it has been seen that individuals with low tolerance for uncertainty perceive uncertainty more, experience excessive physical arousal when a threat arises [54], and exhibit cognitive, emotional, and behavioral responses. Individual anxiety caused by uncertainty at the individual level complicates decision making and behavior. An increase in uncertainty levels in social life also reduces the rational behavior skills of individuals [55] and results in more reflexive behavior aimed at survival. On the other hand, a low level of social anxiety can lead to the outbreak of the epidemic, which in turn increases anxiety levels. A study on the H1N1 epidemic showed that individuals who are unsuccessful in managing uncertainty carry more anxiety [56].

Pandemic situations increase uncertainty and can lead to heightened risk perception and fear, which in turn affects behavior etkilenir [57]. The COVID-19 pandemic, with its uncertain duration and outcome, can also be characterized as a pandemic of anxiety and fear. The uncertainty of when it will end and the possibility of recurrence contributes to a sense of helplessness, inadequacy, pessimism, entrapment, worry about the future and hopelessness among individuals.

During the COVID-19 pandemic, people attempted to gain accurate information about the pandemic and its outcomes in order to control their emotional states such as fear and anxiety, and to protect their health. Individuals who struggled with uncertainty increased their internet searches and paid close attention to social distancing [58]. As a result, there was a transition period in accepting the reality of COVID-19 and complying with institutional measures.

Impact Of The COVID-19 Pandemic On Individual Life

Global outbreaks have negatively impacted the economic, social, cultural, and political structures of societies and caused changes. The COVID-19 pandemic also affected the world's economies and the fundamental dynamics of community life, disrupted face-to-face communication, relationships and cooperation, and made it necessary to change established habits with new ones.

The COVID-19 crisis must be evaluated in terms of its impacts on individuals, families, communities, and the workforce, and its resulting uncertainty and dimensions of vulnerability.

Impact on Psychological Structure

The COVID-19 pandemic has caused people to experience psychological isolation during the period of continued social isolation restrictions. In this situation, people feel inadequate, trapped feelings, helplessness, inadequacy, and uncertainty, while experts also expressed concerns about an increase in panic, depression, and paranoia [59]. As a result of people becoming intolerant in the face of insecurity and uncertainty, psychological and emotional traumas have arisen.

Trauma is caused by a catastrophic reaction that results from a threat to a person's life or physical integrity. Individuals can cope with ordinary negative experiences, but in traumatic events, these skills are ineffective. The emotional field is restricted, angry outbursts occur and difficulty in concentration is experienced. The memory recordings of the individual change after trauma and the normal flow of life is disrupted. Goals become meaningless and the future becomes uncertain [60]. The COVID-19 crisis has rapidly turned into an existential crisis for humanity and society. Bozkurt explains that in times of crisis, people's existential anxieties increase and their calm/analytical thinking skills deteriorate [25].

During pandemics, the first emotions that commonly arise among people are intense anxiety and fear [45]. This can lead to changes in attitudes and behaviors. For example, the increased fear of death and widespread panic caused by the outbreak can affect people's self-esteem and lead them to turn more to cultural values and belief systems in order to repair it [25,45,60], or cause the adoption of negative beliefs and inflexible attitudes [61]. It was expected that mild to moderate fear and concern would arise due to the pandemic, but it has been seen that it has gone beyond that for many people.

Early findings [62] indicate that the COVID-19 pandemic and related restriction measures have negatively impacted mental well-being. The uncertainty experienced has directly impacted people's perception and behavior. Because intolerance to uncertainty leads to an inability to handle negative uncertain situations, avoidance, and a tendency to exaggerate threats and develop psychopathological symptoms [63].The COVID-19 pandemic has resulted in prolonged staying at home, limited social interaction, leading to an increase in anger, tension, communication problems, and family conflicts, exacerbation of previous mental health issues, and an increase in anxiety among those with anxious personality structures. People are facing

significant stressors such as the threat of death, fear of infecting loved ones, loss of living standards, inability to meet basic needs, and financial responsibilities, which lead to feelings of helplessness and hopelessness [45].

Many people felt trapped at home by the virus and considered it as the restriction of their freedom. According to Bozkurt, the feeling of being restricted can create anger and violence. In Bozkurt's study, 52% of the participants felt trapped, and 32% reported becoming more irritable and easily frustrated [25].

The fear of death became more prevalent and people began to live with the fear of death, leading to significant transformations in consumption and spending practices with some restrictions imposed by governments. Although everyone has different tolerances to the uncertainty in life, fear and uncertainty can make people feel stressed, anxious, and vulnerable [64].

According to Bozkurt's research, 65% of the participants reported an increase in restlessness, 52% reported a decline in sleep quality, about one-third felt they had lost control of their life, 42% reported difficulties in their daily activities, 41% reported constant fatigue/exhaustion, 41% were afraid of getting infected, 30% felt helpless, and 27% felt lonely [25]. Studies have shown that demographic variables have weaker explanatory power over fear responses compared to general avoidance of risk and intolerance to uncertainty [65]. Uncertainty and the meaning of life are significant contributors to depression and anxiety [66–68]. The intolerance to uncertainty plays a key role in elevating anxiety and its persistence [69]. The increased fear and terror of death caused by the pandemic can lead to people becoming more attached to their cultural values and belief systems. Stimuli related to death (funeral cars, cemeteries, etc.) can cause people to display traditional and conservative thoughts and behaviors, but can also lead to negative attitudes towards individuals from different cultures açabilir [7,25].

The COVID-19 pandemic has caused numerous negative impacts not only on physical death but also on psychological well-being. Social isolation and loneliness are related to both the objective experiences of being alone and subjective feelings of loneliness, and can generate fear, anxiety, worry, hopelessness, and pessimism. Anxiety sensitivity and intolerance to uncertainty may increase along with social isolation and loneliness, and this can contribute to the increase in suicidal thoughts during the pandemic [70,71]. Nowadays, it is a reality that digital technology is rapidly advancing and permeating almost every aspect of human life. During the pandemic, the knowledge that every online activity can be monitored, can undermine privacy feelings and trigger feelings of being watched and controlled, resulting in the development of fear and anxiety, and even paranoid-schizoid personalities [60].

The pandemic has made it necessary for us to question important issues such as freedom, physical comfort and anxiety about the future. If the individual's philosophy of life does not change, social alienation, loneliness, increase in fear, increase in anxiety, and increase in hostile feelings could lead to the breakdown of social peace due to alienation from oneself and others for a few years [59]. Studies have shown that individuals with existing mental health problems during the Covid-19 quarantine conditions had their problems exacerbated and faced greater difficulties in adjusting after the lifting of the quarantine, leading to a negative impact on their psychological well-being [72]. Along with these negative outcomes, psychological vulnerability has also triggered community-oriented behaviors. At this point, the importance of social capital in society is highlighted. It can be said that a strong social capital has a positive effect on psychological well-being and has both positive effects on community-oriented behavior and individual resilience.

The Impact on Bio-Physiological Structure

The COVID-19 pandemic has not only created various new illnesses but also directly led to death. As a result of the virus spread, pre-existing chronic illnesses have worsened, and a multi-faceted treatment necessity has arisen. COVID-19 is a virus that causes respiratory infections and directly affects human respiratory systems, making breathing difficult. It also weakens the immune system, increases stress levels, disrupts sleep quality, affects the brain and nervous system, causing headaches and fatigue, increases the risk of heart disease by affecting the cardiovascular system, and slows down body functions due to reduced physical activity.

On the other hand, the continued security measures, especially the curfew, have a negative effect on individuals' movements [73]. The decrease in resistance to infection due to the lack of mobility caused by isolation has resulted in cardiovascular and musculoskeletal disorders. The risk of respiratory system disorders has arisen due to the constant use of masks [74]. The use of various disinfectants for personal hygiene has caused skin disorders. These effects vary depending on the length, intensity, and personal compliance with COVID-19 measures, and can differ from person to person.

Impact on Behaviors

The global outbreak has negatively impacted people's psychological and physiological structures. Aktay [75] and other researchers [71] state that it is clearly visible that the outbreak has brought new forms of relationships into our lives and revealed consciousness levels and behavioral patterns. As a result of the isolation process, which is an inseparable part of the measures taken against the COVID-19 pandemic, many psychological

and medical problems have arisen [60]. During this period, it can be said that individuals with high levels of intolerance to uncertainty and anxiety struggle with accepting the security measures taken [76].

Due to the uncertainty and insecurity, people's emotions, thoughts, beliefs, attitudes and habits, as well as their usual behavior have been affected by this process. Isolation, closure of workplaces and schools, restrictions on going out and traveling have confined people to their homes. The compulsory conditions of the applied measures have directed people's behavior and created dependence and abnormality in some aspects. During the pandemic, negative and disruptive reactions were seen in people's behavior, such as overstocking, acting without considering others, attacking healthcare workers, escaping quarantine, etc. [7,45].

Miller states that quarantine can lead to a loss of boundaries in people's behaviors, for example, some individuals may engage in activities they wouldn't normally do, such as drinking in the middle of the day due to changing social norms in the background [45,71]. These behaviors may become a difficult-to-break habit during social isolation and can potentially lead to health problems. Research shows that staying in limited areas with a heightened perception of disease risk for a long time during quarantine can result in increased sleep and eating disorders.

The mediating effect of intolerance of uncertainty on compulsive buying behavior has been evaluated and it has been proven that intolerance of uncertainty has a partial mediating role [77]. The prioritization of hygiene and cleanliness issues after the COVID-19 pandemic is a positive gain, and it can offer an opportunity for individuals with obsessive-compulsive disorder to rationalize their behavior, but it can also carry the risk of developing obsessive-compulsive behaviors [44]. The pandemic can cause specific psychologic stress-related suicide thoughts and deaths, while physical distancing measures and other consequences can generally pose a risk [70,71].

The closure of workplaces due to measures to prevent the spread of the virus has caused many people to face economic difficulties, leading to a transition from an active social life to a passive individual loneliness, causing depression in some young people, causing some to resort to illegal ways to cope with and forget their problems. Afterwards, some of these young people were found to have used drugs and alcohol excessively, and some even committed suicide. In individuals with or without existing psychiatric disorders, factors such as loneliness and financial stress can cause more severe symptoms, and behavioral problems such as family violence, smoking, alcohol, drug and gambling addiction may increase [60,78].

During the COVID-19 pandemic, intolerance of uncertainty and internet addiction increased, especially among adolescents, causing an increase in internet usage [79]. Individuals struggling with increased stress and depleted psychological resources during the process were caused to resort to cyber idleness as a coping mechanism [80]. Addiction such as shopping addiction, food addiction may develop among individuals who are prone to use and become addicted to internet and social media technologies due to limited access impulse and hygiene concern [7,81].

Impact on Private Life

The COVID-19 pandemic affected family life in various ways. People were forced to spend more time at home and this increased communication and interaction among family members. At the same time, the need for work and education at home increased due to the closure of schools and workplaces. The increased stress and strain of working parents at home due to the need for time management and cooperation has increased. The loss of jobs and economic concerns during the process have also increased stress and tension. The closure of schools and educational programs has increased the worries of parents about their children's future as it hinders their access to education.

The long-term stay at home, the need for limited behavior, financial stress and other factors have led to feelings of loneliness, an increase in anger, tension, communication problems, and family conflicts, a flare-up of mental health issues and an increase in anxiety for those with anxious personality structures [60,78,82]. During the process, preschool children showed more sleep difficulties, outbursts of anger, and dependencies, while adolescents mainly showed reactions related to COVID-19 worries and uncertainty [83]. Due to the obligation of staying at home, the decrease in opportunities for social activities [84], the restriction of communication with family and friends, and the disruption of intergenerational solidarity have resulted.

The COVID-19 pandemic has reduced the frequency of face-to-face communication between elderly parents and adult children while increasing the frequency of phone and video calls [85]. There has been a decrease in financial assistance provided by adult children to elderly parents and a decrease in daily assistance (cleaning, cooking, shopping, etc.) provided by elderly parents to adult children [86]. Additionally, the number of elderly parents caring for their grandchildren has also decreased [87]. The COVID-19 pandemic has caused a decrease in the marital satisfaction of mothers, an increase in conflicts with their spouses, difficulties in emotional regulation, and an increase in depression, anxiety, and stress levels [88,89]. According to Bozkurt, communication problems among family members have

increased due to over-crowding and decreased income [25].

A research conducted has found that 29.7% of participants experienced problems with their close partners during the pandemic, 16.83% reported that the issue of domestic violence has worsened since the start of the pandemic, and some resorted to using drugs or alcohol to cope with relationship problems edilmiştir [90].In Turkey, it has been seen that women use positive strategies more than men, and the levels of using emotional and behavioral positive strategies are higher [91]. According to a study by Bozkurt, 54% of participants during the quarantine period reported that their family bonds were strengthened, 17% reported that the family communication problems increased. It is noted that the general increase in trust in people and the abundance of friends that can be talked to, even if it is over the phone or internet (i.e. the abundance of social capital), helps to strengthen family bonds [25,92].

The Socioeconomic Impact of Life

Pandemics, despite having different geographic impact areas and causes, create lasting, widespread and similar effects in every aspect of social life. The pandemic has brought the concepts of isolation and social/health distance to the forefront in social life in the short and medium term getirmiştir [7]. Social isolation, physical distance, and monotonous activities imposed can negatively affect social relationships [45].Dangerous situations can cause individuals to experience fear and panic, there can be an increase in violence tendencies among trapped individuals and crime rates can increase. The COVID-19 pandemic is described as a threat that has globally ended social life by confining people to their homes and isolating the individual from everything else [85]. Before the COVID pandemic, people were pursuing a routine life, but after the pandemic, that routine life was disrupted and life practices changed. These changing behaviors can eventually become the norm and negatively affect the society's moral values [93].

Humans are naturally part of social structure and have a need for attachment. People who can't fulfill the need for attachment and belongingness cannot feel safe [59]. Interpersonal social interaction, an important component of which is trust and distrust, is also related to brain function [94,95]. The COVID-19 pandemic has also caused a weakening of the sense of trust needed for people to maintain social relationships and make connections.

The Effect on Human Relationships in Social Life

At the beginning of every social relationship (marriage, friendship, neighborhood, business partnership, worker-employer, etc.), there is an approach towards trusting. At the beginning of communication and relationships, a trust-based "psychological contract" is made between the parties. If the established communication and relationship affects the individual psychologically and physiologically, the trust feeling is damaged and the individual's decisions and actions are negatively affected.

Social relationships are very important for people's mental, emotional, and physical health. Reliable social connections both strengthen the physical immune system and increase mental and emotional resilience [45].

Trust between individuals is one of the necessary social resources used in cooperation and problem solving [96]. Trust is the determinant in the formation, regulation, and continuation of human relationships [26]. Therefore, if the feeling of trust weakens, it will be difficult for an individual to cooperate and show solidarity with someone they do not know. Safe, satisfying communication and interaction between individuals spreads the feeling of trust in social life and creates a common ground of agreement [97]. Strong social bonds not only reduce fear and anxiety, but also help people cope with psycho-social problems more easily during crisis periods. Trust creates trust and loneliness is as frightening a concept as death in the depths of the human mind [25].

Trust plays a critical role in many aspects of social life [98] and an individual's level of trust influences their social relationships. High levels of trust positively affect socioeconomic relationships and cooperation, while low levels of trust negatively impact these relationships. The breakdown of relationships due to distrust can have negative effects on mental health. A study [99] described psychosis as a social interaction problem and highlighted that trust is an important factor for disrupted social interactions in psychosis [100].

Lack of trust can cause changes in hormonal levels in the human brain and individuals may face challenges such as decision-making difficulties, thought disorder, skeptical approach, and emotional disturbances. When faced with difficulties (economic, social, etc.), cooperation and assistance may break down and individuals may seek individual solutions. Relationships between people form a society and every factor that affects these relationships results in social, economic and political consequences. During the pandemic, different reactions, attitudes and ways of thinking emerged in the psychology of individuals and societies compared to before the pandemic.

The pandemic caused the suspension of traditional actions that express love and respect, which are dominant in cultures where contact and touch are prevalent. Especially in important family and close events (wedding, circumcision, funeral etc.), individuals were isolated and kept away from their closest ones (spouse, mother, father, child etc.). During the pandemic, people couldn't visit their sick relatives and

couldn't perform necessary rituals for the deceased. Behaviors that could cause contamination (such as not wearing a mask, violating hygiene rules, and participating in gatherings) became dangerous due to the fear of catching the virus from others or transmitting it to others. This situation can lead to deterioration of relationships between people, increase in insecurity and anxiety. Prolonged stay at home, weakening of social relationships, limited physical interaction and increased use of internet/phone can cause deterioration of cognitive and emotional abilities. Health measures that restrict social mobility and contact during the COVID-19 period have seriously impacted not only human health but also all aspects of economic and social life. These impacts have caused a slowdown, suspension, and contraction of economic and social activities.

Impact on Economic Activities and Work Life

It is often stated that nothing will be the same after the coronavirus pandemic. It is expected that significant changes will occur in the economic field. The uncertain environment created by the coronavirus pandemic not only caused a loss of confidence in financial markets, but also created a trust issue in every area of life. Confidence has decreased between countries and global cooperation has been replaced by countries trying to solve problems on their own. Loss of confidence weakens not only social life but also economic developments, and ultimately slows down or retards democratic development [1]. After the pandemic, the prices of assets were revalued. The prices of stocks and real assets initially decreased. This caused asset owners to reduce their consumption, considering that their wealth was declining and the possibility of being less wealthy in the future [2]. Countries affected by the crisis have introduced fiscal, monetary, and social policy measures to alleviate the economic and social effects of the pandemic. As the weight of the government in the economy increases with the coronavirus, it can be said that nationalist approaches will emerge instead of globalization [1].

Following the COVID-19 pandemic, there has been a decline in economic activities due to the necessity of observing social distance and the widespread digitalization. Research shows that the fear of COVID-19 is positively related to conformity and panic buying intentions [101]. Anxiety explains the relationship between these variables and panic buying behavior [102]. The unexpected emergence of an economic downturn caused great uncertainty for consumers and increased economic risk perceptions. Consumers' decreased demand for consumption and the direct impact of uncertainty caused economic data to deteriorate [103]. Thus, the COVID-19 pandemic was seen to directly affect and shape people's shopping habits and economic situation. The economic risk perception also reflected on interpersonal relationships. A study found a negative relationship between economic risk perception and social distance, but a positive relationship between health risk perception and social distance [14].

During the pandemic, people rushed to markets due to fears of uncertainty and scarcity. At the beginning of the pandemic, panic prevailed in some areas, and people tried to shop without respecting each other's rights. Physiological needs overshadowed other needs [7]. Studies have revealed that families with children and female consumers have a higher tendency to stock up during the pandemic. Due to limited access and hygiene concerns, consumers turned to online shopping. During this period, all kinds of shopping increased over the internet and continued intensively even after the pandemic. As a result of the COVID-19 pandemic, social life was restricted by the ban on going out in many countries. As a result, the closure of public spaces resulted in a large wound to the demand for goods, and a decrease was seen in national income accounts and world trade. The restriction of human mobility and the narrowing of activities in sectors, such as tourism, civil aviation, and transportation, led to the complete cessation of some services offered by small businesses and artists [6,7].

The closure of service businesses with high social interaction such as barbershops, hairdressers, restaurants, and cafes during the pandemic period caused consumers to find ways to meet these needs on their own. Manufacturing, trade, tourism, entertainment, and the arts sectors are among the sectors most affected by the economic crisis. Economic slowdown due to the global crisis caused employment to decline, many people lost their jobs in various sectors, and employment conditions worsened. The COVID-19 crisis has led to an increase in remote work systems and the implementation of flexible working systems in many sectors due to health measures. The COVID-19 pandemic period has caused people to consider both their health concerns and their worries about unemployment caused by the economic crisis. The mental health of young people has also been affected by the crisis. According to a study, 53% of young men and 60% of women have stated that they have uncertainties and fears about their careers, and 50% have reported experiencing anxiety disorders and depression [6].

A study conducted by Öztürk [74] highlighted that there could be significant impacts on every level in the economy outside of the healthcare sector, in both public and private sectors. In particular, topics such as remote work, alternating work, and remote education deeply affected working methods during the pandemic. Remote work, also known as teleworking, is a flexible working method where the entire or a part of the

worker's hours can be performed outside of the workplace, with flexibility in location and time. Communication and relationships are established through the use of computer, phone, and internet [6]. Bozkurt found that 56% of work-from-home/online workers saw a decrease in productivity and 14% saw an increase, leading to the possibility of offering more support for online work in case of a weakening of workplace attachment [25].

Remote work can also bring with it problems such as increased hiring and training costs for businesses, management and control issues, and health and safety concerns. Additionally, for workers, remote work may result in loss of job-based learning and advancement opportunities, conflicts between work and family roles, reduced loyalty to work and the company, social isolation, relatively low job security, and inadequate working conditions. Other drawbacks include an increase in the underground economy, tax losses, increased social expenses, regulatory and enforcement difficulties, and problems related to social order and health [6].

Impact on Education, Culture, and Arts Life

The Covid-19 pandemic has affected educational activities worldwide. Schools and universities were closed and education shifted to an online environment. As a result, the use of e-learning technologies increased and schools and universities reorganized their learning processes. Also, during the pandemic, access to educational activities was unevenly distributed, and students' online learning experiences were different. During the pandemic, issues and challenges in the education sector also arose. A study conducted by Berument and colleagues [89] found a significant decrease in academic interest and attachment to school among primary and secondary school students and an increase in aggressive behavior and non-compliance with rules. However, there was also a decrease in helping and supporting others.

According to some research results, the sectors that are most affected by the pandemic include the cinema-culture, event, and entertainment industry, tourism industry, transportation services, and hotel-accommodation services [6,7]. During the pandemic, cultural centers, theaters, cinemas, museums, concert halls, and other cultural and art venues were closed in many countries. Similarly, sporting events were also canceled or postponed. During the pandemic, the working conditions of artists, musicians, and other cultural and art sector workers became more difficult, and many projects or performances were canceled. The number of online events increased, and people tried to follow art and cultural events from their homes through the internet.

Conclusion/Suggestion

Uncertainty intolerance is a discomfort felt by an individual due to the lack of clarity or certainty in a situation they perceive or encounter. This can lead to an increase in the need for information about an uncertain outcome or future and result in feelings of anxiety, stress, concern, and fear, as well as negative reactions and behaviors due to the lack of information. Trust is a component that strengthens and solidifies relationships between individuals. It helps a person form a deep and strong connection in their relationships with others. Trust also increases mutual respect and tolerance among individuals. In a relationship with insecurity, people tend to feel more anxious, closed off, and skeptical. This negatively affects the quality and durability of the relationship.

The COVID-19 pandemic, which is considered a health crisis, has become a life crisis that encompasses all aspects of human life due to its inherent uncertainty and undermining of trust. Measures have been taken in the individual sphere to prevent the spread of the virus, such as using masks, maintaining physical distance, and paying attention to personal hygiene. In the social sphere, many businesses have been closed in various sectors, a curfew has been imposed at certain times, travel restrictions have been imposed, schools have been closed and switched to online education, and gatherings and demonstrations have been banned.

Like any pandemic, the Covid-19 pandemic has caused various and significant negative effects on all individuals and societies globally. It has been a source of multi-dimensional problems not only in people's bio-psychological structure but also in their socioeconomic lives. The rapid spread of the virus, the inability to find definite ways of transmission and protection for a long time, and the sudden deaths it causes have thrown people into the struggle to cope with anxiety, depression, loneliness, fear, stress, and losses, ultimately causing trauma.

The preventive measures imposed at the societal level due to the pandemic have restricted social relationships, such as spending time with neighbors, friends, and relatives, and have weakened communication and attachments. Social isolation has caused increased feelings of loneliness, anger, anxiety, sleep disturbances, eating disorders, domestic violence, and increased usage of substances among individuals who were forced to stay at home. The closure of workplaces and suspension of work has reduced the motivation and commitment of workers and increased unemployment and loss of income, causing economic-based anxieties to rise. The closure of schools and the shift of education to digital platforms have decreased the efficiency of education and teaching and have negatively impacted children's knowledge and skills.

However, it has been pointed out that many people have developed new values, believe in the need for more empathy, loyalty, and solidarity, and that it has caused them to re-evaluate their lives and needs. The picture caused by the Covid-19 pandemic has been referred to as "the new normal," and it is widely accepted and stated by most scientists that individual and social life after the pandemic will be much different from before. It can be said that new applications will be needed to overcome the negatives that occur in every aspect of individual and social life, as it is believed that the habits formed during the pandemic will be permanent.

Health authorities globally state that the virus continues with variants that are not as dangerous as in the first years of the pandemic. To reduce the negative impact of the pandemic on individuals and society in the "new normal," we can suggest some measures.

- Strengthening social support resources to enhance the weakened sense of security in individuals during the pandemic and establish trust in social relationships through planned cooperation, mutual aid, and solidarity activities,
- Implementing psychological support programs by public institutions to address emotional disruptions (fear, anxiety, concern, anger, etc.), sleep and eating disorders, excessive consumption and hoarding habits, and substance dependencies during the process,
- Implementing rehabilitation programs to prevent internet technology and social media addiction in children and youth,
- Organizing educational, cultural, artistic, and sports programs to develop socialization and social belongingness in children and youth and ensuring their participation
- Priority should be given to supporting and helping disadvantaged individuals who were negatively impacted both psychologically and economically during the pandemic,
- The necessary infrastructure and personnel should be determined, and necessary measures should be taken in the health sector,
- Consideration should be given to the fact that education and teaching activities are moving towards an online environment every day due to the advancing internet technology, and efforts should be made to strengthen the infrastructure and resources used,
- The negative aspects of remote and flexible work methods favored in many sectors of the working life should be identified, and developing preventive measures should be taken,
- Emphasis should be placed on encouraging and supporting cooperation and partnerships between individuals and organizations in economic activities by relevant public institutions.

This study, based on a literature review, can be considered to take the lead in field surveys related to the subject, taking into account its limitations.

This study does not require ethical committee approval.

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Social Phobia Prevalence among Academicians in Cumhuriyet University

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History

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ABSTRACT

Introduction and Aim: Social phobia; It is a public health problem that can lead to failure in working life, major depression and even suicide. The aim of this study was to investigate the prevalence of social phobia and related factors in academicians from Cumhuriyet University.
Materials and Methods: This cross-sectional study was performed in 2017. The universe of the study consists of 1605 academicians working in the health, science and social science fields of Cumhuriyet University. The sample size was determined as 216. A two-part questionnaire was applied to the participants. The first part consists of questions that investigating the sociodemographic characteristics and the variables that we think are related to social phobia. The second part consists of Liebowitz Social Anxiety Scale (LSAS).
Results: The frequency of social phobia was found to be 9.0%. While the prevalence of mild and significant social phobia was 3.6%, the prevalence of social phobia was 1.8%. More than half of the academicians show avoidance behavior in situations requiring performance. Conclusion: As a result, it has been found that social phobia has a significant frequency of 9.0%. This situation, which may adversely affect the professional performance and mental health of academicians, is important for public health.
Conclusions: As a result, it has been found that social phobia has a significant frequency of 9.0%. This situation, which may adversely affect the professional performance and mental health of academicians, is important for public health.

Keywords: Social Phobia, Academician, Cumhuriyet University

Cumhuriyet Üniversitesi'nde Görevli Akademisyenlerde Sosyal Fobi Düzeyi

Süreç

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ÖZ

Giriş ve Amaç: Sosyal fobi; kişinin yaşam kalitesini olumsuz yönde etkileyen bir hastalıktır. Çalışma hayatında başarısızlığa, majör depresyona ve hatta intihara neden olabilen bir halk sağlığı sorunudur. Bu çalışmanın amacı; Cumhuriyet Üniversitesi'nde görevli akademisyenlerde sosyal fobi sıklığı ve ilişkili faktörlerin araştırılmasıdır.
Materyal ve Metod: Bu kesitsel çalışma 2017 yılında gerçekleştirilmiştir. Araştırmanın evrenini, Cumhuriyet Üniversitesi merkez kampüsünde çalışan sağlık, fen ve sosyal bilim alanlarında görev yapan 1605 öğretim elemanı oluşturmaktadır. Örnek büyüklüğünün sayısı 216 olarak belirlenmiştir. Katılımcılara iki bölümden oluşan bir anket uygulanmıştır. Birinci bölüm sosyodemografik özellikleri ve sosyal fobi ile ilişkili olduğunu düşündüğümüz değişkenleri sorgulayan sorulardan oluşmaktadır. İkinci bölüm Liebowitz Sosyal Anksiyete Ölçeğinden (LSAÖ) oluşmaktadır.
Bulgular: Akademisyenlerde sosyal fobi sıklığı %9.0 olarak tespit edilmiştir. Hafif ve belirgin düzeyde sosyal fobi sıklığı %3.6 iken ciddi düzeyde sosyal fobi sıklığı %1.8 olarak tespit edilmiştir. Akademisyenlerin yarıdan fazlası performans gerektiren bazı durumlarda kaçınma davranışı göstermektedir.
Sonuç: Sonuç olarak akademisyenlerde sosyal fobinin %9.0 gibi önemli bir sıklıkta olduğu tespit edilmiştir. Akademisyenlerin yarıdan fazlası performans gerektiren bazı durumlarda kaçınma davranışı göstermektedir. Fobi düzeyinde olmasa da akademisyenlerin mesleki performanslarını ve ruh sağlıklarını olumsuz etkileyebilecek bu durum halk sağlığı açısından önem arz etmektedir.

Anahtar sözcükler: Sosyal Fobi, Akademisyen, Cumhuriyet Üniversitesi

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Introduction

In 1621, Robert Burton described the symptoms of anxiety attacks in socially anxious people in his book "The Anatomy of Melancholy"[1]. Anxiety disorders are the most prevalent psychiatric disorders. According to epidemiological surveys, one third of the population is affected by an anxiety disorder during their lifetime. There are several types of anxiety disorders, including generalized anxiety disorder, panic disorder, specific phobias, agoraphobia, social anxiety disorder and separation anxiety disorder[2].

Social anxiety disorder (SAD), also known as social phobia is classified in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Diagnostic criteria for social anxiety disorder according to DSM-5 are as follows.

a. Persistent fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others. The individual fears that he or she will act in a way (or show anxiety symptoms) that will be embarrassing and humiliating.

b. Exposure to the feared situation almost invariably provokes anxiety, which may take the form of a situationally bound or situationally predisposed Panic Attack.

c. The person recognizes that this fear is unreasonable or excessive.

d. The feared situations are avoided or else are endured with intense anxiety and distress.

e. The avoidance, anxious anticipation, or distress in the feared social or performance situation(s) interferes significantly with the person's normal routine, occupational (academic) functioning, or social activities or relationships, or there is marked distress about having the phobia.

f. The fear or avoidance is not due to direct physiological effects of a substance (e.g., drugs, medications) or a general medical condition not better accounted for by another mental disorder[3].

SAD; is a disease that affects one's quality of life negatively. It can cause failure in the working life, major depression and even suicide. For these reasons SAD is considered as public health problem. Preexisting SAD increases the risk for "early-onset" depression[4-6]. Stein et al. reported that SAD in the adolescent period is a strong risk factor for depressive illness in young adulthood. Depression and SAD combination in the adolescent period evidently increases the risk for subsequent depressive disorder[7].

Decline in various indicators of the quality of life, severely impaired everyday functioning not only in social, but also in educational and occupational aspects can be seen. SAD is associated with higher risks of suicide and of developing other psychopathology[8].

The aim of this study is to investigate the frequency of SAD and related factors in academicians at Cumhuriyet University.

Material and Methods

This cross-sectional study was conducted in 2017. The population of the study consists of 1605 academic staff working on the central campus of Cumhuriyet University. Number of the sample size was determined to be 216 (α=0,05, d=±0,035, p=0,10, q=0,90 t=1,96).

The academic staff included in the study were selected with stratified random sampling method. The number of academicians working in health sciences, natural sciences and social sciences were determined at Cumhuriyet University. Each field of science was stratified according to academic level. Academics were informed about the research. Questionnaire forms were given in closed envelopes.

The questionnaire consists of two parts. The first part contains of questions about sociodemographic characteristics and the variables we think are related to SAD. The second part consists of Liebowitz Social Anxiety Scale (LSAS)[9].

LSAS was developed by Liebowitz in order to evaluate the severity of anxiety and avoidance in social environments and situations requiring performance. The validity and reliability study of LSAS in our country was performed by Dilbaz and Güz and the internal consistency (Cronbach alpha) was found to be 0.96[10]. The scale is composed of 24 items divided into 2 subscales, 13 of them concerning performance anxiety, and 11 of them pertaining to social situations. The 24 items are rated on a Likert Scale from 1 to 4 first on anxiety felt during the situations, and then regarding the avoidance of the situations. The total score could be calculated by collecting the points obtained by the academicians from each section. 55-65 moderate SAD, 66-80 significant SAD, 81-95 severe SAD, ≥96 has been evaluated as very severe SAD. Since each question was 4 points in Turkish version, the total score was adapted according to the original version. SPSS (ver 23) program was used for statistical evaluation of the data. The data obtained were expressed with descriptive statistical criteria such as arithmetic mean, standard deviation, and percentage, and the Chi-square test was used for comparisons. The statistical significance level in the analyzes was accepted as $p<0.05$.

Ethical Aspect of Research

Cumhuriyet University Non-Interventional Clinical Research Ethics Committee approval (Ethics Committee Decision No: 2017-03/11) was obtained to conduct the study. Verbal consent was obtained from the individuals participating in the study after the explanation about the research was given.

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Results

Of the participants, 69 were female (41.1%) and 99 were male (58.9%). The mean age was 42.9±11.1years. 44 (26.7%) of them were professors, 24 (14.6%) were associate professors, 48 (29.1%) were assistant professors, 6 (3.5%) were prelectors and 43 (26.1%) were employed as research assistants. 132 (78.6%) of the participants were married. Fifty-two of the participants (32.7%) were employed in medicine, 30 (18.9%) in engineering and 32 (20.1%) in faculty of science (Table 1).

The prevalence of SAD was found to be 8.7 percent in females and 9.1 percent in males. The frequency of phobia was found to be 18.6 and most frequently under the age of 30 years (Table 1).

Table 1. Social phobia prevalence in academicians according to some socio-demographic characteristics.

	Normal		Social phobia	
	N	**%**	**n**	**%**
Gender; X^2=0.01, p>0.05				
Female	63	91.3	6	8.7
Male	90	90.9	9	9.1
Age groups; X^2=6.16 p>0.05				
<30	26	81.3	6	18.6
30-39	34	89.5	4	10.5
40-49	36	97.3	1	2.7
50-59	46	93.9	3	6.1
60>	11	91.7	1	8.3
Mean Age *(X±SD)*		42.9±11.1		
Marital status X^2=0.40, p>0.05				
Married	120	90.9	12	9.1
Bachelor	29	90.6	3	9.4
Divorced	4	100.0	-	-
Faculty X^2=16.80, p>0.05				
Medicine	44	84.6	8	15.4
Science	30	93.7	2	6.3
Engineering	29	96.7	1	3.3
Theology	16	94.1	1	5.9
Health Science	14	100.0	0	0.0
Literature	10	90.9	1	9.1
Dentistry	2	100.0	0	0.0
Junior Technical College	0	0.0	1	100.0
Academic Degree, X^2=11.08, p>0.05				
Professor	43	97.7	1	2.3
Associate Professor	23	95.8	1	4.2
Assistant Professor	45	93.8	3	6.3
Prelector	5	83.3	1	16.7
Research Assistant	35	81.4	8	18.6

While the frequency of SAD was the most common among the academicians belonging to the Faculty of Medicine, there was no person with SAD in the Faculty of Health Sciences. While the frequency of SAD was the most common research assistant, the lowest frequency was found in the professors and associate professors. Considering the place where they lived longest until 18 years of age, the frequency of SAD has been found to be 17.9% for the academicians who lived in the town and 6% for those who lived in the city center (Table 2). The frequency of phobia has been found to be 13.5% in the academicians diagnosed with chronic disease and 7.6% in those without the disease (Table 3). SAD was not detected in those who described their socioeconomic status as low in childhood (Table 2).

Table 2. Social phobia prevalence in academicians according to some familial and environmental characteristics.

	Normal		Social phobia	
	N	**%**	**N**	**%**
Living place until the age of 18, *X^2=4.57, p>0.05*				
Provincial center	110	94.0	7	6.0
District	23	82.1	5	17.9
Village	19	86.4	3	13.6
How many siblings you are, *X^2=1.99, p>0.05*				
1	3	100.0	0	0
2	37	86.0	6	14.0
3+	113	92.6	9	7.4
What is your birth order?, *X^2=1.473, p>0.05*				
1	54	88.5	7	11.5
2	44	91.7	4	8.3
3+	54	94.7	3	5.3
How do you evaluate your socioeconomic status in your childhood? *X^2=2.97, p>0.05*				
Good	41	93.2	3	6.8
Moderate	93	88.6	12	11.4
Poor	18	100.0	0	0.0
How do you describe the family environment in which you grew up? *X^2=3.11, p>0.05*				
Democratic / tolerant	89	92.7	7	7.3
Authoritative	49	92.5	4	7.5
Over protective	13	86.7	2	13.3
Indifferent parent	2	66.7	1	33.3
Have you ever been beaten by your parents in your childhood? *X^2=0.182, p>0.05*				
Yes	70	92.1	6	7.9
No	83	90.2	9	9.8
How often have you been beaten by your parents? *X^2=0.689, p>0.05*				
Rarely	65	92.9	5	7.1
Often	5	83.3	1	16.7
Have you ever been beaten by your teacher in elementary school? *x2=0.621, p>0.05*				
Yes	61	92.4	5	7.6

No	92	90.2	10	9.8
How often have you been beaten by your teacher? *X^2=0.262, p>0.05*				
Rarely	57	91.9	5	8.1
Often	2	100.0	0	0.0
Continuously	1	100.0	0	0.0
How many close friends did you have in childhood? *X^2=14.78, p<0.05*				
None	5	55.6	4	44.4
1	16	94.1	1	5.9
2 and more	132	93.0	10	7.0

Table 3. Social phobia prevalence in academicians according to lifestyle characteristics.

	Normal		Social phobia	
	N	**%**	**N**	**%**
Do you smoke cigarettes ? *X^2=0.128, p>0.05*				
Smoker	35	89.7	4	10.3
Non smoker	98	91.6	9	8.4
Quitter	19	90.5	2	9.5
The amount of cigarettes smoked? (pocket/year) *X^2=0.613, p>0.05* ***Do you smoke?***				
Less than 20	27	90.0	3	10.0
20-39	3	100.0	0	0.0
40+	5	83.3	1	16.7
Do you consume alcohol ? *X^2=0.821, p>0.05*				
Yes	49	90.7	5	9.3
No	100	91.7	9	8.3
How often do you consume alcohol? *X^2=3.93, p>0.05*				
Rarely	24	96.0	1	4.0
Often	20	90.9	2	9.1
Continuously	5	71.4	2	28.6
Do you participate in social activities with your family? *X^2=0.439, p>0.05*				
Yes	132	91.7	12	8.3
No	21	87.5	3	12.5
Do you participate in social activities with your friends? *X^2=0.277, p>0.05*				
Yes	139	91.4	13	8.6
No	14	87.5	2	12.5
How many close friends do you currently have? *X^2=5.50, p>0.05*				
None	8	72.7	3	27.3
1	5	83.3	1	16.7
2 and more	140	92.7	11	7.3
Do you exercise regularly? *X^2=0.00, p>0.05*				
Yes	40	90.9	4	9.1
No	113	91.1	11	8.9
Do you play musical instrument? *X^2=0.00, p>0.05*				
Yes	31	91.2	3	8.8
No	120.	9.9	12	9.1
What is the TV program you watch mostly? *X^2=34.96, p<0.05*				
Competition	4	50.0	4	50.0

program				
Marriage	0	0.0	1	100.0
Program				
Film	19	82.6	4	17.4
Series movie	24	100.0	0	0.0
Documentary	30	100.0	0	0.0
News	55	91.7	5	8.3
Sports	10	100.0	14	9.0
Do you have any chronic diseases ? *X^2=1.227, p>0.05*				
Yes	32	86.5	5	13.5
No	121	92.4	10	7.6
Do you have any diagnosed mental illness? *X^2=0.582, p>0.05*				
Yes	3	100.0	0	0.0
No	148	90.8	15	9.2
Do you have any mental illness in your family? *X^2=0.582, p>0.05*				
Yes	9	90.0	1	10.0
No	144	91.1	14	8.9

According to LSAS scoring the means of anxiety, avoidance and the total scores were detected as 38.64±10.82, 35.41± 9.01 and 74.18 ± 18.49 respectively.

When the LSAS scores were evaluated according to related factors the mean of anxiety was higher in women (39.1±11.2) while the mean of avoidance (35.8±9.3) was higher in men.

The mean anxiety score in the 30-year-old group was 40.7±12.5. The mean anxiety score was decreasing with increasing age. The mean score of avoidance under 30 years and over 60 years of age was higher than other groups. The mean score of anxiety (39.1±10.9) and avoidance score (35.5±8.8) were higher in married academicians. The lowest avoidance (35.3±12.2) and anxiety score averages (32.0±4.7) were detected in divorced academicians.

When the mean score was compared according to faculties, there was higher anxiety and avoidance scores in the Faculty of Medicine. The Faculty of Health Sciences had the lowest mean score of anxiety (35.3±5.3) and avoidance (31.1±6.4).

The anxiety (36.1±7.9) and avoidance (34.0±6.90) scores of the professors were lower than the other academic staff. In Research assistants the anxiety and avoidance scores were detected as 40.3±12.1 and 38.1±12.3 respectively.

The anxiety and avoidance scores of the patients living alone at home were determined as 36.7±10.1 and 34.7±6.5 respectively. Those who lived in the subprovince until the age of 18 had higher anxiety scores than those living in the city. The anxiety score was 42.7±12.8 in patients with chronic disease and 37.5±10.0 in patients without chronic disease. The anxiety and avoidance scores were 31.3±4.9 and 31.0±4.6 in patients with a diagnosed mental illness. However, there was no statistically significant difference. Those who rated their socioeconomic status as good in their childhood had lower anxiety and avoidance scores. The mean score of anxiety and avoidance for those who considered their parents uninterested in their childhood was found to be 47.0±10.68 and 39.3±5.7 respectively. The mean score of anxiety and avoidance for those who considered their parents as overprotective during their childhood was 42.8±8.3 and 38.1±9.0 respectively. The mean anxiety and avoidance scores in academicians who were frequently beaten by their parents during childhood were found as 44.7±11.8 and 42.2±14.1 respectively. Those means were 37.7±10.1 and 34.4±8.5 in academicians who did not get beaten by their parents in childhood, respectively. The means of anxiety and avoidance score in the academicians who were beaten by their teachers in primary school were 38.1±9.7 and 35.0±8.9 while those means in academicians who were not beaten were detected as 39.0±11.5 and 35.5±9.2 respectively.

In smokers the anxiety and avoidance scores were found as 39.2±10.4 and 36.1±9.1, while for nonsmokers 37.2±10.6 and 34.6±9.2 respectively.

Anxiety and avoidance score were found as 36.5±8.1 and 33.8±6.5 for those who consume low amounts of alcohol, and 45.0±24.7 and 33.9±11.1 for those who consumed excessive amounts.

Anxiety and avoidance scores were 38.4±10.6 and 35.1±8.9 for academicians participated in social activities with their families, and 40.2±12.5 and 37.4±9.7 respectively for those did not.

It was seen that anxiety and avoidance scores were lower in who had more than two close friends in childhood. The anxiety scores were higher in the academicians who interested in sports (40.7±12.0) and played musical instruments (40.0±13.1).

The lowest anxiety (32.8±6.8) and avoidance (31.7±5.8) scores were observed in the followers of the documentary TV channels.

Two point five percent of the academicians stated that they had severe anxiety in case of getting up and talking in a meeting without being ready. Trying to meet someone to establish a romantic or sexual relationship has caused severe anxiety in 10.7% of academicians. Movement, demonstration or speech in front of the audience causes anxiety in 7.7% of academicians.

When the anxiety subscale is evaluated, ability skill or knowledge testing leads to severe anxiety in 4.2% and avoidance behavior in 2.4% of academicians. Looking directly into the eyes of strangers resulted in moderate anxiety in 8.9% of academicians, while caused avoidance behavior for %6.5 of academicians. Speaking with strangers resulted in moderate anxiety in 5.4% of academicians, while 6% of academicians mostly avoided this situation.

According to LSAS 23.2% of academicians showed avoidance behavior when trying to meet someone in order to have a romantic or sexual relationship. 6.5% of the academicians stated that they always showed avoidance behavior in this case. When they had to get up and talk in a meeting without pre-preparation, 24.4% of the academicians stated that they often show avoidance behavior, while 6.0% stated that they always show avoidance behavior.

The frequency of phobia in academicians is given in Table 4.

Table 4. The frequency of social phobia in academicians

	N	%
Normal	153	91.0
Moderate social phobia	6	3.6
Marked social phobia	6	3.6
Severe social phobia	3	1.8
SAD Total	15	9.0

The prevalence of SAD was found to be 9.0%. The prevalence of moderate and marked SAD was 3.6% and the frequency of severe SAD was 1.8% (Table 4). No significant relationship has been found between smoking and alcohol use and SAD frequency. Social phobia was more common in academics who did not have close friends in childhood (44.4%) (p<0,05) (Table 2). While the frequency of social phobia was detected as 7.3% in academicians who have two or more close friends this frequency was 27.3% in who have no close friends. No significant relationship has been found between regular sporting, musical instrument playing and the frequency of SAD. When the most watched television program was analyzed, it was seen that there were no social phobic academicians among those who watch documentary and TV series. The frequency of SAD was higher in academicians who were watching competition program and movies (p<0,05) (Table 3). No significant relationship was found between SAD and gender, age groups, marital status, faculty, academic degree, place of living until the age of 18, the number of siblings, the number of children of the family, presence of chronic illness, presence of mental illness, presence of mental illness in the family, socioeconomic status in childhood, exposure to family and teacher violence in childhood, parents' tendency to violence against themselves while growing up. SAD was found to be significantly lower in those who had a close friend as a child (p<0,05) (Table 2).

4.DISCUSSION

SAD is a relatively common disorder that affects 7% to 3% of individuals in developed countries throughout their lives[11]. There is no evidence that prevalence rates have changed in recent years. Differences in prevalence rates in different countries and cultures may result from differences in methodology rather than culture-specific factors. Epidemiologic studies may help in planning treatment and prevention programs, and they may also help us to better understand the etiology of these disorders[12]. Researches conducted in Turkey in 1996, according to the profile of Mental Health Research, the prevalence of SAD in the last 12 months, adults were found to be 1.8%[13]. The frequency of SAD was determined as 9.8% in a study conducted in Cumhuriyet University[14]. In a study conducted by Gültekin et al. on 700 students; the prevalence of SAD in the last 12 months and lifetime was 20.9% and 21.7%, respectively[15]. In Kessler's study, prevalence estimates of 12-month-old and lifelong social anxiety disorder (DSM-IV) were reported to be 7.1% and 12.1%, respectively, with higher prevalence rates in women[16],. In a study conducted by Stein et al reported that, the estimated lifetime, 12-month, and 30-day SAD prevalence is highest in high income countries (5.5%, 3.1%, 1.7%), intermediate in upper-middle income countries (2.9%, 2.1%, 1.3%), and lowest in low/lower-middle income countries (1.6%, 1.0%, 0.5%)[17]. In our study, we determined the frequency of SAD as 9.0%. We could not find any study investigating the frequency of SAD in academicians.

This frequency in academicians is consistent with other studies.

SAD is frequently seen in academics who are working as Research Assistants. Being in the lowest level of the academic hierarchy may have been a cause of this phenomenon.

In our study, SAD was most frequently seen in academicians working in the Faculty of Medicine. In addition to the hierarchical order within the faculty, this situation may also be caused by living in isolation from social life due to the nature of the profession.

Asher et al. argued that female gender was more disadvantageous for SAD[18]. Studies supporting the hypothesis of Asher et al. are predominant[11,19-21]. There are also studies that suggest that social phobia is more common in male gender. Cakin et al. Found a significant difference in SAD scores according to gender. Men had higher SAD total and subscale scores (excluding social avoidance and distress general subscale) than girls[22]. In our study, the mean score of anxiety in women was higher in males but the mean score of avoidance was higher in men. In terms of frequency of SAD, we could not find a significant difference between men and women. This result in our study may be caused by the high level of education and social status of the individuals participating in our study. This situation is similar to other studies in the literatüre[23-27].

While the frequency of SAD was the most common among the academicians belonging to the Faculty of Medicine, there was no person with SAD in the Faculty of Health Sciences. The frequency of SAD was determined to be least in professors and associate professors. As academic career increases, the frequency of phobia decreases. We think that this may be due to the decrease in the frequency of SAD with age and the decrease in the concern about the professional career.

Izgic et al. reported that the family of young adults with SAD had a higher family history of psychiatric disorder[14]. In our study, we could not detect any differences in the family history of mental illness among those with and without SAD. When the child rearing styles of parents with social phobic children were examined, it was seen that these parents were overprotective or uninvolved against their children[28]. In our study, we found that the prevalence of phobia was higher in the academicians who considered their family as overprotective and uninvolved. Studies on individuals and their families with social anxiety suggest that the behavioral patterns learned from the family could play an important role in the transfer of SAD[29].

In many studies has been reported a significant increase of alcohol use in the presence of SAD[19,21,30,31]. Although the frequency of phobia was higher in academicians who consumed alcohol frequently no significant relationship was found between smoking and SAD in our study. In a study conducted by Villorosa et al, it was stated that the presence of SAD did not increase alcohol use and would only increase the outcomes of alcohol use[32].

Wells stated that individuals with SAD were married at a lower rate than the general population in 1994[33]. Wittchen defined not being married as a risk factor for SAD (34). In our study, no significant relationship was found between marital status and SAD. This result is consistent with another study conducted in a similar time period[35].

In a study conducted by Gültekin et al showed a significant relationship between birth order and SAD[21]. However, no significant relationship has been found between SAD and birth order in our study. The difference between the two studies may have been caused by the mean age and current social position of the research population.

In our study, no significant relationship has been found between the presence of chronic disease and SAD. In a study conducted by Adams et al in 2016, it was concluded that there was a relationship between the presence of chronic disease and SAD[36]. The difference between the two studies may have been caused by differences in used methods.

In the study, no significant relationship was found between SAD and settlement (village or city) in childhood. This study is consistent with other studies in the literature[35]. A study conducted by Gültekin et al on university students found a significant relationship between city-district-village settlement and SAD[21]. This difference may be caused by the age group of the study population.

There are many studies in the literature regarding the presence of relationship between mental illness and SAD[19,37,38]. In our study, no significant relationship was found between the presence of mental illness and SAD.

It was found that the group with SAD reported more negative life events related to their childhood. Childhood experiences such as exposure to peer bullying, degrading experiences in the school, abuse, etc. are higher in the group with SAD[39]. In our study, we did not find any statistical difference regarding this issue.

Many studies emphasize the relationship between the socio-economic status of the family and the frequency of SAD in childhood. Karlsen et al found a strong relationship between social anxiety and mental health problems in the group of parents representing the low socioeconomic status[40]. In our study, we could not find any relationship between socioeconomic status and SAD.

In the literature, there is a significant increase in nicotine use in the presence of SAD[21,30,41,42]. Potential causal links between social anxiety symptoms and nicotine dependence in adolescence should be investigated[43]. Social anxiety was found to be significantly associated with nicotine dependence in both cross-sectional retrospective and prospective-longitudinal analyses. It is suggested that social anxieties could lead to heavy tobacco use as smoking is a socially acceptable behaviour that relieves anxiety in social situations. Possible differential effects of social anxiety on the early stages of smoking behaviour compared to effects on nicotine dependence are discussed. These findings should stimulate a continued search into potentially causal links between social anxiety symptoms and the development of tobacco consumption and nicotine dependence in adolescence[43].

In our study, it was concluded that having a close friend significantly reduced SAD. This is consistent with other studies in the literatüre[19,44]. In the light of these results, it can be said that the presence of close friends is an important factor for coping with SAD.

Regarding interventions for SAD, the extent of life intervention will be affected by a number of factors such as the severity of social anxiety and the individual's age, gender, life goals and culture[45].

Another challenge lies in the improvement of access to the health care system both on the side of patients and on that of caregivers through optimizing, for example, the detection rates of SAD. As a first step, data on pathways of individuals with SAD to mental health services are urgently needed to provide starting points for effective structural interventions[8].

In a study conducted by Eun reported that the low parental care and high maternal control were associated with a number of past-year mental disorders in adolescents. In the same study emphasized the complexity of the relationship between parenting style and mental health[46].

Limitations of the Research

The present study was conducted among academicians in Cumhuriyet University, and the information obtained can not reflect the knowledge of other universities or the general population. It is recommended that researchs in this area be carried out with larger populations.

5.Conclusion

In our study, the frequency of SAD in academicians is quite high. Support should be given to academics regarding this mental disorder, which has a negative impact on occupational success and leads to continuous anxiety and avoidance behavior. Starting from childhood, education should be planned to prevent social phobia. We think that participatory and active education models will prevent the development of social phobia at an early age. For this reason, education models that give students the opportunity to express themselves should be adopted. Training programs will be useful to increase SAD's awareness in the society.

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Evaluation of Cognitive Functions of Patients with Anemia

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Research Article

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ABSTRACT

Abstract: Iron deficiency anemia is the most common anemia type in the population. It causes many systemic clinical symptoms, including the effects on cognitive functions. Therefore, this study aimed to observe individuals' cognitive changes resulting from iron deficiency, a preventable cause.

Method: Cognitive scores of 112 individuals (56 with iron deficiency anemia-test group and 56 healthy individuals-control group) from the Montreal Cognitive Assessment (MoCA) were recorded. In addition, hemoglobin levels and recurrent anemia histories of individuals with iron deficiency anemia were also considered.

Results: Cognitive functions were lower in the test group (individuals with iron deficiency anemia) compared to the control group. The mean MoCA score of the test and control groups were 19.80±3.46 and 22.98±3.59, respectively, and the difference was significant ($p<0.05$). In addition, a direct relationship ($R=0.22$) was found between the hemoglobin level and the MoCA score in individuals with anemia.

Conclusion: Cognitive functions and B12 deficiency come to the fore in these patients whose cognitive functions are also affected by iron deficiency. Therefore, besides examining cardiovascular and gastrointestinal systems, these patients' neurological systems should also be evaluated in terms of cognitive functions.

Keywords: Anemia, iron deficiency anemia, Montreal cognitive assessment scale, cognitive functions

Demir Eksikliği Anemisi Olan Bireylerin Control Grubuyla Karşılaştırılmalı Montreal Bilişsel Değerlendirme Ölçeği İle Bilişsel Fonksiyonlarının Kıyaslanması

Süreç

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ÖZ

Giriş ve Amaç: Demir eksikliği anemisi toplumda en sık görülen anemi türüdür. Bilişsel işlevler üzerindeki etkileri de dahil olmak üzere birçok sistemik klinik belirtiye neden olur. Bu nedenle bu çalışma, önlenebilir bir neden olan demir eksikliğine bağlı olarak bireylerin bilişsel değişimlerini gözlemlemeyi amaçlamıştır.

Materyal ve Metod: 112 kişinin (56 demir eksikliği anemisi test grubu ve 56 sağlıklı birey-kontrol grubu) Montreal Cognitive Assessment (MoCA) kognitif skorları kaydedildi. Ayrıca demir eksikliği anemisi olan bireylerin hemoglobin düzeyleri ve tekrarlayan anemi öyküleri de dikkate alındı.

Bulgular: Bilişsel işlevler test grubunda (demir eksikliği anemisi olan bireylerde) kontrol grubuna göre daha düşüktü. Test ve kontrol gruplarının ortalama MoCA puanları sırasıyla 19,80±3,46 ve 22,98±3,59 olup aradaki fark anlamlıdır ($p<0,05$). Ayrıca anemisi olan bireylerde hemoglobin düzeyi ile MoCA skoru arasında direkt bir ilişki ($R=0,22$) bulundu.

Sonuç: Bilişsel işlevleri demir eksikliğinden etkilenen bu hastalarda bilişsel işlevler ve B12 eksikliği ön plana çıkmaktadır. Bu nedenle bu hastaların kardiyovasküler ve gastrointestinal sistemlerini incelemenin yanı sıra bilişsel işlevleri açısından nörolojik sistemlerini de değerlendirmek gerekir.

Anahtar sözcükler: Anemi, demir eksikliği anemisi, Montreal biliş değerlendirme skalası, bilişsel fonksiyonlar

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Introduction

Anemia is a prevalent health problem in society. According to the World Health Organization (WHO), it is defined as the hemoglobin level being below 13 in men and 12 in women. The majority of anemia is iron deficiency anemia, which is preventable [1,2].

Iron deficiency anemia affects more than one system; thus, patients' clinical symptoms are very diverse; skin changes, weakness, fatigue, shortness of breath, tachycardia, headache, slowing of memory and movements, and syncope can be observed. Although the cause has not been fully elucidated, cognitive functions decline due to the decrease in the oxygen transport capacity resulting from decreasing number of hemoglobin and the decrease in the iron capacity [3-5]. Therefore, many methods have been developed to evaluate individuals cognitively, among which standardized scales are the most commonly used in the clinic. The Montreal Cognitive Assessment (MoCA) is one of them. It allows the evaluation of fully integrated functions such as attention and concentration, executive functions, memory, language, abstract thinking, calculation, and orientation. It was developed by Nasreddine et al. and is recommended for mild cognitive functions. The validity and reliability studies of the scale in Turkey were carried out by Selekler et al. It helps compare healthy people with individuals having mild cognitive disorders [6,7].

Material and method

The study was designed prospectively. First, data collection was started after getting the approval of the local ethics committee. Then, it was conducted by applying MoCA after getting the consent of the patients who applied to the internal medicine clinic of our hospital and were diagnosed with iron deficiency. A total of 112 patients 18-65 years old, 56 in the test group (diagnosed with iron deficiency anemia) and 56 in the control group, were included in the study.

The patient's age, gender, education, and recurrent anemia histories were questioned, and their hemoglobin levels were recorded. Finally, the MoCA was applied to the test and control groups, and their scores were recorded.

SPSS (Statistical Package for the Social Sciences) 22.0 was used to analyze the data. Categorical measurements were given as numbers & percentages, and continuous measurements as mean & standard deviation (median and min-max where appropriate). The data fulfilled parametric test assumptions (Kolmogrev-Simirrow). The significance test was used to analyze the difference between the means of two independent groups, and the Chi-square test was used for correlations and categorical data.

Results

56 patients with iron deficiency anemia (test group) and 56 healthy people (control group) were included in the study. MoCA was applied to evaluate their cognitive functions. There was no statistically significant difference between these two groups regarding gender, age, and education ($p>0.05$).

Regarding the cognitive functions of the groups, the test group scored lower than the control group; the test group's mean score was 19.80±3.46, whereas the control group's mean score was 22.98±3.59, and the difference between them was significant ($p<0.05$).(table.1)

A direct relationship ($R=0.22$) was found between the hemoglobin level and the MoCA score in individuals with anemia. However, this relationship is statistically insignificant ($p>0.05$). In addition, no statistical correlation was found between the MoCA score and the hemoglobin level in individuals with a history of recurrent anemia ($p>0.05$). (table 2)

Table 1. The results of the groups according to the Montreal cognitive assessment scale

	n (person)	mean	Std deviation	result
Anemia	56	19,8036	3,46105	p = 0,001*
Control	56	22,9821	3,59035	
Total	112			

*p<0,05 significant

Table 2. Montreal cognitive assessment scale results according to the history of recurrent anemia

Story	n (person)	mean	Std. deviation	result
Yes	29	19,8276	3,25213	p = 0,958
No	27	19,7778	3,73480	

p > 0,05 insignificant

Discussion

Cognitive functions are affected by many conditions and diseases. Age comes first. Physical and mental slowing down with age is a physiological process of old age. There is a certain level of intellectual regression. For this reason, individuals under 65 were included in the study to exclude the effect of age, which is the most significant factor [8,9].

It is known that individuals regress cognitively in neurological disorders such as Alzheimer's and Parkinson's. This criterion is used in many studies for diagnosing diseases. Some studies addressed multisystem involvement, such as multiple sclerosis and systemic lupus erythematosus. In addition, studies aiming to see the mental integrity of individuals with chronic diseases such as heart failure and diabetes have also evaluated cognitive functions [10-15]. In iron deficiency anemia, oxygen capacity decreases, resulting from both anemia and the decrease of iron in the body. It is thought that this change may affect cognitive functions directly and through hypoxia. The study's results were also in this direction, and a correlation was found between the degree of anemia and the MoCA score.

The study's results, aiming to show the cognitive impairment caused by iron deficiency anemia, are as follows; The MoCA average score of the test group without any history of other disease was 19.80±3.46; The mean score of the control group was 22.98±3.59. This result proves that cognitive functions are affected by anemia. Furthermore, a similar study conducted on individuals with sickle cell anemia supports these results [16].

Iron deficiency can be seen with low intake, malabsorption or loss of iron. Low intake can be a problem that starts from childhood. Iron deficiency, which is very common in children with nutritional problems, causes chronic damage and negative effects on mental health in the future [17]. For this reason, awareness and screening programs on anemia should be expanded from childhood. Thus, a transition should be made with mental health in adulthood. In another iron deficiency disorder, absorption disorder, many diseases such as intestinal pathologies and diseases can be listed. In fact, when they observed the intestinal microbiota, cognitive dysfunction attracted the attention of scientists [18,19]. Losses are especially frequent in young women, but when they go with menstrual cycles, gastrointestinal system diseases in advanced age should be considered. There is chronic vaccinia disease in cases of reducing hemin absorption and spreading the loss. Although there are many anemia factors in people followed up with hemodialysis treatment due to stage-5 chronic birth pregnancy; One preventable cause of anemia is iron deficiency. Since hemodialysis and peritoneal dialysis can be compared, cognitive functioning is better preserved in peritoneal dialysis. During anemia is one of the causative factors of cognitive function assignment in hemodialysis [20]. In iron deficiency anemia, it is very important to reduce iron for mental operations without affecting the daily lives of individuals.

Conclusion

Iron deficiency is prevalent in society, and our study showed that both this deficiency and anemia affect cognitive functions. Iron deficiency anemia, a preventable type of anemia, occurs mainly in the retrospective period. Therefore, life expectancy, early diagnosis and treatment of iron deficiency are very important to protect people's cognitive functions for many years and let them spend their remaining life most efficiently.

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Colonic Slow Transit in Patients with Dyssynergic Defecation and Effectiveness of Biofeedback Therapy

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Research Article

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ABSTRACT

Background: Colonic transit is delayed in two-thirds of patients with dyssynergic defecation. As a result, dyssynergic defecation and slow transit constipation may be linked. There is a scarcity of research on the coexistence of dyssynergic defecation and slow transit constipation, as well as the efficacy of biofeedback therapy in this group of patients.
Methods: The results of anorectal manometry and MR defecography were used to diagnose dyssynergic defecation. The colon transit time was measured with the help of 24 specially marked markers. All of the patients were given biofeedback therapy.
Results: The study's average age of 17 patients with dyssynergic defecation was 45.6 years. The colon transit time was prolonged in seven patients (41.2%). In anorectal manometry, seven patients (41.2%) had improved dyssynergic defecation after dyssynergic defecation. When the relationship between colon transit time and biofeedback therapy was investigated, it was discovered that normal colon transit time was found in 85.7 percent of those who benefited from biofeedback therapy. In contrast, slow transit constipation was found in 60% of those who did not.
Conclusion: In patients with pre-biofeedback therapy and dyssynergic defecation, colon transit time should be evaluated, and it should be kept in mind that these patients may be resistant to treatment in the case of accompanying slow transit constipation.

Keywords: Anorectal manometry, Biofeedback therapy, Colonic Slow Transit, Constipation, Dyssynergic Defecation

Dissinerjik Defekasyonlu Hastalarda Kolonik Yavaş Geçiş ve Biofeedback Tedavisinin Etkinliği

Süreç

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ÖZ

Amaç: Dissinerjik dışkılama olan hastaların üçte ikisinde kolonik geçiş gecikir. Sonuç olarak, dissinerjik dışkılama ve yavaş geçişli kabızlığın bağlantılı olması mümkündür. Dissinerjik dışkılama ile yavaş geçişli kabızlığın bir arada bulunmasının yanı sıra bu hasta grubunda biofeedback tedavisinin etkinliğine ilişkin araştırma sayısı azdır.
Yöntemler: Dissinerjik dışkılama tanısı için anorektal manometri ve MR defekografi sonuçları kullanıldı. Kolon geçiş süresi, özel olarak işaretlenmiş 24 işaret yardımıyla ölçüldü. Tüm hastalara biofeedback tedavisi verildi.
Bulgular: Çalışmada dissinerjik dışkılama şikayeti olan 17 hastanın yaş ortalaması 45,6 idi. Yedi hastada (%41.2) kolon geçiş süresi uzamıştı. Anorektal manometride, 7 hastada (%41.2) dissinerjik defekasyondan sonra dissinerjik defekasyon düzeldi. Kolon geçiş süresi ile biofeedback tedavisi arasındaki ilişki incelendiğinde biofeedback tedavisinden fayda görenlerin yüzde 85,7'sinde kolon geçiş süresinin normal olduğu, fayda görmeyenlerin ise yüzde 60'ında yavaş geçişli kabızlık bulunduğu keşfedildi.
Sonuç: Prebiofeedback tedavisi ve dissinerjik dışkılama olan hastalarda kolon geçiş süresi değerlendirilmeli ve bu hastaların eşlik eden yavaş geçişli kabızlık durumunda tedaviye dirençli olabileceği akılda tutulmalıdır.

Anahtar sözcükler: Anorektal manometri, Biofeedback tedavisi, Kolonik Yavaş Geçiş, Kabızlık, Dissinerjik Defekasyon

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Introduction

Constipation is a common ailment in the community, with a prevalence of 14–18%, and it significantly impacts the quality of life. Furthermore, constipation increases the use of healthcare services as well as direct and indirect economic costs. The three types of functional constipation are normal transit constipation (NTC), slow transit constipation (STC), and rectal evacuation disorders or dyssynergic defecation (DD) [1,2]. Accurate categorization is critical for treating and managing constipation (1). One-third of all chronic constipation patients have DD, which is one of the most common causes of chronic constipation. This acquired behavioral problem is caused by the coordination disorder of the abdominal and pelvic floor muscles during stool evacuation (1-3). Meanwhile, STC is one of the other causes of chronic constipation. It is characterized by changes in the number of myenteric plexus neurons or Cajal interstitial cells, as well as causing myopathy or neuropathy, which prolongs colonic transit time [4].

Because two-thirds of patients with difficult defecation also have delayed colonic transit, DD and STC may be linked [1,5]. It has been discovered in some studies using radio opaque or scintigraphy that there is a delay in DD not only in the rectosigmoid region but also throughout the colonic transit [5,6]. Treatments (lifestyle changes, medical treatments, diet, and so on) are frequently insufficient in patients with DD and STC, and many patients continue to suffer from symptoms [1,2]. Biofeedback therapy (BFT) is a low-cost, non-invasive, and easy-to-use treatment for DD that has been shown to be one of the most effective. Both clinical symptoms and the DD pattern can be improved with BFT [2,3]. Hence, BFT is recommended as first-line therapy for DD by both the American Neurogastroenterology and Motility Society and the European Society of Neurogastroenterology and Motility (ANMS - ESNM) [7]. For patients with STC who do not respond to diet and lifestyle changes, pharmacological stimulating agents (bisacodyl, neostigmine, etc.) are recommended as first-line therapy. However, in STC patients who have failed to respond to first-line treatments, treatment options are limited. A total colectomy may be necessary in severe cases, but this is rare [8]. BFT has been shown in studies to normalize colon transit time (CTT) in patients with STC, in addition to being the most effective treatment for DD [9]. As a result, BFT is also recommended for STC patients who are not responding to medical treatment.

The literature on the coexistence of DD and STC, as well as the effectiveness of BFT in this group of patients, is limited.

MATERIALS AND METHODS

Patients

Patients who received BFT treatment for DD and whose CTT was studied were included in the study after our study protocol was scrutinized and approved by the Local Ethics Committee (protocol number: 72300690-799). From January 2010 to February 2019, patient data were collected retrospectively. The information was gathered from the motility laboratory's medical records as well as our hospital's digital database.

Patients between the ages of 18 and 75 who presented to our clinic with constipation symptoms that did not respond to conservative treatments (diet, laxatives, enemas, stool volume-enhancing treatment, or laxatives), those who had symptoms for more than 12 months, those whose organic and metabolic causes of chronic constipation were excluded by colonoscopy and laboratory tests, and those who fully met the DD criteria for Rome IV (10) were included. The results of anorectal manometry (ARM) and MR (magnetic resonance) defecography were used to make the diagnosis of DD.

All of the patients were given BFT. BFT was completed for those who benefited from the treatment for 10-15 sessions, but for those who did not benefit, the treatment was stopped after the 6th session. Patients whose DD pattern disappeared in ARM after BFT were considered successful, as were patients who achieved adequate anal relaxation (> 20% sphincter relaxation) and had clinically complete improvement in their symptoms. Even if bowel and defecation symptoms improved, BFT was considered unsuccessful in those whose DD pattern persisted in ARM.

Demographic and characteristic features of the patients, their history, as well as ARM results at baseline and after BFT were evaluated.

Colon transit time

The patient swallowed 24 specially marked capsules of 5 mm in diameter, which can be seen on x-ray (radiopaque), after breakfast in the morning. X-rays were taken on the 3rd and 5th days. The test was discontinued if more than 80% of the rings were not seen on the X-ray on the 3rd day. A colon transit time of 3 days is considered normal. If 80% of the rings were still visible on the 3rd-day x-ray, the control x-ray was taken on the 5th day. If less than 20% of the rings were visible on the 5th day, the colonic transit time was considered five days or normal. If more than 20% of the rings are seen on the 5th day, the colonic transit time is considered > 5 days or abnormal. In this situation, the distribution of the remaining rings in the column was evaluated. A diagnosis of STC was made if there was a homogeneous distribution of the rings in the colon or if they accumulated on the right side of the vertebra. As the diagnosis of DD was definite by ARM and MR defecography in all patients in the study, if the rings were collected in the last part of the rectosigmoid colon and were absent in the right colon, it was considered secondary to DD and colonic motility was accepted as

normal. Yet, these patients were considered to have anorectal dysfunction.

Anorectal physiological tests

A seven-channel water perfusion system (Dent-sleeve International) was used for ARM [11]. The anal resting pressure, anal maximum squeezing pressure, anal pressure during coughing, anal relaxation or paradoxical contraction during defecation and rectoanal inhibitory reflex were recorded. The rectal sensation was assessed by measuring the first sensation, the desire to defecate, and the maximum tolerated volume.

Biofeedback Therapy

Bowel habits, exercise, laxatives, dietary fiber, fluid intake, and timed toilet training were all discussed with patients. Patients were taught how to improve defecation efforts using postural and diaphragmatic breathing techniques by an experienced team of motility specialists (gastroenterologist, nurse, and physiotherapist), with education on the anatomy of normal defecation, advice on correct toilet positioning, and postural and diaphragmatic breathing techniques, and they were asked to perform these maneuvers at home at least 2 to 3 times a day for 15 minutes. The physiotherapist showed the patients visual and verbal exercises to relax the pelvic floor muscles, strengthen the abdominal and pelvic muscles, and allow abdominopelvic coordination. Patients were also given visual and written documents that they could use to exercise at home.

For BFT, the EMG-BFT technique was used [12]. Surface EMG probes were stitched to the skin in the bilateral anal canal while the patient was lying in the left lateral ducubit position. On a computer monitor, patients watched manometric tracings from surface EMG probes around the bilateral anal canal. With visual and verbal feedback, patients were taught to follow the nurse's commands to control the sphincter and pelvic floor muscles and improve abdominopelvic coordination. BFT had been applied to patients for at least six sessions under the supervision of a motility nurse. The BFT sessions lasted anywhere from 30 to 45 minutes.

Statistical analysis

In the computer environment, the data were analyzed using IBM SPSS Statistics 20.0 Windows 10 software. Patients' continuous numerical variables with normal distribution were expressed as mean SD values, whereas those with non-normal distribution were expressed as median, minimum-maximum values. Ratios were used to express nominal data (percentages). Because of the small number of patients, no comparisons were made.

Results

Of the 17 patients with DD included in the study, 14 (82.4%) were female, three were male, and the mean age was 45.6 17.9 years. The patients had symptoms for a median of 84 months [12–30]. The most common complaints among the patients were constipation (82.4%) and an inability to completely defecate the stool (17.6%). CTT was normal in 10 (58.8%) patients and prolonged in 7 (41.2%) patients. When the rectal sensations were evaluated with a rectal balloon, 9 (52.9%) of the patients had normal rectal sensations, 7 had rectal hyposensitivity, and 1 had rectal hypersensitivity. The rectal resting pressures of the patients decreased after BFT, whereas an increase was observed in the anal squeezing pressures. Following BFT, DD improved in 7 patients (41.2%) in ARM and the patients' clinical complaints disappeared. However, the DD pattern in ARM continued in 10 patients (58.8%). The demographic and clinical characteristics of the patients are presented in Table 1.

Table 1: Characteristic and demographic features of patients

Age, years, mean, (range), min-max.	45.6±17.9 (19-80)
Male, n (%)	3 (%17.6)
Female, n (%)	14 (%82.4)
Primary symptom	
• Constipation, n (%)	14 (82.4)
• Inability to fecal evacuation, n (%)	3 (17.6)
Primary symptom duration, median (min-max)	84 (12-300)
Biofeedback number of sessions, median (min-max)	10 (7-15)
Primary symptom	
• Constipation, n (%)	14 (82.4)
• Inability to fecal evacuation, n (%)	3 (17.6)
Resting Pressure	
• Before BFT (mmHg, mean)	73.6 ± 24.4
• After BFT (mmHg, mean)	66.1±16.1
Squeeze Pressure	
• Before BFT (mmHg, mean)	133.4 ± 36.5
• After BFT (mmHg, mean)	154.7 ± 23.6
Rectal sensitivity	
• Normal, n (%)	9 (52.9)
• Decreased, n (%)	7 (41.2)
• Increased, n (%)	1 (5.9)
Colon transit time	
• Normal colon transit time,n (%)	10 (58.8)
• Slow colon transit time, n (%)	7 (41.2)
Response to biofeedback therapy	
• Responders, n (%)	7 (41.2)
• Non-responders, n (%)	10 (58.8)

BFT; Biofeedback therapy

Table 2: Comparison by colon transit time of patients

	Normal colon transit time, (%)	Slow colon transit time, (%)
Male, (n:3)	66.7	33.3
Female, (n:14)	57.1	42.9
Primary symptom		
• Constipation, (n:14)	57.1	42.9
• Inability to fecal evacuation, (n:3)	66.7	33.3
Resting Pressure		
• Before BFT (mmHg, mean)	80.5±25.8	63.8±19.9
• After BFT (mmHg, mean)	72.9±13.9	56.4±14.6
Squeeze Pressure		
• Before BFT (mmHg, mean)	155±11.5	154.2±35.9
• After BFT (mmHg, mean)	158±11.2	156.2±35.8
Rectal sensitivity		
• Normal, (n:9)	77.8	22.2
• Decreased, (n:7)	28.6	71.4
• Increased, (n:1)	100	
Response to biofeedback therapy		
• Responders, n (%)	85.7	14.3
• Non-responders, n (%)	40	60

BFT; Biofeedback therapy

When the CTTs of the patients were evaluated (Table 2), it was found that SCT accompanied DD in 33.3% of males and 42.9% of females. SCT was detected in 6 (42.9%) patients who presented with constipation and in 1 (33.3%) patient who presented with the inability to fecal evacuation. A decrease in rectal resting pressures and an increase in anal squeezing pressures

The DD pattern improved in 41.2% of the patients in our study after BFT, and only one of the patients with STC improved the DD pattern after BFT, whereas 85.7% (6/7) did not benefit from BFT.

Only 2 patients had a history of surgery due to an anal fissure. One of these two patients had prolonged

were observed after BFT in all patients with normal or slowed CTT. In 71.4% of patients, SCT was accompanied by decreased rectal sensation. When the relationship between CTT and BFT was analyzed, it was found that NCTT was detected in 85.7% of those who benefited from BFT, while SCT was observed in 60% of patients who did not respond to BFT.

CTT and normal rectal sensitivity. This patient did not benefit from BFT. In the other patient, transit time was normal, rectal hyposensitivity was observed, and the DD pattern improved following BFT.

Discussion

A significant proportion of patients with DD also have a delay in the delivery of colon contents, according to our research. Patients with a long CTT are more likely to have abnormal rectal sensitivity. Patients who have both DD and STC benefit less from BFT.

In 55-64 percent of adults with DD and 12 percent of adolescents with DD, delayed CTT with scintigraphy or radiopaque markers has been reported [6,11]. Tanner et al. (13) found DD in 9% of patients with constipation who did not respond to first-line treatment, STC in 42%, DD and STC overlap in 13%, and NTC in 36% of patients in a study of 230 patients with constipation who did not respond to first-line treatment. A total of 50 patients were diagnosed with DD, with 30 of them (60 percent) also having STC. 30 (24%) of the 127 patients with STC also had DD. Grotz et al. [14] found that patients with STC had longer left colon and rectosigmoid transit times than patients with DD when using radiopaque markers.

Furthermore, Nullens et al. [5] used the scintigraphy method to measure CTT in patients with DD, patients with STC with normal ARM, and healthy control groups. They discovered that in patients with DD and STC coexistence, the overall CTT time was longer than in the control group, and that overall colon transit was slower in patients with STC than in patients with DD. In contrast to Grotz et al. [14], Nullens et al. [5] found that patients with STC had slower right colon emptying than patients with DD. This disparity, however, could be due to the different methods they used to measure CTT. In CTT measurements made with a radiopaque marker, radiopaque materials may accumulate in the rectosigmoid region due to anorectal dysfunction in patients with DD [15]. Furthermore, Nullens et al. [5] used the scintigraphy method to measure CTT in patients with DD, patients with STC with normal ARM, and healthy control groups. They discovered that in patients with DD and STC coexistence, the overall CTT time was longer than in the control group, and that overall colon transit was slower in patients with STC than in patients with DD. In contrast to Grotz et al. [14], Nullens et al. [5] found that patients with STC had slower right colon emptying than patients with DD. This disparity, however, could be due to the different methods they used to measure CTT.

Although the etiology of DD is unknown, inadequate relaxation of the anal sphincter, paradoxical contraction of the anal sphincter, or a disorder in the forward evacuation of stool in the rectum are blamed as the mechanisms causing its pathophysiology. With BFT, it is aimed to restore the abdominopelvic coordination ability (1-3) and in numerous randomized studies demonstrating the effectiveness of BFT, response rates have been reported as 70-80% [16-18]. Meanwhile, in our study, 41.2% of patients benefited from BFT. This variability in treatment success may be due to the differences in BFT methods (manometry-based biofeedback, EMG biofeedback, balloon defecation training, and home-based training biofeedback) applied to patients as well as to the differences between patient groups [16-19].

The exact reason for DD and STC coexistence is unknown. The improvement of STC after BFT has been attributed to the theory that DD causes reflex inhibition in the proximal colon and colonic transit [20,21]. Patients with STC who do not respond to diet or stimulant treatments have limited treatment options, and the role of BFT in these patients is uncertain [20,22]. According to another study, BFT helps with both normal and slow transit constipation [23-25]. However, it was suggested in a later controlled study that while BFT was beneficial only for patients with DD and NTC, it was not beneficial for patients with STC [26]. Improvements in anal sphincter tone at rest, functions of the anal sphincter and puborectalis muscles, abdominopelvic coordination during defecation, and rectal sensation functions may occur as a result of the BFT [18,27]. A decrease in resting pressures, improvement in patients' first sensations, and a slight increase in squeezing pressures were all observed after BFT in one study, but their predictive effect on BFT success was not found [3]. In our research, however, we found that after BFT, anal squeezing pressure increased while anal resting pressure decreased. Furthermore, DD may be accompanied by 50–60% impaired rectal sensation. While some studies found changes in rectal sensation after BFT, others found no difference in rectal sensation after BFT [2, 28-30]. The rectal sensations of the patients after BFT were not assessed in our study, but we did find that 47.1 percent of the patients had impaired rectal sensation, with the majority (62.5 percent) of those with impaired rectal sensation having a prolonged colon transit time.

It was revealed that the symptom profiles of patients with chronic constipation did not differ depending on the subgroups of constipation, and the symptom profiles of patients with STC and DD overlap were largely similar to those of patients with NTC, STC, or DD alone [13]. In our study, although all patients had DD, constipation was the primary symptom in 82.4% of them, rather than the inability to fecal evacuation. ARM and colon transit time should be studied for the treatment plan of patients who do not respond to diet and laxatives, considering the fact that their symptom profile cannot distinguish subgroups of constipation.

The limitations of the study are that the study was retrospective, relatively small number of patients, colon transit time and rectal sensation were not checked after BFT, and the effectiveness of BFT was not compared in patients with only STC.

Conclusion

Patients with DD might also have a prolonged total colon transit time. In the coexistence of DD and STC, response rates to BFT might decrease. Hence, colon transit time should be evaluated in patients with pre-BFT and DD, and it should be kept in mind that these

patients may be resistant to treatment in the case of accompanying STC. A more multidisciplinary approach and combining treatments with BFT could increase the chance of success in these patients. However, more randomized studies with large patient groups are needed for the treatment of DD and STC coexistence.

Ethics Committee Approval: The Ankara City Hospital of Medical Sciences Ethics Committee granted approval for this study (protocol number: 72300690-799)

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

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Quality of Life of Patients with Postoperative Hypothyroid and Hashimoto Thyroiditis

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Research Article

History

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ABSTRACT

Purpose: The symptoms and findings resulting from thyroid dysfunctions display a picture similar to mood disorders such as depression and anxiety, which are the most common psychiatric disorders in the community. Therefore, this study aims to examine patients with Hashimoto thyroiditis receiving L-thyroxine therapy and euthyroid patients with postoperative hypothyroidism receiving L-thyroxine therapy in terms of their susceptibility to anxiety and depression and the change in their quality of life, as well as determine the role of the thyroid gland in psychosocial life.

Material and method: For evaluating anxiety, depression, and quality of life, Beck depression and Beck anxiety inventories and SF-36 were administered to 120 people (20-80 years old) who were applied to the Department of Endocrinology of Sivas Cumhuriyet University. Forty of them had developed hypothyroidism after thyroid surgery, 40 were diagnosed with Hashimoto thyroiditis at some point in their life and are now in a euthyroid state after L-thyroxine treatment, and 40 were healthy people in the control group. Data were analyzed on SSPS.

Results: The reasons for these psychosocial effects on individuals were evaluated in multiple ways, such as thyroid dysfunction, having a chronic disease, the idea of constant drug use, the effect of autoimmunity, and endogenous hormone secretion. Thyroidectomy patients' anxiety and depression were higher, and their quality of life was poorer than both Hashimoto thyroiditis patients and the control group ($p<0.05$). In addition, patients with Hashimoto thyroiditis and thyroidectomy had higher anxiety and depression and poorer quality of life than the control group ($p<0.05$).

Conclusion: It was concluded that patients with Hashimoto thyroiditis are more depressed and anxious and have a lower quality of life than the control group due to the autoimmunity effect. On the other hand, patients with thyroidectomy were psychologically more affected than Hashimoto thyroiditis patients, and their quality of life was poorer due to the lack of endogenous hormone secretion and/or the undiscovered physiological effect of the thyroid gland. It was concluded that maximal protection of the thyroid gland is crucial.

Keywords: Hypothyroidism, Thyroidectomy, Beck Anxiety Inventory, Beck Depression Inventory, SF-36

Postoperatif Hipotiroidi ve Hashimoto Tiroiditi Olan Hastaların Yaşam Kalitesi

Süreç

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ÖZ

Amaç: Tiroid işlev bozukluklarına bağlı belirti ve bulgular toplumda en sık görülen psikiyatrik bozukluklar olan depresyon ve anksiyete gibi duygudurum bozukluklarına benzer bir tablo sergilemektedir. Bu nedenle bu çalışma, L-tiroksin tedavisi alan Hashimoto tiroiditli hastalar ile L-tiroksin tedavisi alan ötiroidili postoperatif hipotiroidili hastaların anksiyete ve depresyona yatkınlıkları ve yaşam kalitelerindeki değişimi incelemek ve tiroid bezinin psikososyal yaşamdaki rolü.

Gereç ve yöntem: Sivas Cumhuriyet Üniversitesi Endokrinoloji Anabilim Dalı'na başvuran 120 kişiye (20-80 yaş) anksiyete, depresyon ve yaşam kalitesini değerlendirmek için Beck depresyon ve Beck anksiyete envanterleri ve SF-36 uygulandı. 40'ı tiroid ameliyatından sonra hipotiroidizm geliştirmişti, 40'ı hayatının bir noktasında Hashimoto tiroiditi teşhisi kondu ve şimdi L-tiroksin tedavisinden sonra ötiroid durumda ve 40'ı kontrol grubundaki sağlıklı insanlardı. Veriler SSPS'de analiz edildi.

Bulgular: Bireyler üzerindeki bu psikososyal etkilerin nedenleri, tiroid disfonksiyonu, kronik bir hastalığa sahip olma, sürekli ilaç kullanma düşüncesi, otoimmünitenin etkisi ve endojen hormon salgılanması gibi birçok yönden değerlendirildi. Tiroidektomi hastalarının anksiyete ve depresyonları hem Hashimoto tiroiditi hastalarına hem de kontrol grubuna göre daha yüksek, yaşam kaliteleri daha kötüydü ($p<0,05$). Ayrıca Hashimoto tiroiditi ve tiroidektomili hastalarda kontrol grubuna göre daha yüksek anksiyete ve depresyon ve daha kötü yaşam kalitesi saptandı ($p<0.05$).

Sonuç: Hashimoto tiroiditli hastaların otoimmünite etkisinden dolayı kontrol grubuna göre daha depresif ve kaygılı oldukları ve yaşam kalitelerinin daha düşük olduğu sonucuna varıldı. Öte yandan, tiroidektomi yapılan hastalar, Hashimoto tiroiditi hastalarına göre psikolojik olarak daha fazla etkilenmiş ve endojen hormon salgılanamaması ve/veya tiroid bezinin keşfedilmemiş fizyolojik etkisi nedeniyle yaşam kaliteleri daha düşük olmuştur. Tiroid bezinin maksimum korunmasının çok önemli olduğu sonucuna varıldı.

Anahtar Kelimeler: Hipotiroidizm, Tiroidektomi, Beck Anksiyete Envanteri, Beck Depresyon Envanteri, SF-36

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Introduction

The most common disease related to the thyroid gland is hypothyroidism, which causes a slowing of metabolism due to thyroid hormone deficiency or dysfunction at the tissue level [1]. The symptoms observed in these patients include dry skin, feeling cold in the body, pale skin color, physical fatigue, muscle pain and cramps, changes in voice, especially coarseness and slow speech, constipation, rhythm disturbances, bradycardia, swelling in the eyes and face, enlargement of the tongue, loss of appetite and despite this weight gain, decrease in sweating, hair loss, decrease in hearing, and menstrual irregularities. Moreover, there may be signs such as slowing down in thinking, lack of concentration, mental fatigue, irritability, sleep disorders, and forgetfulness. Similar symptoms are also observed in mood disorders such as depression and anxiety, the most common psychiatric disorders in society. This study aimed to examine the susceptibility to anxiety and depression and the change in the quality of life of patients with Hashimoto thyroiditis receiving L-thyroxine treatment and postoperative hypothyroidism patients receiving L-thyroxine treatment and reveal the thyroid gland's role in psychosocial life [1-4]. Beck Depression Inventory was developed by Beck et al. in 1961 to measure behavioral symptoms of depression in adolescents and adults. In 1978, the scale was fully revised, and patients were asked to mark their last week's status, including today. The Beck Anxiety Inventory determines the frequency of anxiety symptoms experienced by individuals on a Likert scale. Its validity and reliability studies in Turkey have been performed. This test consists of 21 items; the responder is requested to answer as none, mild, moderate, and severe regarding their feelings and thoughts in the last week. The SF-36 is one of the most commonly used tools. It was developed and launched by the Rand Corporation in 1992. Koçyiğit et al. carried out the reliability and validity of its Turkish version. This survey is used to measure an individual's health status. It is a self-assessment tool; its short form consisting of 36 questions and eight subscales is known as SF-36 [5-8].

Method

Sample Groups

The study's sample group is patients who applied to Sivas Cumhuriyet University Faculty of Medicine Endocrinology Polyclinic. They were 20-80 years old, and their thyroid function tests were in the normal range. 40 patients were diagnosed with Hashimoto Thyroiditis, and 40 received L-thyroxine therapy for postoperative hypothyroidism; 40 were healthy people used as the control group. Individuals with thyroidectomy secondary to malignancy were excluded. The test and control groups had similar sociodemographic characteristics. Beck depression and Beck anxiety inventories and SF-36 were administered to these 120 people.

40 individuals who received postoperative L-thyroxine therapy, except for individuals who had secondary thyroidectomy for malignancy.

Required permission was granted from the ethics committee of Sivas Cumhuriyet University Faculty of Medicine, with the decision number 2017-07/23, dated 26.07.2017.

Materials

1. Sociodemographic Data Form: It was prepared by the doctor who conducted the study to collect patients' information such as age, gender, educational status, and BMI, to evaluate their clinical characteristics better.

2. Beck Depression Inventory: The scale consists of 21 statements sorted from mild to severe. Each statement is scored between 0-3. A score of 0–9 is considered the minimal range; 10–18 is mild, 19–29 is moderate, and 30–63 is severe depression[5,7].

3. Beck Anxiety Inventory: Each item is scored between 0 and 3. Evaluation of the scale; 0–7, normal or no anxiety; 8–15, mild to moderate anxiety; 16–25, moderate to severe anxiety; and 26–63, severe anxiety [6,7].

4. Quality of Life and SF-36: The subscales are physical functioning (10 items), role physical (4 items), role emotional (3 items), bodily pain (2 items), vitality (4 items), social functioning (2 items), mental health (5 items) and general health perceptions (5 items) Evaluation of the scale; Each subscale is scored between 0-100, where "0" indicates the poorest and "100" the best quality of life [8].

5. Statistical Method: The data was loaded into the SSPS=22.0 program; parametric test assumptions were tested and found to be met (Kolmogorov-Smirnov). The following tests were used to find the group/groups with significant differences: One-way ANOVA to compare measurements obtained from two and more independent groups, the Mann-Whitney U test to reveal the source of the significant difference, and the chi-square test for the data obtained by counting. The error level was taken as 0.05.

Results

83.3% of the participants were female, and 11.7% were male. In the group with Hashimoto Thyroiditis, the female percentage was significantly higher than in the groups. This result aligns with the literature and supports that Hashimoto thyroiditis is more common in women. The gender distribution of the groups was similar ($p>0.05$)(Table 1).

Table 1. Demographic Features

			Gender		
			Woman	Man	Total
Groups	Control	S	35	5	40
		%	87.5%	12.5%	100.0%
	Hashimoto	S	37	3	40
		%	92.5%	7.5%	100.0%
	Thyroidectomy	S	34	6	40
		%	85.0%	15.0%	100.0%
Total		S	106	14	120
		%	88.3%	11.7%	100.0%

According to the table, there are significant differences between groups Physical functioning, physical role functioning, bodily pain, and general health perceptions. The pairwise comparison showed that the differences between thyroidectomy & control groups and Hashimoto Thyroiditis & thyroidectomy groups were significant. Meanwhile, the differences between the control & Hashimoto thyroiditis groups were insignificant (p>0.05). The differences among groups were insignificant for emotional role functioning, vitality, mental health, and social functioning (p>0.05). Regarding the pairwise comparison between Beck Depression and Beck Anxiety Inventories between groups, the difference between the control & thyroidectomy groups was significant. In contrast, the differences between the control & Hashimoto thyroiditis and Hashimoto thyroiditis & thyroidectomy group were insignificant (p>0.05). (Table 2)

Table 2. Pairwise comparison between Beck Depression and Beck Anxiety Inventories between groups

		n	Average	Standard deviation	middle	Minimum	Maximum	Conclusion
SF-36	Control	40	90.13	12.42	95.00	50	100	KW=23.58
Physical	Hashimoto	40	79.25	20.86	85.00	20	100	**p=0.001***
Functioning	Thyroidectomy	40	64.13	26.57	65.00	5	100	
SF-36	Control	40	75.00	31.00	87.50	0	100	KW=10.72
Physical Role	Hashimoto	40	65.00	39.95	87.50	0	100	**p=0.005***
Functioning	Thyroidectomy	40	44.38	42.92	25.00	0	100	
SF-36	Control	40	63.33	39.80	66.67	0	100	KW=1.00
Emotional Role	Hashimoto	40	60.84	41.26	66.67	0	100	p=0.605
Functioning	Thyroidectomy	40	54.17	41.81	33.33	0	100	
SF-36	Control	40	55.87	16.63	55.00	20.00	90.00	KW=4.93
Vitality	Hashimoto	40	48.37	19.68	50.00	5.00	80.00	p=0.085
	Thyroidectomy	40	45.87	23.39	45.00	.00	95.00	
SF-36	Control	40	63.10	16.95		24.00	92.00	F=1.37
Emotional health	Hashimoto	40	59.00	17.55		4.00	92.00	p=0.258
	Thyroidectomy	40	56.40	20.06		12.00	96.00	
SF-36	Control	40	55.56	23.59	50.00	12.50	100.00	KW=4.04
Social Functioning	Hashimoto	40	64.06	19.85	62.50	25.00	100.00	p=0.132
	Thyroidectomy	40	61.25	22.07	62.50	12.50	100.00	
SF-36	Control	40	70.62	20.26	57.60	30.00	100.00	KW=7.47
Bodily Pain	Hashimoto	40	58.81	20.10	57.50	22.50	90.00	**p=0.024***
	Thyroidectomy	40	58.25	24.47	57.50	.00	100.00	
SF-36	Control	40	60.13	15.99		25.00	90.00	F=4.99
General Health	Hashimoto	40	54.87	17.59		10.00	80.00	**p=0.008***
Perception	Thyroidectomy	40	47.14	21.49		5.00	95.00	
Beck Depression	Control	40	9.10	9.55	7.50	.00	45.00	KW=5.74
	Hashimoto	40	12.60	10.80	11.00	.00	43.00	p=0.047
	Thyroidectomy	40	16.00	14.25	12.50	.00	49.00	
Beck Anxiety	Control	40	9.35	9.10	6.50	.00	36.00	KW=9.46
	Hashimoto	39	14.07	10.02	10.00	1.00	36.00	**p=0.009***
	Thyroidectomy	40	16.97	13.20	13.50	1.00	44.00	

Regarding the significance test of the Beck Depression Inventory, the difference was insignificant (p>0.05). Although the difference is insignificant, moderate and severe depression is more common in individuals with thyroidectomy (Table 3). The analysis of the Beck Anxiety Inventory revealed a significant difference (p<0.05). The control group was not anxious; individuals with Hashimoto thyroiditis tend to have mild anxiety, and those with thyroidectomy tend to have severe anxiety (Table 4).

Table 3. Comparison of the Groups' Depression Levels According to the Beck Depression Inventory

BECK DEPRESSION INVENTORY			0-9 Normal	10-18 Mild depression	19-29 Moderate Depression	30-63 Severe depression
Groups	Control	S	23	13	2	2
		%	57.5%	32.5%	5.0%	5.0%
	Hashimoto	S	17	15	4	4
		%	42.5%	37.5%	10.0%	10.0%
	Thyroidectomy	S	14	11	7	8
		%	35.0%	27.5%	**17.5%**	**20.0%**
Total		S	54	39	13	14
		%	45.0%	32.5%	10.8%	11.7%

Table 4. Beck Anxiety Inventory

BECK ANXIETY INVENTORY			0-7 minimal anxiety	8-15 mild anxiety	16-25 moderate anxiety	26-63 severe anxiety	Total
Groups	Control	S	25	7	3	5	40
		%	**62.5%**	17.5%	7.5%	12.5%	100.0%
	Hashimoto	S	12	14	8	6	40
		%	30.0%	35.0%	20.0%	15.0%	100.0%
	Thyroidectomy	S	14	8	8	10	40
		%	35.0%	20.0%	20.0%	**25.0%**	100.0%
Total		S	51	29	19	21	120
		%	42.5%	24.2%	15.8%	17.5%	100.0%

Discussion

Thyroid dysfunction is a common endocrinological pathology and creates some psychological effects in people. Many studies reported that anxiety and depression are closely related to thyroid dysfunctions. The study by Hickie et al. found that treatment-resistant depression could be caused by hypothyroidism [9]. Ordas et al. examined primary thyroid functions of people with major depression without any diagnosed thyroid dysfunction, and thyroid function problem was found in 21% of them [10]. Sapini et al. examined the relationship between hypothyroid and hyperthyroid patients' psychiatric diseases. Depression, psychosis, and cognitive dysfunction were more common in hypothyroid patients, while psychosis, aggression, anxiety, and cognitive impairment were more prominent in hyperthyroid patients [11]. Kathol et al. found that psychiatric diseases are associated with thyroid dysfunction in individuals. The incidence of depression increased from 9% to 44%; anxiety disorder increased from 6% to 47% [12]. In another study, the percentage of people diagnosed with depression during their lifetime was 10-25%, but

this rate was 5-12% in men [13]. In addition, depression can be seen in 31-69% of patients with thyroid dysfunction; on the other hand, thyroid dysfunctions were observed in 5-10% of patients diagnosed with depression [12]. Another study examined the patients applied to the nuclear medicine unit and followed up after the diagnosis of thyroid dysfunction. The effect of their past lives and the efficacy of the treatment for endocrinological diseases in the emergence of problems related to their mental states were questioned. 41% of hypothyroid patients had depression, and 59% had anxiety symptoms [14]. Similarly, in this study, the depression rate was two times higher among patients with Hashimoto thyroiditis and four times higher in patients with thyroidectomy compared to the control group. On the other hand, although anxiety was variable, it was higher in people with Hashimoto thyroiditis and thyroidectomy than in the control group. This study supports the relationship between thyroid dysfunction and psychiatric diseases. Therefore, in the clinic, care is needed for thyroid dysfunction in patients resistant to antidepressant treatment and psychological effects in noncompliant patients receiving hypothyroidism treatment.

Regarding the effects of having a chronic illness, it can be said that factors such as constant drug use, frequent doctor check-ups, and frequent blood tests affect patients' psychology, regardless of the illness. In Katon's study, depression rates were higher in chronic patients, and it was shown that a pre-existing medical disease could cause this symptom to increase. [15]. Rogers et al. examined the relationship between some medical diseases and anxiety, and the peptic ulcer was more common in people with anxiety. In addition, regarding gender, they reported that angina in men and thyroid diseases in women were associated with anxiety [16]. Numerous studies reported that constant drug use increases psychiatric disorders, especially anxiety disorders [17, 18]. In this study, anxiety and depression rates were higher in patients with Hashimoto thyroiditis and thyroidectomy than in the control group due to the adverse psychologic effects of having a chronic disease.

Studies examining the effectiveness of thyroid replacement therapy on mood and quality of life in patients with hypothyroidism did not reach a consensus. Hypotheses have been proposed for the effect of triiodothyronine on central noradrenergic receptors [19]. It is thought that its replacement would make improvements in central systems. However, some studies argue that psychological symptoms improve with treatment, while others show that the psychological effect is permanent and improvement with treatment is impossible [20,21]. Some studies reported partial improvements. Making the patient's euthyroid as soon as possible is very important; many studies focused on the dose and application forms required for this underlining the importance of early diagnosis [22,23].

As it is known, the L-thyroxine needs of hypothyroid people with and without thyroid tissue are not the same. The need for L-thyroxine depends on age, gender, and body weight. An endogenous thyroid hormone secretion occurs in patients with Hashimoto thyroiditis; their daily L-thyroxine need is 75-100 ug/day (1,6 ug/kg/day), while the daily L-thyroxine need of people with thyroidectomy is 100-250 ug/day[2]. In this study, there was no statistical difference between groups regarding age, gender, and BMI ($p<0.05$). Obviously, the need for thyroid replacement is proportional to the remaining thyroid tissue. Besides this, the dose difference and the unexplained effects of the remaining thyroid tissue are also mentioned. Ito et al. reported that the T3 values of people who recently undergone total thyroidectomy were lower than their preoperative levels, showing the role of the thyroid gland in regulating the hormonal balance. In addition, this study indicated that people with thyroidectomy were deprived of maintaining hormone balance [24]. In another study, Ito et al. examined the relatively low T3 levels in patients with thyroidectomy under L-thyroxine treatment and showed the effect of endogenous secretion [25]. Gullo et al. tested the knowledge that the patients receiving L-thyroxine monotherapy treatment meet their needs at the tissue level by transforming the thyroid hormone in the peripheral tissues. Regarding whether all patients can achieve adequate transformation, more than 20% of the patients' TSH was in the normal range. However, their T4 and T3 were outside the reference ranges, showing that peripheral deiodination was insufficient.

For this reason, it was thought that further physiological treatment is needed in some hypothyroid patients [26]. This study has led to considering whether it is possible to close the physiological deficit with the treatment given. It revealed that the depression and anxiety of patients with Hashimoto thyroiditis are lower than those with thyroidectomy. The unknown physiological effect of the existing thyroid tissue-derived endogenous hormone secretion or the presence of thyroid tissue that has not yet been elucidated may have a positive effect on psychology. The lack of thyroid tissue may not be felt at all in the future with treatment options that can complete this physiological deficiency.

Up to now, many studies have assessed the quality of life. As hypothyroidism symptoms reminded the diseases that impair the quality of life, these studies addressed hypothyroidism, one of society's most common endocrinological diseases. It affects people's quality of life due to the disease's symptoms and mental health deterioration [27,29]. Published studies show that some individuals' quality of life does not improve even with treatment [27,29]. Winter et al. administered the SF-36 to the patients with

hypothyroidism before the L-thyroxine treatment, during the sixth week of the treatment, and during the sixth month. After the treatment, some improvement was reported in the patient's quality of life [27]. McMillan et al. administered the Thyroid Dependent Quality of Life Questionnaire and the Thyroid Treatment Satisfaction Questionnaire to patients with hypothyroidism. Nevertheless, patients' quality of life did not increase after the treatment [29]. Samuel et al. reported no improvement in the symptoms of patients who received thyroid replacement therapy [30]. In this study, SF-36 sub-scales showed that patients with thyroidectomy had a poorer quality of life than patients with Hashimoto thyroiditis. Both groups' quality of life is poorer compared to the control group. This result shows that the diagnosis and treatment of hypothyroidism should be started without affecting the quality of life and psychological state.

Limitations Of The Study

Our study has the same limitations. The first is that since we are in the pandemic period, the number of our patients is limited. Another limitation is many factors that affect mental health. More extensive studies are needed on this subject.

Conclusion

The anxiety and depression of the patients with Hashimoto thyroiditis and thyroidectomy were higher than those in the control group. It may be related to the generic patient's psychology, chronic disease psychology, and continuous drug use. Mental health may have been affected during the period of thyroid dysfunction, which may have caused permanent problems, even if they are now euthyroid. It was concluded that patients with Hashimoto thyroiditis were more depressed and anxious and had a poorer quality of life than the control group due to the autoimmunity effect. On the other hand, people with thyroidectomy are more affected than those with Hashimoto's thyroiditis. Their quality of life is poorer due to the lack of endogenous hormone secretion and/or the undiscovered physiological effect of the thyroid gland. It has been concluded that maximal protection of the thyroid gland is vital.

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Effect of Coronavirus Disease 2019 on Fluorine-18 fluorodeoxyglucose Uptake of Endocrine Organs

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Research Article

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ABSTRACT

Purpose: The new type of Coronavirus (SARS-CoV-2) damages cells by using the angiotensin converting enzyme-2 (ACE2) as a receptor to adhere and go through the cell membrane. It is known that some of the endocrine organs express ACE2 and these organs are potential targets for Coronavirus 2019 disease (Covid-19). This study aimed to investigate the effect of Covid-19 on Fluorine-18 fluorodeoxyglucose (18F-FDG) uptake of endocrine system organs.

Material and method: Sixteen patients who had Covid-19 underwent 18F-FDG positron emission tomography/computed tomography (PET/CT) later, 77 patients who did not have Covid-19 underwent 18F-FDG PET/CT between March 2020-October 2021 were included. SUVmax and SUVmean of the pituitary, thyroid, adrenal gland, pancreas, and testis measured from the PET/CT of the patients who had Covid-19 were compared with SUVmax, and SUVmean measured from the same organs in PET/CT images of the patients who had not Covid-19.

Results: Pancreatic mean SUVmax was significantly higher in patients who had Covid-19 than in patients who did not (p= 0.035). Pancreatic mean SUVmean was slightly higher in patients who had Covid-19 than in patients who did not, but this difference was not statistically significant (p= 0.072). No significant difference was found between the SUVmax and SUVmean values of the pituitary gland, thyroid gland, adrenal gland, and testis in patients who had Covid-19 and did not have.

Conclusion: : It was thought that the pancreas might have been affected in the course of Covid-19 due to the higher mean SUVmean values of the pancreas in patients who had Covid- 19.

Keywords: Covid-19, endocrine system, FDG, PET/CT.

Coronavirüs Hastalığı 2019'un Endokrin Organların Flor-18 florodeoksiglukoz Alımına Etkisi

Süreç

Geliş: 22/12/2022
Kabul: 13/02/2023

ÖZ

Amaç: Yeni tip koronavirüs (SARS-CoV-2) anjiyotension dönüştürücü enzim-2'yi (ACE2)reseptör olarak kullanarak hücreye tutunur, hücre membranı geçer ve hücreye zarar verir. Bazı endokrin organların ACE2 eksprese ettiği bilinmektedir ve bu organlar Covid-19 için hedef olma potansiyeline sahiptir. Bu çalışmada Covid-19'un endokrin sistem organlarındaki F-18 florodeoksiglukoz (18F-FDG) tutulumuna etkisini araştırmak amaçlanmıştır.

Gereç ve yöntem: Mart 2020-Ekim 2021 arasında Covid-19 geçirip sonrasında 18F-FDG PET/BT çekimi yapılan 16 hasta ile Covid-19 geçirmeyen 77 hastanın verileri analiz edildi. Covid-19 geçiren ve geçirmeyen hastaların hipofiz bezi, tiroid bezi, adrenal bez, pankreas ve testislerinden ölçülen SUVmax ve SUVmean değerleri karşılaştırıldı.

Bulgular: Covid-19 geçiren hastaların ortalama pankreas SUVmax değeri Covid-19 geçirmeyen hastalarınkinden daha yüksekti (p= 0.032). Covid-19 geçiren hastaların ortalama pankreas SUVmean değeri Covid-19 geçirmeyen hastalarınkinden daha yüksekti ancak bu fark istatistiksel olarak anlamlı düzeyde değildi (p= 0.072). Covid-19 geçiren ve geçirmeyen hastaların hipofiz bezi, tiroid bezi, sürrenal bez ve testislerinden ölçülen SUVmax ve SUVmean değerleri arasında anlamlı düzeyde farklılık saptanmadı.

Sonuç: Covid-19 geçiren hastalarda ortalama pankreas SUVmean değerinin daha yüksek olması nedeniyle pankreasın Covid-19 sürecinde etkilenmiş olabileceği düşünüldü.

Anahtar Kelimeler: Covid-19, endokrin sistem, FDG, PET/BT.

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Introduction

The Coronavirus 2019 disease (Covid-19), which emerged in Wuhan, China, in December 2019, spread all over the world in a short time, and the World Health Organization (WHO) declared Covid 19 a pandemic on March 11, 2020. The new type of coronavirus (SARS-CoV-2), the causative agent of Covid-19 (1), has mutated, revealing new, more infectious variants (Alpha, Beta, Gamma, Delta, and Omicron, etc.) (2-4).

Covid-19 usually manifests itself with mild upper respiratory tract infections or is asymptomatic in people with a robust immune system and no severe comorbidities (5). The most frequently affected organ system in severe cases is the pulmonary system (5,6). However, many extrapulmonary manifestations have been reported (5). Hyperthyroidism and hypothyroidism are among the best-known effects of Covid-19 on the endocrine system (7,8). The effect of Covid-19 on other endocrine organs other than the thyroid gland has not yet been clarified. It was revealed in the severe acute respiratory syndrome (SARS) epidemic in 2003 that the coronavirus could affect the endocrine system (9). It is known that Fluorine-18 fluorodeoxyglucose (18F-FDG) has a high uptake in inflammatory pathologies. Therefore, in the presence of inflammation in endocrine organs, 18F-FDG uptake of these organs may increase. This study aims to investigate the effect of Covid-19 on 18F-FDG uptake/glucose metabolism of endocrine system organs.

Materials

All patients who underwent 18F-FDG positron emission tomography/computed tomography (PET/CT) in our hospital and applied to the Covid-19 pandemic outpatient clinic between March 2020 and October 2021 were identified. Among these patients, those who did not have a real-time polymerase chain reaction (RT-PCR) test were excluded. Finally, 93 patients who underwent 18F-FDG PET/CT in our clinic and whose RT-PCR was studied from nasal and throat swabs for Covid-19 for any reason were included in the study. During this period, PET/CT was not performed on patients with symptoms such as fever, cough, sore throat that may be associated with Covid-19, unless the RT-PCR result was negative. Maximum standardized uptake value (SUVmax) and mean standardized uptake value (SUVmean) (threshold of 40% of SUVmax) were calculated by placing a circular region of interest (ROI) in the localization of the pituitary fossa, thyroid gland, pancreas, adrenal gland and testes of male patients on PET/CT fusion images (Figure 1). Patients with 18F-FDG uptake suggest primary malignancy associated with the aforementioned endocrine organs or metastasis in these organs were excluded. Since four of 16 patients who had Covid-19 had pancreatic cancer, SUV measurements were not made from the pancreas of these patients. Since the hypothalamus, pineal gland, parathyroid glands, and ovaries could not be distinguished in 18F-FDG PET/CT, SUV values of these organs could not be calculated.

18F-FDG was injected intravenously at a 3.7 MBq/kg (0.1 mCi/kg) dose to patients with blood glucose levels below 200 mg/dl after fasting for at least 4-6 hours. Before PET/CT, the patients rested for 60 minutes in a single room. PET/CT images were obtained from the vertex to the upper thigh (Siemens Biograph mCT 20). First, low-dose CT images were acquired with 120 kVp, 50 mAs, iodine-containing oral contrast, and free-breathing protocol. Then, PET images were obtained with a 2 min/bed position in three-dimensional mode. A nuclear medicine physician with five years of oncological PET/CT experience evaluated images at the Siemens syngo.via workstation.

SUVmax and SUVmean values of the pituitary, thyroid, adrenal gland, pancreas, and testis measured from the first PET/CT performed in the post-Covid-19 period of patients had Covid-19 were compared with SUVmax, and SUVmean values measured from the areas mentioned earlier in PET/CT images performed during the staging phase of the diseases of the patients who had not Covid-19. The independent sample t-test was used to make this comparison. Only eight patients with Covid-19 had PET/CT images before Covid-19. The SUVmax and SUVmean values obtained from the previously mentioned endocrine organs from these patients' pre- and post-Covid-19 images were compared. The Wilcoxon test was used to make this comparison. In all statistical tests, $p < 0.05$ was considered statistically significant. All Statistical analyzes were performed with SPSS version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Results

Post-infective PET/CT imaging of patients who had Covid-19 was performed median of 130 (45-284) days after nasal and throat swab RT-PCR results. Detailed demographic information is in table 1.

Table 1. Patient characteristics

	Patients infected by Covid-19 n (%)	**Patients not infected by Covid-19** n (%)	**p-value**
Gender			0.479
- Female	6	22	
- Male	10	55	
Primary malignancy	- Lung 5 (31) - Pancreas 4 (25) - Breast 3 (19) - Others 4 (25)	- Lung 24 (31) - Unknown primary 9 (11) - Larynx 6 (7) - Others 39 (51)	
	Mean ± SD	Mean ± SD	
Age (years)	65 ± 11	66 ± 13	0.737
Blood glucose level during FDG injection (mg/dL)	104 ± 15	102 ± 18	0.624
Dose of FDG (MBq)	274 ± 85 (7.4 ± 2.3 mCi)	285 ± 48 (7.7 ± 1.3 mCi)	0.416

Covid-19: Coronavirus disease 2019, FDG: Fluorodeoxyglucose

Only six of 16 patients who had Covid-19 did not receive oncological treatment at the time of post-Covid-19 PET/CT, and imaging was performed for cancer staging in these patients; In the other ten patients, the imaging purpose was to evaluate treatment response. Pancreatic mean SUVmax value was slightly higher in patients who had Covid-19 (2.1 ± 0.3) than in patients who did not (1.9 ± 0.4) (p= 0.035) (Table 2). In addition, pancreatic mean SUVmean value was slightly higher in patients who had Covid-19 (1.6 ± 0.3) than in patients who did not (1.4 ± 0.3), but this difference was not statistically significant (p= 0.072). SUVmax and SUVmean values of the pituitary gland, thyroid gland, adrenal gland, and testis did not differ between the patients who had Covid-19 and who did not (Table 2).

Table 2. SUVmax and SUVmean values of the endocrine organs

	Patients infected by Covid-19 (n=16) Mean ± SD	**Patients not infected by Covid-19 (n=77)** Mean ± SD	**p-value**
Pituitary gland			
- *SUVmax*	2.6 ± 0.6	2.8 ± 0.7	0.506
- *SUVmean*	1.8 ± 0.4	2.0 ± 0.5	0.361
Thyroid gland			
- *SUVmax*	2.2 ± 0.8	2.0 ± 0.5	0.228
- *SUVmean*	1.6 ± 0.7	1.6 ± 0.4	0.964
*Pancreas			
- *SUVmax*	2.1 ± 0.3	1.9 ± 0.4	**0.035**
- *SUVmean*	1.6 ± 0.3	1.4 ± 0.3	0.072
Adrenal gland			
- *SUVmax*	2.1 ± 0.4	2.3 ± 0.6	0.175
- *SUVmean*	1.6 ± 0.4	1.6 ± 0.4	0.948
Testicle			
- *SUVmax*	2.9 ± 0.8	3.2 ± 0.6	0.598
- *SUVmean*	1.9 ± 0.6	2.5 ± 0.8	0.207

SUV: standardized uptake value, Covid-19: Coronavirus disease 2019, SD: Standard deviation

* n=12 for postinfected group, because 4 patients had focal F-18 FDG uptake in pancreas due to pancreatic cancer.

No significant difference was found between the SUVmax and SUVmean values of the pituitary gland, thyroid gland, adrenal gland, pancreas, and testis before and after Covid-19 in 8 patients who had Covid-19 and had PET/CT examination both before and after infection (Table 3).

Table 3. Comparison of the Groups' Depression Levels According to the Beck Depression Inventory

Table 3. SUVmax and SUVmean values of the endocrine organs befere and after Covid-19 (n=8)

	Before Covid-19		**After Covid-19**		**p-value**
	Mean ± SD	Median (min - max)	Mean ± SD	Median (min-max)	
Pituitary gland					
- *SUVmax*	2.7 ± 0.6	2.7 (1.9 – 3.4)	2.6 ± 0.5	2.3 (2.0 – 3.2)	0.596
- *SUVmean*	1.9 ± 0.4	1.9 (1.5 – 2.6)	1.8 ± 0.3	1.6 (1.5 – 2.2)	0.129
Thyroid gland					
- *SUVmax*	1.8 ± 0.4	2.0 (1.2 – 2.4)	2.1 ± 0.6	2.3 (1.2 – 2.6)	0.416
- *SUVmean*	1.6 ± 0.3	1.6 (1.1 – 1.9)	1.6 ± 0.4	1.7 (1.1 – 2.0)	0.414
Pancreas					
- *SUVmax*	1.9 ± 0.5	1.9 (1.4 – 2.7)	2.1 ± 0.3	2.1 (1.6 – 2.5)	0.115
- *SUVmean*	1.0 ± 0.6	1.2 (0.9 – 1.7)	1.6 ± 0.3	1.5 (1.3 – 2.1)	0.114
Adrenal gland					
- *SUVmax*	2.1 ± 0.5	2.1 (1.4 – 2.8)	2.2 ± 0.4	2.2 (1.7 – 2.7)	0.734
- *SUVmean*	1.7 ± 0.5	1.6 (1.2 – 2.5)	1.8 ± 0.4	1.7 (1.1 – 2.3)	1.000
Testicle					
- *SUVmax*	3.5 ± 0.8	3.7 (2.4 – 4.3)	2.9 ± 0.7	3.0 (2.1 – 3.4)	0.068
- *SUVmean*	2.3 ± 0.4	2.4 (1.8 – 2.7)	1.9 ± 0.4	2.0 (1.5 – 2.3)	0.144

SUV: standardized uptake value, Covid-19: Coronavirus disease 2019

Discussion

In the light of the information obtained from the SARS epidemic, the endocrine system was thought to be a potential target for SARS-CoV-2 (9). Findings of central adrenal insufficiency in some patients after SARS epidemic, thyroid follicular epithelial damage and decrease in TSH in some patients, presence of SARS-CoV in pancreatic tissue, increase in prolactin, luteinizing hormone and follicle-stimulating hormone, decrease in estrogen and progesterone after SARS in women, the demonstration of microscopic damage to the testicular tissue of patients who died from SARS and the high angiotensin converting enzyme-2 (ACE2) expression in the testis were the basis of this thought (9). Clinically, it has been shown in some retrospective studies that Covid-19 can cause endocrinological problems, especially thyroid dysfunction (10,11).

Considering this information, we examined whether there were changes in the glucose metabolisms of the endocrine system organs after Covid-19. Pancreatic SUVmax values of patients who had Covid-19 were slightly higher than those who did not (p= 0.035). Among these patients, SUV measurement was not performed in the pancreas of 4 patients with primary pancreatic cancer. None of the remaining 12 patients had focal or diffuse pathological ^{18}F-FDG uptake in their pancreas. Five of these patients had lung cancer, but none had a history of immunotherapy that could cause pancreatitis. Wang et al. reported elevated pancreatic enzymes in 17% of 52 patients hospitalized for Covid-19 pneumonia (12).

Bruno et al. showed that 8.5% of the 70 patients hospitalized for Covid-19 who had pneumonia had elevated pancreatic enzymes (13). In the two studies mentioned, the patients had no acute pancreatitis clinically. Since the patients in our study applied to the hospital for cancer staging or oncological treatment response evaluation, amylase and lipase laboratory test results were not available in the routine clinical evaluation of the patients. Therefore, the relationship between pancreatic ^{18}F-FDG uptake after Covid-19 and pancreatic enzymes could not be examined. The expression of ACE2 messenger RNA in pancreatic tissue (14,15) and the presence of case reports about Covid-19-associated acute pancreatitis (16-18) suggest that the pancreas may be vulnerable to the attack of SARS-CoV-2. We think that the mild SUVmax elevation, which we detected after Covid-19 in the pancreas, may be related to mild inflammation.

Lania et al. detected thyrotoxicosis in 20.2% of the 287 Covid-19 patients who were hospitalized (10). They found overt thyrotoxicosis in 53.4% of the patients with thyrotoxicosis. They reported an inverse correlation between thyroid stimulatin hormone (TSH) and interleukin 6 (rho= −0.41; $p < 0.001$). The same study reported that 5.2% of the patients had hypothyroidism. Our study showed no difference between SUVmax and SUVmean of the thyroid glands of patients who had and did not have Covid-19. There was no difference between pre-Covid-19 and post-Covid-19 SUVmax and SUVmean of the thyroid glands of the eight patients who underwent PET/CT before and after Covid-19. The patients' history of hospitalization due to Covid-19, serum TSH, free T3 and free T4 hormones, and thyroid antibody levels during the PET/CT were unknown. The small number of patients in this study and perhaps the fact that most of the patients had mild Covid-19 may be the reason why there was no diffuse increase in ^{18}F-FDG uptake in the thyroid gland. Also, Albano et al. reported that the most common pathology in patients with diffuse ^{18}F-FDG uptake in the thyroid gland is thyroiditis (19). However, not all patients with thyroiditis may show diffuse ^{18}F-FDG uptake. For this reason, we think that the presence of thyroiditis cannot be definitively excluded in the patients in our study who had Covid-19.

In their review, Frara et al. reported that cases of pituitary apoplexy, syndrome of inappropriate antidiuretic hormone, and hypophysitis associated with Covid-19 have not yet been proven (20). Similarly, Covid-19-related adrenal gland and testicular inflammation have not been proven. In our study, there was no difference between the SUVmax and SUVmean values of the pituitary, adrenal gland, and testis in patients who had and did not have Covid-19.

Study Limitations

Our study has some limitations. The small number of patients who had and did not have Covid-19 is is an important factor that may affect the results of statistical analysis. The high number of patients who had PET/CT scans both before and after Covid-19 could have enabled us to more accurately evaluate the effect of Covid-19 on ^{18}F-FDG uptake of endocrine organs. Because the laboratory test results associated with organs whose SUV values were measured were absent in our study, we could not evaluate the glucose uptake of these organs parallel to test results such as thyroid function tests, amylase, and lipase. In addition, among the patients included in our study, asymptomatic carriers who did not have a PCR test and the presence of patients with false negative or false positive PCR test results cannot be definitively excluded.

Conclusion

The pancreas, like many organs, is a possible target for SARS-CoV2 due to its expression of ACE2 mRNA. In our study, the slightly higher ^{18}F-FDG uptake in pancreas in the patients who had Covid-19 compared to those who did not have the disease suggested that the pancreas may have been affected in the course of Covid-19.

Ethics Committee Approval: This study was approved by Ethics Committee and conducted according to the principles of the Declaration of Helsinki (Decision date: 14.12.2021, approval number: 2021/213).

Informed Consent: The ethical committee waived the requirement for informed consent as the study was retrospective.

Authors' Contributions: Concept- O.B. ; Design- O.B., S.G., D.N. ; Supervision: O.B. ; Data Collection and Processing: O.B., S.G., D.N. ; Analysis and/or Interpretation: O.B, S.G., D.N. ; Literature Search: O.B., ; Writing: O.B.

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Evaluation of Endometrial Bcl-2 Expression and Ki-67 Proliferative Index in Infertile Patients with and without Polycystic Ovary Syndrome

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Review Article

History

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ABSTRACT

Background: In women of reproductive age, polycystic ovary syndrome (PCOS) is the most prevalent cause of infertility. The purpose of this study is to compare the Bcl-2 and Ki-67 values between infertile patients with and without PCOS.

Methods: The study included 27 infertile patients diagnosed with PCOS and 28 infertile patients who did not meet the criteria for PCOS. Smoking, pelvic infection symptoms, endometrial polyps and submucosal myomas during a transvaginal ultrasound, pituitary insufficiency, hyperprolactinemia, congenital adrenal hyperplasia, having had adnexal surgery and having a male factor that will result in infertility are all considered exclusion criteria. All patients' data were collected, including age, the length of their infertility, body mass index (BMI), waist-to-hip ratio (WHR), hirsutism score, blood pressure, total testosterone, triglyceride, total cholesterol, LDL, and HDL values, as well as Homa-IR and Hs-CRP readings. The pathology specialist in the examples evaluated Bcl-2 and Ki-67 levels.

Results: We found that BMI, WHR, total testosterone level, blood pressure, total cholesterol, HOMA-IR, and hs-CRP values were significantly higher in infertile cases with PCOS. We also found that the Ki-67 and Bcl-2 values were higher in endometrial cells in sterile PCOS cases than in the control group.

Conclusions: Ki-67 and Bcl-2 levels rise in PCOS patients, preventing apoptosis, limiting the formation of a suitable endometrial environment, and preventing embryo implantation. PCOS patients frequently experience infertility and recurrent pregnancy losses. The cause of this problem may be the increased activity of estrogen. The primary treatment for PCOS will depend on further investigation into the variables that affect GnRH release, and the care plan should be built around this goal.

Keywords: Apoptosis, Bcl-2, Infertility, Ki-67, Polycystic Ovary Syndrome

Polikistik Over Sendromu Olan ve Olmayan İnfertil Olgularda Endometrial Bcl-2 Ekspresyonu ve Ki-67 Proliferatif İndeksinin Değerlendirilmesi

Süreç

Geliş: 12/02/2023
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ÖZ

Amaç: Polikistik over sendromu üreme çağındaki kadınlarda görülen en sık infertilite nedenidir. Bu çalışmanın amacı; polikistik over sendromu olan ve olmayan infertil hastalardaki Bcl-2ve Ki-67 değerleri arasındaki farkı biyokimyasal parametreler eşliğinde incelemektir.

Yöntem: Çalışmaya polikistik over sendromu teşhisi konulan 27 infertil hasta ve polikistik over sendromu kriterlerine uymayan 28 infertil hasta dahil edildi. Çalışmadan dışlanma kriterleri; kişinin sigara kullanması, pelvik enfeksiyon bulgusu, transvajinal ultrasonografide endometrial polip, submüköz myom vb. saptanması, hipofizer yetmezlik, hiperprolaktinemi, konjenital adrenal hiperplazisi olmak, adneksiyal cerrahi geçirmiş olmak ve infertiliteye sebep olacak erkek faktörü bulunmaktır. Tüm bireylerin yaş, infertilite süresi, vücut- kitle indeksi, bel-kalça oranı, hirsutizm skoru, kan basınçları, total testesteron, trigliserit, total kolesterol, LDL, HDL değerleri, HOMA-IR ve hsCRP değerleri not edildi. Patoloji uzmanı tarafından Bcl-2ve Ki-67 düzeyleri değerlendirildi.

Bulgular: PKOS'lu infertil olgularda vücut- kitle indeksi, bel-kalça oranı, total testosteron seviyesini, kan basıncını, total kolesterol değerini, HOMA-IR ve hs-CRP değerlerini belirgin yüksek bulduk. Ayrıca Ki-67 ve Bcl-2 değerinin PKOS'lu infertil olgularda endometriyal hücrelerde kontrol grubuna oranla daha yüksek olduğunu bulduk. **Sonuç**: Ki-67 ve Bcl2 değerleri, PKOS'lu olgularda artarak, apopitozisin gerçekleşmesini engeller ve uygun endometriyal ortam oluşmasını engelleyerek de embriyonun implantasyon sürecini kısıtlar. Bu durum PKOS'lu olgularda infertilite ve tekrarlayan gebelik kayıplarına neden olmaktadır. Bu soruna artmış östrojen aktivitesi sebep olmaktadır. PKOS'un ana tedavisinde özellikle GnRH salınımını düzenleyici ajanlar üzerine çalışmalar yapılmasına ihtiyaç duyulduğu ve tedavinin bu hedef üzerine şekillenmesi gerektiği kanaatindeyiz.

Anahtar sözcükler: Apopitozis, BCL-2, İnfertilite, Kİ-67, Polikistik Over Sendromu

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in reproductive women[1]. Although its prevalence varies according to societies and diagnostic criteria, it is between approximately 8 % to 13 % [2]. Clinical features of PCOS on ultrasound include polycystic ovarian appearance, oligo/anovulation, hormonal and clinical hyperandrogenemia, and infertility [3]. It is a complex disease whose etiopathogenesis is accused of factors such as genetic sensitivity, epigenetic mechanisms, insulin resistance, environmental effects, steroid metabolism changes, and lifestyle [4]. Etiology is still not fully illuminated. Due to all these etiological reasons, the frequency and amplitude of luteinizing hormone (LH) release increase due to insensitivity to gonadotropin-releasing hormone (GnRH) at the pituitary level. The rise in LH is more significant than the increase in follicle-stimulating hormone (FSH). This increase in LH stimulates androgen synthesis in theca cells. Impaired FSH synthesis and release leads to insufficient stimulation of granulosa cells, resulting in decreased aromatase activity. This condition causes an increase in circulating androgen levels [5]. Hirsutism and obesity resulting from hyperinsulinemia and hyperandrogenemia are among the leading physiological causes of PCOS [6]. PCOS may be associated with severe health issues such as diabetes, coronary heart disease, and cancer. Furthermore, endometrial hyperplasia and endometrial cancer are more likely to develop when estrogen is not balanced by progesterone[7,8]. PCOS, a metabolic syndrome, is an essential reproductive disorder and is the most common cause of infertility due to anovulation in reproductive women [9].

It has been shown that PCOS increases preeclampsia in pregnancy by 3 to 4 times and causes miscarriages, gestational diabetes mellitus (DM), and premature births. This situation suggests that PCOS disrupts the placentation process [10,11]. Fauser et al. have noted changes in many biochemical markers associated with chronic inflammation, endothelial dysfunction, hyperandrogenism, dyslipidemia, obesity, and insulin resistance in patients with PCOS [7]. Studies show that hyperandrogenism and insulin elevation can cause changes in endometrial functions by affecting the expression of growth factor receptors and steroid receptors [10,12].

Due to these changes caused by PCOS, homeostasis is disrupted throughout the body. Programmed cell death is an essential regulatory mechanism in achieving homeostasis; apoptosis is also affected. Achieving a normal endometrial cycle depends on the balance between apoptosis and mitosis [13]. The increase or decrease of apoptosis can cause many diseases. Patients diagnosed with PCOS increased the Bcl-2/Bax ratio due to the release of estrogen not being met with progesterone in the endometrium [8].

This study aimed to compare histopathological Bcl-2 and Ki 67 expression rates in endometrial tissue samples in infertile patients with and without PCOS and to evaluate them biochemically in light of hormonal parameters.

Materials and Methods

The Balıkesir University Faculty of Medicine Clinical Research Ethics Committee approved this prospective study. Sixty patients aged 18-45 who applied to Balıkesir University Obstetrics and Gynecology Clinic with infertility complaints were selected for the study. A detailed informed consent form was obtained from all participants. Each patient was given detailed information about the study to be performed, and their verbal and written informed consents were obtained. 2 patients were removed from the control group, and three patients were removed from the study group because they did not meet the research criteria. Patients were divided into two groups 27 infertile patients diagnosed with PCOS and 28 infertile patients with normal endometrial findings who did not meet the requirements of PCOS. The study group of infertile patients diagnosed with PCOS based on 2003 Rotterdam ESHRE/ASRM consensus criteria [14]. Patients with normal endometrial findings diagnosed with unexplained infertility constituted the control group criteria for excluding volunteers from research. Cases with endometrial polyps, submucosal myoma, pathology detection in transvaginal ultrasonography or endometrial sampling by the patient, endocrine diseases such as pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia, ovarian or adnexal surgery and male factor that will cause infertility were accepted as cases with male factors that would cause infertility and the instances that voluntarily left the study were excluded from the study. In these cases, age, body mass index (BMI), waist-hip ratio (WHR), hirsutism score, blood pressure, and duration of infertility were recorded.

Biochemical evaluation

5cc venous blood was taken from all patients for basal hormonal evaluation. After centrifugation (850g, 10 min), serum samples were obtained, emptied into Eppendorf tubes (Eppendorf, Hamburg, Germany), and stored in a deep freezer at 80 degrees Celsius until biochemistry analysis was performed. Serum samples were analyzed by a complete quantitative method. Follicular Stimulant hormone (FSH, mIU/mL), Luteinizing hormone (LH, mIU/mL), Estradiol (E2, pg/mL), Total testosterone (ng/dl), Triglyceride (mg/dl), Total cholesterol (mg/dl), LDL (mg/dl), HDL (mg/dl) were analyzed by complete quantitative method (Cobas Integra 800; Roche Diagnostics GmbH; Mannheim, Germany), hs-CRP was evaluated using the chemiluminescent immunoassay method (ADVIA Centaur XP, Siemens Healthcare Diagnostics, NY, USA).

Insulin values were studied with hormone auto-analyzer devices (Beckman Coulter; Unicel DXI 600; Access Immunoassay System). HOMA-IR (mg/dl) (fasting insulin x fasting glucose)/ (constant) was calculated with the formula. Since the fasting glucose value was calculated as mg/dl, the constant was taken as 450, and the limit value was accepted as 2.4.

Hysteroscopic endometrial biopsy sampling

Hysteroscopic endometrial biopsy sampling with sedation was taken from all the patients who participated in the study. Sample endometrial tissues were first followed by light microscopy. Then an immunohistochemistry study was performed to mark them with hematoxylin-eosin (H-E) stain and Ki-67 and Bcl-2.

Light microscopy and Immunohistochemical evaluation

1. Endometrial tissue samples were fixed 24 hours daily in 10% formaldehyde.
2. Sections were passed through increasing ethanol series.
    - 90% ethanol.....1 day
    - 96% ethanol.....1 day
    - Pure ethanol ..... 1 day
    - Toluol .....1 hour
3. The tissues were kept in liquid paraffin at 35°C temperature twice for 1 hour to prepare for cross-section. The tissues were then embedded in paraffin inside square-shaped iron blocks. The paraffin blocks with tissue were removed from the iron blocks after cooling. 4-micron endometrial tissue sections taken from paraffin blocks using microtome were taken separately for each staining on 1/10 poly-L-lysine treated slides. The sections were kept in a 56°C oven for the night to remove the paraffin. They were secondarily put through xylene and decreasing ethanol series to remove the paraffines. Toluol 2x30 minutes, Pure ethanol 10 minutes, 96% ethanol 10 minutes, 90% ethanol 10 minutes, 70% ethanol 10 minutes, distilled water 2x5 minutes, sections were kept in the dark for 20 minutes in 1% H2O2 prepared with methanol to prevent endogenous peroxidase activities. The sections were then renewed in phosphate buffer containing 0.1 % triton-X-100 and kept times for 5 minutes. The sections were transferred to a plastic shawl containing ten10 mM citrate buffer (pH:6) to ensure antigen reversal, and the citrate buffer was renewed each time in the microwave oven and kept three times for 2 minutes. The sections removed from the microwave oven were allowed to cool for 20 minutes at room temperature. After threats, the protein block solution containing 0.1% triton-X-100 was renewed in PBS and kept three times for 5 minutes. The sections were placed in the immunohistochemistry and were drawn with a hydrophobic pencil. To prevent non-specific attachment to this area, chitin that closes the epitopes was administered in PBS for 5 minutes. Commercially available primary Ki-67 and Bcl-2 antibodies (1:100 dilution) were added to the sections without removing PBS and refrigerated at four °C for 12 hours. The sections were then renewed each time on PBS and three times for 5 minutes. The sections were then administered a biotin secondary antibody for 1 hour. The sections were then restored on PBS and times for 5 minutes each time. After the sections, streptavidin peroxidase was administered for 30 minutes. The cells were then renewed on PBS and kept twice for 5 minutes. Diaminobenzidine (DAB) chromogen amino ethyl cortisol prepared for sections was administered under a microscope in the dark. In the last stage, Mayer's hematoxylin was applied to threads for 5 minutes for posterior area painting. The sections were soaked in tap water for 15 minutes, then taken into distilled water. After removing water from the sections, the DAB-compatible mounting medium was dripped onto them and covered with lamellae. The sections were imaged using a digital camera and counted using a light microscope. In evaluating Bcl-2 expression, the intensity of positive staining in glandular cells was assessed through the assessment Ki-67, and areas with the highest proliferative activity were selected, which did not include hemorrhage and necrosis from the materials. Ki67 and Bcl-2 expression were examined and scored semi-quantitatively according to staining intensity and size; Ratings were recorded as non-staining (0), (1+) for weak positive staining, and (2+) for intense positive staining.

Statistics

SPSS 22.0 for the Windows program was used for statistical analysis. In assessing the data obtained from the survey, Chi-square, Man Whitney U, and independent T-test were used to determine the difference between the two means in separate groups. The Spearman Rank test evaluated the correlation between the groups. $P < 0.05$ level was significantly assessed.

Results

Patients were divided into two groups (PCOS and control group); descriptive data, including age, BMI, WHR, hirsutism score, blood pressure, and FSH, LH, E2 total testosterone, triglycerides, total cholesterol, LDL,

HDL, HOMA-IR, and hs-CRP values are compared in Table 1.

Table 1. Comparison of PCOS and Control group descriptive parameters

	PCOS group (n:27)	Control group (n:28)	*P*
Age (min-max)	25 (18-34)	26 (21-37)	0.1579
BMI (kg/m^2)	25.3 (19.9-37.8)	21.9 (17.5-27.4)	**< 0.001**
WHR	0.87 (0.70-0.95)	0.74 (0.65-0.97)	**< 0.001**
Hirsutism score	11 (2-14)	1,6 (1-7)	**< 0.001**
Systolic blood pressure (mmHg)	130 (90-140)	100 (90-120)	**< 0.001**
Diastolic blood pressure (mmHg)	80 (60-90)	60 (50-80)	**< 0.001**
FSH (mIU/mL)	6.7 (3.3-10.3)	6.2 (3.2-10.5)	0.9664
LH (mIU/mL)	6.4 (2.2-20.8)	4.8 (2.8-10.0)	**< 0.001**
E2 (pg/mL)	41 (7-120)	29 (14-92)	**< 0.001**
Total Testosteron (ng/dL)	0,52 (0.24-3.26)	0.11(0.07-0.25)	**< 0.001**
Triglycerides (mg/dL)	124 (45-276)	67(44-109)	**< 0.001**
Total cholesterol (mg/dL)	179 (109-257)	173 (107-226)	0.3813
LDL (mg/dL)	99 (64-158)	96 (61-138)	0.2965
HDL (mg/dL)	50 (37-77)	63.5 (36-82)	**0.001**
HOMA-IR	2,62 (0.95-17.02)	1.44 (0.46-5.36)	**0.001**
hs-CRP	3.1(0.3-39.8)	1.1 (0.2-9.8)	**< 0.001**

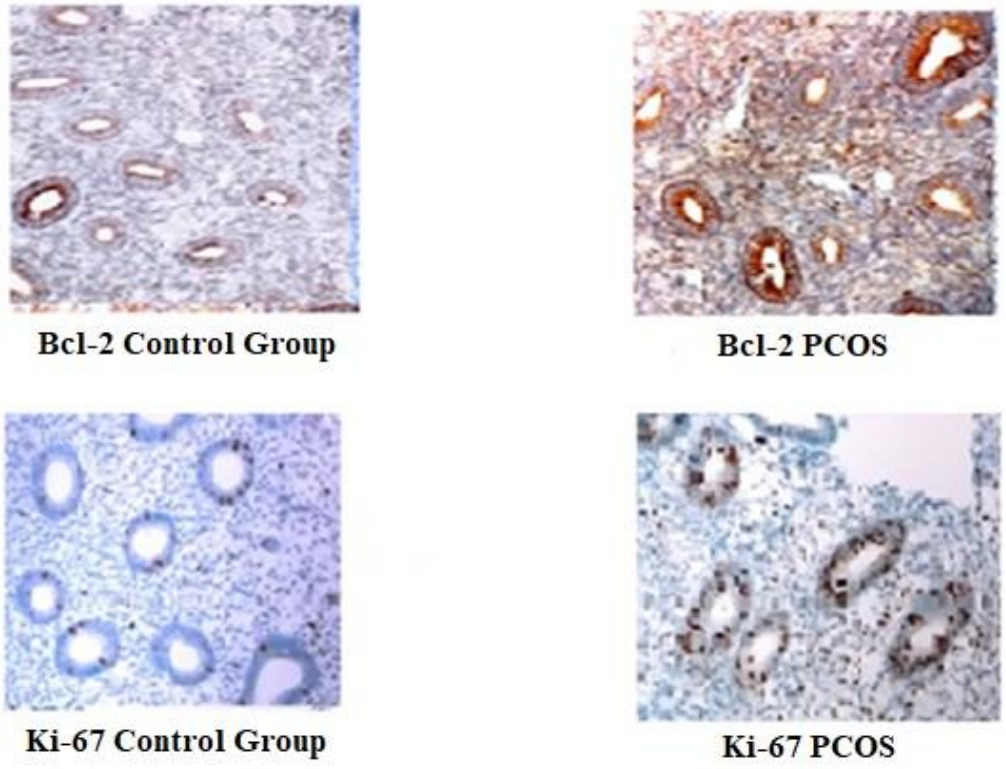
Mann-Whitney test, x^2 test, mean±SEM or median (min-max)

Twenty PCOS patients were during the proliferative phase, while seven were during the secretory phase. There were also nineteen cases in the proliferative phase and nine in the secretory phase in the control group. No statistically significant difference also existed between the groups (p=0.2956).

Light microscopic images of Bcl-2 gene expression and Ki-67 proliferative index in endometrial tissue sampling of patients in both groups are given in Figure 1.

Even while the duration of infertility was 3 (1-6) years in patients with PCOS, it was calculated as 2 (1-8) years in the control group. In terms of the duration of infertility, there was no significant difference between the groups. (p=0.2738).

Figure 1: Bcl-2 gene expression and Ki-67 proliferative index in endometrial biopsy



Light microscopy imaging in PCOS and Control Group

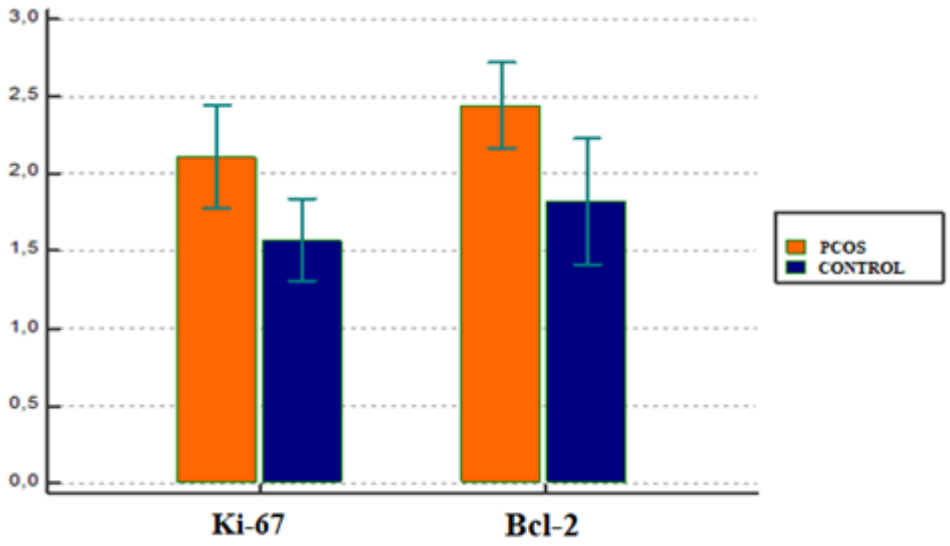
The mean Ki-67 value was determined as 2.1±0.16 in PCOS patients, compared to 1.57±0.13 in the control group. When both groups reached the Ki-67 proliferative index value, the Ki-67 proliferative index value was statistically significantly higher in the PCOS group (p=0.0122). The mean Bcl-2 value in patients with PCOS was 2.46±0.12. In the control group, the mean Bcl-2 value was calculated as 1.78±0.20. Bcl-2 antiapoptotic gene expression was statistically significantly higher in the PCOS group compared to the control group (p=0.0059) (Table 2; Figure 2).

Table 2: Bcl2 and Ki-67 expression values in PCOS and Control groups

	PCOS	Control group	*P*
Kİ-67 expression	2.11±0.16	1.57±0.13	**0.0122**
Bcl-2 expression	2.46±0.12	1.78±0.20	**0.0059**

Independent t-test

Figure 2: Ki-67 and Bcl-2 gene expression in the PCOS group and Control group



Spearman's correlation analysis of PCOS and Control group descriptive parameters shows a positive and significant association between BMI, WHR, Total testosterone, HOMA-IR, and hs-CRP with Bcl-2 antiapoptotic gene expression and Ki-67 proliferative index; the important negative relationship was observed with HDL. The data for the analysis are shown in Table 3.

Table 3. Spearman correlation of descriptive parameters with Ki 67 and Bcl2 expression

	Ki-67		Bcl-2	
	$r_{spearman}$	*p*	$r_{spearman}$	*p*
Age (years)	0.260	0.0550	0.159	0.2463
BMI (kg/m²)	0.5689***	**<0.001**	0.349**	**0.0090**
WHR	0.366**	**0.006**	0.359**	**0.0071**
Hirsutism score	0.226	0.0974	0.263	0.0528
Systolic blood pressure (mmHg)	0.136	0.3212	0.269*	**0.0467**
Diastolic blood pressure (mmHg)	0.229	0.0929	0.199	0.1456
Total testosterone (ng/dL)	0.307*	**0.0225**	0.340*	**0.0111**
Triglycerides (mg/dL)	0.254	0.0611	0.4687***	**<0.001**
Total cholesterol (mg/dL)	0.0523	0.7048	0.0800	0.5615
LDL (mg/dL)	0.0764	0.5794	0.0915	0.5065
HDL (mg/dL)	- 0.252	0.0635	- 0.389**	**0.0033**
HOMA-IR	0.415**	**0.0016**	0.474***	**<0.001**
hs-CRP	0.269*	**0.0470**	0.630***	**<0.001**

* p<0.05. ** p<0.01. *** p<0.001

Discussion

In our study, we examined the effects of Bcl-2 antiapoptotic gene expression and Ki 67 antigen, a marker of mitotic activity, on prognosis in the light of hormonal parameters and compared Bcl-2 antiapoptotic gene expression in endometrial tissue samples taken from infertile patients with and without PCOS.

In obese women with PCOS, insulin resistance changes the expression of endometrial estrogen, androgen, and progesterone receptors [9]. Palomba et al. found endometrial resistance to progesterone in PCOS patients and abnormal gene expression associated with increased estrogen activity due to increased estrogen receptor expression [15]. So it has been reported that hyperinsulinemia can impact the endometrium, resulting in inadequate epithelial differentiation during the first few weeks of pregnancy [16]. Yet it has been shown that obesity, insulin resistance, or diabetes increases the risk of endometrial thickness, irregular uterine bleeding, and even endometrial cancer in PCOS patients who are not pregnant [17]. Android-type obesity is a known significant risk factor for developing Type 2 DM and cardiovascular disease (CVD) in the long term. Solomon et al. showed that the transition from impaired glucose tolerance to type 2 DM increased 2–5 times in obese PCOS patients in the USA and Australia [18]. Also, in a research of 1741 cases evaluating the prevalence of obesity in PCOS, 38% of the patients had the disease [19]. According to the literature, in our study, patients with PCOS had significantly higher BMI levels than the control group and HOMA-IR and E2 values as indicators of insulin resistance. Menstrual irregularity and BMI were found to be strongly correlated. Although these features are the main factor in the aggravation of the symptoms of PCOS patients, they indicate that PCOS is both an endocrinological and metabolic disease.

Insulin resistance and hyperinsulinemia in PCOS cause a decrease in SHBG (sex hormone binding globulin) levels and increase androgen synthesis and free testosterone levels in the ovary. In a study conducted on 264 women with PCOS in India, patients were divided into two groups: those with glucose intolerance and those without, using the OGTT 2nd hour value, and HOMA-IR and total testosterone values were found to be higher in the group with abnormal glucose tolerance [20]. In this study, total testosterone levels in infertile patients with PCOS were significantly higher than in the control group, consistent with the literature. The increased thecal thickness in PCOS leads to an increase in testosterone activity and expression, as well as the inhibitory effect of high Anti Müllerian Hormone release (AMH) on aromatase activity caused by FSH, which leads to a decrease in testosterone conversion to estrogen and an increase in testosterone levels [21]. In conclusion, this situation contributes to the development of infertility with the mechanisms that occur due to anovulation and insulin resistance. However, further studies at the molecular level are needed.

PCOS patients are at risk for many chronic diseases, especially CVD and DM. C-reactive protein (CRP), an acute phase reactant, is a highly sensitive marker of chronic low-grade inflammation and is closely associated with CVD and DM [22]. In the literature, many studies have shown increased CRP values in PCOS and suggested that PCOS is an inflammatory disease [23,24]. Karoli et al. stated that there was no increase in CRP values in women with PCOS compared to the control group [25]. Again, Ganie et al. did not find a significant difference in CRP values compared to the control group in their study with 160 PCOS patients [22]. Studies show that ovarian androgen levels increase in infertile patients with PCOS in correlation with CRP levels, which are markers of inflammation [26]. Increased inflammatory activity in patients with PCOS may affect folliculogenesis and lead to infertility by anovulation [27]. In our study, supporting this theory, the hs-CRP value was statistically significantly higher in the infertile group with PCOS than in the control group. Nevertheless, considering the small sample size in our study, there is a need for studies with a higher number of patients in this regard.

It is known that the endometrial cycle occurs with the harmonious functioning of the mechanisms of apoptosis and antiapoptotic activity. Many studies have also shown that the action of estrogen and progesterone 4,10 hormonally regulates these mechanisms. Estrogen and progesterone regulate the uterine epithelium's proliferation, differentiation, and death. Nawaz et al. stated that a significant increase in the apoptotic index of the endometrium was observed after oophorectomy in rabbits, which could be prevented by exogenous estrogen replacement therapy [28]. Maliqueo et al. stated that in their study of women with PCOS, the synthesis of antiapoptotic Bcl-2 was significantly higher in the endometrium with PCOS due to the effect of estrogen not being met with progesterone [4]. Vaskivuo et al. stated that Bcl2 gene expression in human endometrium with regular menstrual cycle findings increases in the proliferative period due to cyclic hormonal changes and decreases with the onset of menstruation in the secretory period. The Ki-67 protein is a marker monitored in the cell nucleus during the proliferative phase of the cellular cycle. They reported that in their study, an increase in the Ki-67 index was observed due to the effect of increased estrogen in the proliferative phase of the cycle [29]. Our study evaluated cases in the proliferative phase of the menstrual cycle in infertile instances with and without PCOS. Both groups were compared in terms of the Bcl-2 and Ki-67 proliferative index. Accordingly, to the literature, increased Bcl-2 gene expression and increased Ki-67 index was detected in cases with PCOS compared to the control group.

Implantation pathologies are also efficient in infertility and early-week losses because the mechanism of apoptosis cannot be achieved because of elevated Bcl-2 expression in infertile patients with PCOS. Additionally, it should be emphasized that PCOS patients have a significant long-term risk of developing endometrial cancer due to all this.

In the current study, unlike the studies on PCOS in the literature, infertile patients with and without PCOS in the same age range were evaluated. We found that BMI was significantly higher in infertile patients with PCOS compared to the control group. Total testosterone ratio and HOMA-IR levels were elevated in infertile patients with PCOS. Increased inflammatory activity in patients with PCOS can affect folliculogenesis, cause anovulation, and disrupt the endometrial environment—raising dating the implantation of the pregnancy product and leading to infertility. The effect of increased androgens and disorders in homeostasis caused by insulin resistance contributes to the development of infertility—improved insulin resistance results in impaired glucose tolerance and obesity in patients with PCOS. In addition, this situation increases the risk of CVD in the long term for patients with PCOS. Further studies at the molecular level are needed to prove the mechanisms caused by insulin resistance, which is one of the main problems of PCOS. We found that the acute phase reactant hs-CRP was high in infertile patients with PCOS.

Conclusion

We found that; Ki-67 proliferative index and Bcl-2 antiapoptotic gene expression were significantly higher in the proliferative phase in infertile patients with PCOS. Clinically, amenorrhea and oligomenorrhea are seen in PCOS patients because apoptosis cannot take effect. In addition, this reduces endometrial receptivity and does not allow the normal implantation process of the embryo. This situation causes recurrent pregnancy losses in patients with PCOS. This picture is due to increased estrogen activity. Additionally, we think that to effectively treat PCOS, novel research on GnRH modulatory therapeutic agents, which are crucial, notably in the release of estrogen, is needed.

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Effects Of COVID-19 Isolation Practices on Neusurgical Traumas

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ABSTRACT

Aim: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. Our objective was to investigate the incidence of head and spine injuries due to trauma before and after the pandemic. To identify the situations that should be considered in isolation measures due to the possibility of the occurrence of such epidemics in the globalized world.

Material and Method: Medical records of 2595 patients were accessed, and 1309 patients with missing patient data were excluded from the study. Normal distribution of continuous variables was tested with the Kolmogorov-Smirnov test. Continuous variables were compared with the Mann-Whitney U test, and categorical variables were compared with the Chi-square test or Fisher's exact test.

Results: Data from 1286 patients were analyzed. Some parameters differ before and after the pandemic, including age, injury mechanism, type of lesion, and spinal trauma. The average age is 33.9, and the male-female ratio is 3/2. The most frequent admission to the emergency room was in the summer. Our data showed significant differences in age, gunshot wounds in the trauma mechanism, soft tissue injury and contusion in lesions, and spinal trauma.

Conclusion: The Covid pandemic has caused imperative changes in every aspect of life. This situation also caused a shift in the reasons for applying to hospitals. It has been shown that the isolations performed because of Covid-19 did not affect the patient's diagnosis, treatment, and mortality, although they changed the mechanisms of head and spine trauma. Even with compulsory isolation for public health, the approach to neurosurgical traumas that may occur should not change. It should not be forgotten that other clinical situations continue during the COVID-19 pandemic

Keywords: Emergency, COVID-19, neurosurgical, trauma, isolation, pandemic

COVID-19 İzolasyon Uygulamalarının Beyin Cerrahisi Travmalarına Etkisi

Süreç

Geliş: 25/07/2022
Kabul: 22/03/2023

ÖZ

Amaç: Coronavirüs hastalığı 2019 (COVID-19), şiddetli akut solunum sendromu koronavirüs 2'nin (SARS-CoV-2) neden olduğu bulaşıcı bir hastalıktır ve 11 Mart 2020 tarihinde Dünya Sağlık Örgütü (WHO) tarafından pandemi ilan edilmiştir.Bizde bu çalışmada pandemi öncesi ve sonrasında travmaya bağlı kafa ve omurilik yaralanmalarının sıklığını araştırmayı amaçladık.

Gereç ve Yöntem: 2595 hastanın tıbbi kayıtlarına ulaşıldı ve eksik hasta verileri olan 1309 hasta çalışma dışı bırakıldı. Sürekli değişkenlerin normal dağılımı Kolmogorov-Smirnov testi kullanılarak değerlendirildi. Sürekli değişkenler Mann-Whitney U testi ile karşılaştırıldı ve kategorik değişkenler Ki-kare testi veya Fisher'in kesin testi kullanılarak karşılaştırıldı. Globalleşen dünyada bu tür salgınların ortaya çıkma ihtimalinden dolayı, izolasyon tedbirlerinde dikkat edilmesi gereken durumları ortaya koymak.

Bulgular: 1286 hastanın verileri analiz edildi. Pandemi öncesi ve sonrası, yaş, yaralanma mekanizması, lezyon tipi, spinal travma gibi bazı parametreler farklılık göstermektedir. Yaş ortalaması 33.9 ve erkek-kadın oranı 3/2'dir. Acil servise en sık başvuru yazın oldu. Verilerimizde yaş, travma mekanizmasında ateşli silah yaralanması, lezyonlarda yumuşak doku yaralanması ve kontüzyon ve spinal travma açısından anlamlı farklılıklar vardı.

Sonuç: Covid pandemisi hayatın her alanında zorunlu değişikliklere neden olmuştur. Bu durum hastanelere başvuru nedenlerinde de değişikliğe neden olmuştur. Covid-19 nedeniyle yapılan izolasyonların, baş ve omurga travmasının mekanizmalarını değiştirse de hastaların tanı, tedavi ve mortalitesini etkilemediği gösterildi. Halk sağlığı için zorunlu izolasyona rağmen oluşabilecek beyin cerrahisi travmalarına yaklaşım değişmemelidir. Unutulmamalıdır ki Covid 19 pandemisinin varlığında diğer klinik durumlarda da devam etmektedir.

Anahtar sözcükler: Acil, COVID-19, beyin cerrahisi, travma, izolasyon, pandemi

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INTRODUCTION

Traumatic brain injury (TBI) is a disruption in the normal function of the brain and spinal cord caused by an external mechanical force. TBI has physical and psychological effects on people and also has serious economic consequences for the countries.[1,2]

TBI is also known as a silent epidemic due to the lack of public awareness and accounts for almost half of all injuries admitted to the emergency department[3]. Although spinal traumas are less common than brain traumas, their morbidity rates are relatively higher.[4] Most people with TBI suffer from disability for the rest of their lives.[3]

In all age groups, the annual incidence of TBI in Europe is 326 per 100,000, while the frequency of spinal injuries is 12.1-57.8 per one million.[2,4] In the USA, nearly 50,000 annual deaths are associated with TBI.[3] Motor vehicle accidents mostly cause TBI and falls from heights, followed by exposure to physical violence and concussion sustained because of sports activities (e.g., mountaineering, skiing, football, and basketball), recreational activities, and excessive alcohol consumption.[2] Severe and fatal TBIs often occur due to motor vehicle accidents.[2]

The risk of TBI increases with age.[3] The elderly often have higher mortality and morbidity rates compared to those of young people due to the use of anticoagulant drugs, the presence of comorbidities (orthopedic, neurological, cardiac diseases), and the anatomical changes in the brain structure that occur with age.[3]

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020, after this declaration, WHO recommended that all countries develop their own national risk assessments to combat the COVID-19 outbreak.[5,6] One of the most effective methods in this combat is social isolation, which has changed the trauma mechanisms and the demographic characteristics of TBI patients admitted to hospitals.[6] In this study, we aimed to investigate the frequency of head and spinal injuries caused by low-energy trauma before and after the pandemic.

METHODOLOGY

The retrospective study included TBI patients who applied to our emergency department between January 1, 2019, and January 1, 2021. The study was conducted according to the World Medical Association Declaration of Helsinki for studies on human subjects and was approved by our university's Scientific Research Ethics Committee (dated January 15, 2021; protocol number (2021/01-26). The data of the study were obtained using emergency room notes, neurosurgery consultation notes and, radiological imaging reports, the national disease classification codes (ICD-10) in the electronic data system (Enlil).

Medical records of 2595 patients were accessed, and their data were entered into SPSS software using the following ICD-10 codes: W19, V49.9, S01.0, S01.9, S02.0, S02.1, S02.3, S02.7, S02.9, S04.0, S06.0, S06.9, S07.0, S07.1, S07.8, S07.9, S09.7, S09.9, T01.0, T02.0, T04.0, T06.0, T90.1, T90.2, T90.4, T90.5, T90.8, and T90.9.

Patients were divided into two groups based on their admission date: (i) January-December 2019 and (ii) January-December 2020. Most common mechanisms of injury included falls from heights, motor vehicle accidents, assault, hanging, gunshot injury, injury, electric shock, and drowning. The severity of TBI was categorized into three categories using Glasgow Coma Scale (GCS): (I) mild (GCS score, 13-15), moderate (GCS score, 9-12), and severe (GCS score, ≤8). Cranial lesions, epidural/subdural/subarachnoid hemorrhage, cerebral contusion, cerebral edema, skull fracture, and pneumonia were recorded for each patient.[2] Spinal trauma was categorized as cervical, thoracic, lumbar, and sacral injuries. Isolated soft tissue injury was excluded from the study. The presence of pain alone was soft tissue injury. Patients whose observation periods ended in the emergency and neurosurgical service or operated were included in the study.

Data were analyzed using SPSS for Windows version 27.0 (Armonk, NY: IBM Corp.). Descriptives were expressed as mean, standard deviation (SD), median, minimum-maximum values, frequencies (n), and percentages (%). Normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables were compared using the Mann-Whitney U test, and categorical variables were compared using the Chi-square test or Fisher's exact test.

RESULTS

Data from 1286 patients were analyzed in this study. The average age is 33.9, and the male-female ratio is 3/2. Demographic data are given in table 1. Hospitalizations and follow-ups were made in the emergency department, neurosurgery service, and intensive care unit. Patients who applied to the emergency department after trauma and were planned to be followed for over 6 hours were admitted to the hospital. Except for minor traumas, all suspicious clinical conditions were followed up by hospitalization. Trauma mechanisms, clinical lesions and, mortality rates are given in table 2.

The most common injury mechanism was a fall, and the most common lesion was a bone fracture. Subarachnoid hemorrhage was not seen in any of our cases. The most injured area in spinal trauma was the lumbar region. The surgical treatment rate was 3.51%, and the mortality rate was 0.43.

Admissions to the emergency department by months before and after the pandemic are given in Table 1

Table 1: **Admissions to the emergency department by age, gender, before-after pandemic, and months**

		Before pandemic	After pandemic		Total	
		Mean ± s.s/n-%	Mean ± s.s/n-%	P	Min.-Max. / Median	Mean ± s.s/n-%
Age		35 ± 26,4	32,8 ± 25,2	***0,012***	1,0-97,0 / 31,0	33,9 ± 25,8
Gender	Male	789- 61,4%	786 – 56,6%	0,495		1575- 60,7%
	Female	497 – 38,6%	523 – 37,7%			1020-39,3%
Before pandemic						1286- 49,6%
After pandemic						1309- 50,4%
Months						
January		108-8,4%	67-4,8%			175-6,7%
February		107-8,3%	85-6,1%			192-7,4%
March		101-7,9%	72-5,2%			173-6,7%
April		87-6,8%	106-7,6%			193-7,4%
May		81-6,3%	107-7,7%			188-7,2%
June		119-9,3%	143-10,3%			262-10,1%
July		158-12,3%	160-11,5%			318-12,3%
August		116-9%	140-10,1%			256-9,9%
September		104-8,1%	118-8,5%			222-8,6%
October		101-7,9%	126-9,1%			227-8,7%
November		94-7,3%	103-7,4%			197-7,6%
December		110-8,6%	82-5,9%			192-7,4%

The most frequent admission to the emergency room was in the summer.

The mean age was significantly lower in patients admitted after the pandemic compared with those admitted before the pandemic ($p<0.05$). In contrast, no significant difference was found regarding gender distribution between the two periods ($p>0.05$). The frequency of admissions because of gunshot injury was significantly higher in patients admitted after the pandemic compared to those admitted before the pandemic ($p<0.05$). The prevalence of cerebral contusion and the frequency of admissions due to spinal trauma were significantly higher in patients admitted after the pandemic compared to those admitted before the pandemic ($p<0.05$). The frequency of admissions because of soft tissue injury was significantly higher in patients admitted after the pandemic compared to those admitted before the pandemic ($p<0.05$). Statistical distribution of pre-pandemic and post-pandemic age, gender, trauma mechanisms, clinical lesions, and mortality are given in Table 2.

Table 2. Comparison results by groups

		Pre-Pandemic		Post-Pandemic		*p.
		N	%	N	%	
GENDER	Male	1675	77,8%	478	22,2%	,275
	Female	1061	76,2%	331	23,8%	
TRAUMA MECHANISM	Fall	434	73,8%	154	26,2%	**,045**
	Motor vehicle accident	360	83,1%	73	16,9%	
	Minting	48	81,4%	11	18,6%	
	Vaccine	10	90,9%	1	9,1%	
	ASY	56	77,8%	16	22,2%	
	İnjury	34	75,6%	11	24,4%	
	Electric shock	2	100,0%	0	0,0%	
	Suffocation	2	100,0%	0	0,0%	
	Other	1790	76,7%	543	23,3%	
TRAUMA VIOLENCE	Light	479	81,0%	112	19,0%	,061
	Middle	33	76,7%	10	23,3%	
	Heavy	44	83,0%	9	17,0%	
	Unknown	2180	76,3%	678	23,7%	
SCALP LASERATION	Available	147	77,4%	43	22,6%	,807
	No	83	79,8%	21	20,2%	
	Unknown	2506	77,1%	745	22,9%	
INTRACRANIAL LESION	Available	107	84,3%	20	15,7%	,118
	No	130	79,3%	34	20,7%	
	Unknown	2499	76,8%	755	23,2%	
LESION	Epidural	17	85,0%	3	15,0%	,905
	Epidural, Subdural	2	100,0%	0	0,0%	
	Epidural, Bone fracture	11	84,6%	2	15,4%	
	Subdural	20	80,0%	5	20,0%	
	Subdural, Contusion	3	100,0%	0	0,0%	
	Subdural, Bone fracture	6	60,0%	4	40,0%	
	Subarachnoid	1	50,0%	1	50,0%	
	Contusion	28	80,0%	7	20,0%	
	Contusion, Edema	5	100,0%	0	0,0%	
	Contusion, Bone fracture	12	75,0%	4	25,0%	
	Edema	12	85,7%	2	14,3%	
	Edema, Bone fracture	3	100,0%	0	0,0%	
	Bone fracture	271	77,2%	80	22,8%	
	Bone fracture, Pneumocephalus	3	75,0%	1	25,0%	
	Midline shift	0	0,0%	0	0,0%	
	Pneumocephalus	3	75,0%	1	25,0%	
	Unknown	2339	77,0%	699	23,0%	
SPINAL TRAUMA	Available	223	74,3%	77	25,7%	,148
	No	103	83,1%	21	16,9%	
	Unknown	2410	77,2%	711	22,8%	
CERVICAL	Cervical	48	80,0%	12	20,0%	,390
	Cervical, Thoracic	11	90,9%	1	9,1%	
	Thoracic	45	67,2%	22	32,8%	
	Thoracic, Lumbal	15	68,2%	7	31,8%	
	Lumbar	93	73,8%	33	26,2%	
	Lumbal, Soft tissue	2	100,0%	0	0,0%	
	Sacral	2	66,7%	1	33,3%	
	Soft tissue	150	80,6%	36	19,4%	
	Unknown	2369	77,3%	697	22,7%	
SURGICAL DONE?	Available	26	63,4%	15	36,6%	**,001**
	No	757	81,8%	168	18,2%	
	Unknown	1953	75,7%	626	24,3%	
URGENTLY DISCHARGE	There is	140	75,7%	45	24,3%	,680
	No	370	78,6%	101	21,4%	
	Unknown	2226	77,1%	663	22,9%	
MORTALITY	There is	4	100,0%	0	0,0%	,260
	No	952	78,4%	263	21,6%	
	Unknown	1780	76,5%	546	23,5%	
		Mean	**Std. Dev.**	**Mean**	**Std. Dev.**	***p.**
AGE		34,09	25,93	34,31	26,35	,495

*Chi-square test Statistics **Mann-Whitney U test Statistics

DISCUSSION

The labor loss and the impairment of physical or mental function caused by head and spinal trauma place a serious burden on the patients and countries.[2,7]

Traumatic brain injury (TBI) mostly affects individuals aged 21-40 years and has two peaks of incidence, of which the first peak is at 20-40 years, mostly due to motor vehicle accidents, and the second peak is often seen in the elderly over 65 years of age due to falls.[4] In children, however, the most affected age range is 0-10 years.[8] A study conducted in Iran reported that the incidence of head injuries caused by low-energy trauma had recently increased in individuals over 60 years, particularly those aged between 83-90 years.

The authors also noted that such patients might be asymptomatic.[3]A study conducted in Scotland reported that the rates of hospitalization because of neurosurgical trauma in patients aged over 65 years had increased remarkably.[9] Perez et al. reported that the traumatic spinal injury rate in individuals over 65 years showed no significant change.[4] In the literature, advanced age is accepted as a prognostic factor for head trauma.[10] In this study, the mean age of patients admitted to the emergency department with neurosurgical trauma was 33.9 ± 25.8 years. The mean age of the patients who applied after the pandemic was significantly lower than those who applied before the pandemic. We think this is because patients over 65, who are in the highest risk group in the Covid-19 pandemic, are more isolated in our country.

The incidence of neurosurgical trauma shows wide variation among studies, which could be attributed to the differences in the inclusion criteria of the studies and two, including patients with headache, vertigo, and amnesia, particularly in cases with mild trauma.[11] A previous review analyzed 23 studies conducted in Europe and reported the incidence of head trauma as 23, mortality as 15 per 100,000 population, and traumatic spinal injury as 13.4 (20.4 in men and 4.7 in women) per one million population.[1,4] In Spain, 1000 recent cases of traumatic spinal injury are diagnosed, and 20000 traumatic brain injury patients are hospitalized each year.[4] According to a study conducted in Nigeria, patients with head trauma made up 30.9% of all trauma patients admitted to the hospital.[8] Heydari et al. reported that traumatic brain injury had doubled, and the hospitalization rate had increased 2.5 times over the last 18 years.[3] These studies show that patients with neurosurgical traumas have a high hospitalization rate (Table 2). The rate of those who were followed up in the emergency service, neurosurgery service, and intensive care unit was 72.9%. The reason for this high rate is the prolongation of the follow-up period in case of clinical suspicion since the trauma is a dynamic process.

According to the literature, both head and spinal traumas are more common in males. It is known that men are more involved in business life than women.[2,4,8,9,12] Although the rate of males was higher before and after the pandemic in our study, no statistically significant difference was found between the genders (Table 1). We think this difference does not exist as all genders stay at home as active life is restricted due to Covid-19 isolation.

Falls from height and motor vehicle accidents are the most common causes of neurosurgical trauma in all age groups, followed by assault, domestic injuries, and gunshot injuries.[8] Motor vehicle accidents and falls from heights are more common in younger ages, while falling from the same level is more frequent in elderly individuals.[3,7,9] In our study, no significant difference was found between pre- and post-pandemic periods regarding the frequency of admissions due to motor vehicle accidents, assault, hanging, injury, electric shock, and drowning ($p>0.05$). In contrast, the frequency of admissions because of gunshot injury was significantly higher in patients that were admitted after the pandemic compared to those who were admitted before the pandemic. We think that long quarantine periods cause a tendency to violence by negatively affecting human psychology.

The severity of TBI is commonly assessed using GCS. Most TBIs admitted to the emergency department are often categorized as mild trauma, with a GCS score of ≥13.[13] Heydari et al. and Onwuchekwa et al. evaluated TBI patients admitted to the emergency department and reported that 85.3% and 39% of the patients had mild TBI, respectively.[3,8] Low GCS is associated with high mortality and morbidity and thus can be used in predicting the prognosis of TBI patients.[3,10] However, patients with a history of conditions such as hypotension, alcohol abuse, and medication may be under the influence of drugs[11]. In this study, 85.5% of the cases had mild TBI (Table 2). We want to state that the social quarantine measures implemented in our country due to Covid-19 do not significantly affect the severity of TBI in our patients.

Patients with head and spinal traumas can present with many manifestations ranging from asymptomatic to intracranial lesions or lateralizing signs[1,4]. In patients with minor head trauma, altered mental status, clinical signs of skull fracture, vomiting, and soft tissue injury in the craniofacial region are alarming factors.[12]

A 2020 study evaluated patients who applied to the emergency department and reported that intracranial lesions were detected in 14% of patients with mild trauma and 22.9% of patients with mild-moderate trauma.[3] Fractures of the skull, skull base, and facial bones, focal neurological deficits, seizures, coagulation disorder, and ongoing anticoagulant therapies have been associated with severe TBI.[11] But no relationship has been found between scalp lacerations and intracranial hemorrhage.[3]

In the present study, no significant difference was found between before-after pandemic periods regarding the incidence of intracranial lesions, cerebral edema, bone fracture, scalp lacerations, and pneumocephalus. In contrast, the incidence of contusion decreased, and the incidence of soft tissue injury increased after the pandemic. These findings may be due to the relatively increased frequency of low-energy trauma because of decreased motor vehicle accidents during the quarantine period.

Spinal cord injury can be seen at any age, mostly in the third decade of life.[4,13,14] Knútsdóttir et al. reported the incidence of spinal cord injury per one million as 289 in Finland, 365 in Norway, 534 in Australia, and 750 in the USA(14).

Although the incidence of spinal cord injury has been higher in men in recent years, its incidence is gradually increasing in both genders. [4,14] The incidence of cervical spinal cord injury is higher than that of thoracic/lumbar injury.[14] In our study, although the overall incidence of spinal cord injury was significantly higher in the after-pandemic period, no significant difference was found between the two periods in terms of the incidence of cervical, thoracic, and lumbar injuries (Table 2). We can explain this situation because the trauma mechanism did not change before and after the pandemic.

Neurosurgical traumas often occur during working hours and social activities.[8] A study conducted in Denmark showed that the incidence of neurosurgical traumas increased at the weekend and in summer, and the authors attributed this increase to violence, entertainment, and substance and alcohol abuse.[2] In Turkey, following the identification of the first cases of Covid-19 in March 2020, the incidence of neurosurgical traumas admitted to the emergency department decreased slightly beginning from April 2020, particularly between July and December, compared to the same period in the previous year, mainly due to the intermittent social isolation imposed in the country (Table 1). In our study, most patients presented to the emergency department in summer, both before and after the pandemic, which could be associated with the loosening of isolation policies and travel rules in summer due to the decline in the number of cases in summer.

Treatment of neurosurgical trauma depends on the severity of the trauma and clinical and comorbid conditions. The most effective treatment method is the prevention of trauma.[1] In the case of trauma, the primary goal is to minimize the risk of permanent neurological injury.[10] Computed tomography (CT) is recommended in the presence of clinical signs of increased intracranial pressure and spinal cord compression.[12] The specificity and sensitivity of CT in trauma patients were found to be superior to direct roentgenograms.[15] The patient can be treated surgically or conservatively, depending on the lesion status.[10] In such patients, stroke, cancer, coronary artery disease, and anticoagulant drug use may affect the recovery time.[8,10] It has been shown that patients with chronic conditions are not significantly affected by isolation measures.[16] In the present study, no significant difference was found between pre- and post-pandemic periods regarding the number of surgical procedures performed and the number of patients discharged from the emergency department (Table 2). Since most cases do not require surgical treatment, we think there is no significant difference between before and after the pandemic. Most important parameters affecting mortality after head and spinal trauma include trauma severity, low GCS, advanced age, and comorbidities.[3,8,9]

In the present study, no significant difference in mortality rate was found between before-after pandemic periods.

CONCLUSION

Coronavirus disease 2019 (Covid-19) is a global pandemic affecting the entire world and leading to different lifestyles in social and business settings. With the globalization of the world, the risk of threatening public health from such pandemics continues. The best method to control pandemic processes in isolation. We think these processes will affect individuals, societies, and states. Our findings indicated that, although this isolation changed the mechanisms of head and spinal traumas, it did not affect the patient's diagnosis, treatment, and mortality. More studies are needed to detect and control these effects.

ETHICAL APPROVAL

The study was started after the approval of the local ethics committee of Yüzüncü Yıl University with the number 2021/01-26.

PATIENTS' CONSENT

Informed consent is not required as it is a retrospective study, and patient identity was kept anonymous.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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The Effect of Pregnant Education Classes on the Birth Process of Nulliparous Pregnants

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Research Article

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ABSTRACT

Objective: This study aimed to investigate the effects of antenatal education classes on the type of delivery, duration of labor in vaginal delivery, and birth rates with episiotomy.
Material Method: This retrospective study included 200 nulliparous pregnant women who were admitted to Adana City Hospital Obstetric and Gynecology Outpatients clinic between 2017-2021. Of these, 99 attended antenatal education classes, which the Turkish Ministry Of Health designed, and 101 did not participate in antenatal educational courses. In the retrospective analysis of the cases, cesarean section rates, indications for cesarean section, duration of labor, episiotomy rates, and APGAR scores were compared.
Results: No significant difference was found between the mode of delivery between the pregnant women who attended and did not attend antenatal education classes (p=0.463). A significant difference was determined between the groups regarding cesarean section indications and duration of labor (p=0.007 and p<0.001, respectively). Regarding cesarean indications, the cesarean section rate was higher in the group that did not participate in the antenatal classes (27.7% vs. 10.1%), especially due to non-progressive labor. There was no significant difference between the groups in terms of birth rates with episiotomy (p=0.088).
Conclusion: The study results demonstrated that the pregnant women who participated in the antenatal education classes had shorter labor and a lesser need for cesarean section, indicating non-progressive labor. Although there was no significant difference between the cesarean section rates between the groups, it can be considered important to expand the scope of these antenatal classes and provide the appropriate social and legal groundwork to achieve this goal.
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Keywords: Pregnant Class, Pregnant education program, Birth, Cesarean delivery, Vaginal delivery

Gebe Eğitim Sınıflarının Nullipar Gebelerin Doğum Sürecine Etkisi

Süreç

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ÖZET

Amaç: Bu çalışmamızda gebe eğitim sınıfında alınan eğitimin, doğum şekli, vajinal doğumda travay süreleri ve epizyotomili doğum oranlarına etkisini araştırmayı amaçladık.
Yöntem: Retrospektif olan bu çalışmamıza 2017-2021 yılları arasında Adana Şehir Hastanesi Kadın hastalıkları ve doğum kliniğine rutin kontrol için başvuran ve hastane bünyesindeki gebe okulu eğitimlerinin tamamına katılan 99 ve hiç eğitim almayan 101 nullipar gebe olmak üzere 200 gebe alınmıştır. Olguların retrospektif incelemesinde gruplar arası sezaryen oranları, sezaryen endikasyonları, travay süreleri, epizyotomi oranları ve APGAR skorları karşılaştırıldı.
Bulgular: Çalışmamızda antenatal gebe eğitim sınıflarına katılan ve katılmayan gebeler arasında doğum şekli arasında anlamlı fark bulunmadı (p=0.463). Fakat sezaryen endikasyonları ve travay süreleri arasında gruplar arasında anlamlı fark saptanmıştır (Sırası ile p=0.007 ve p<0.001). Sezaryen endikasyonları açısından da özellikle ilerlemeyen travay nedeni ile sezaryene alınma oranları anteneatal gebe sınıfına katılmayan grupta daha yüksek saptanmıştır (%27.7 vs %10.1). Gruplar arasında epizyotomili doğum oranları açısından da anlamlı fark saptanmadı (p=0.088).
Sonuç: Çalışmamızda özellikle gebe eğitim sınıfına katılan gebelerde kısa travay süreleri ve ilerlemeyen travay endikasyonu ile daha az sezaryen sayıları dikkat çekmektedir. Gruplar arasında sezaryen oranları arasında anlamlı bir fark olmamakla beraber bu hedefe ulaşmak için bu eğitimlerin kapsamının genişletilmesi beraberinde de uygun sosyal ve hukuki zeminin de sağlanmasının önemli olduğunu düşünmekteyiz.

Anahtar sözcükler: Gebe Sınıfı, Gebe eğitim programı, Doğum, Sezaryen Doğum, Vajinal Doğum

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Introduction

Pregnancy, including the birth and postpartum periods, is a time in a woman's life when very important physical and psychological changes occur, and these experiences will never be forgotten. While the majority of these experiences and memories are positive, there can be negative experiences associated with various factors in some pregnancies. In a study conducted in Holland in 2008, when women were asked what they remembered about the birth at three years postpartum, more than 16% negatively remembered the birth process. Most of the patients in that study comprised primiparity or women who had undergone an operative birth[1]. Previous negative experiences of pregnancy, lack of information and support for the pregnancy and birth, and various environmental factors are sources of stress and fear for mothers-to-be[2-4]. The first advocate for antenatal education was the English doctor Dick-Read, who stated that the fear and stress experienced by women could increase their perception of pain, which could affect the normal progression of labor. Therefore, antenatal education is of great importance. The rate of cesarean section (CS) deliveries in 2017 was reported to be 27.9% in OECD countries[5] and 53% in Turkey[6]. Although there are other components in the increase in CS rates, such as medicolegal stress, hospital conditions, and physician-related factors, informing and supporting pregnant women to eliminate the fear of birth is crucial for healthy labor. There is an increasing demand for antenatal classes, which started at the beginning of the 20th century and has continued in various forms, such as the Dick-Read, Bradley, Leboyer, Mongan, Pam England, Gaskin, Odent, and Lamaze methods. To reduce the increasing CS rates and achieve active participation of the mother in the birth by providing the necessary information and support and to be able to reduce complications that can develop during the delivery and in the early postpartum period, the Public Health Institute of Turkey published a circular in 2014. This circular aimed for public hospitals to open antenatal classes, and education in these classes is still ongoing today. In these antenatal classes, education is given on many subjects, such as the anatomy of the female urogenital system, the pregnancy-related physiological changes that occur, nutrition during pregnancy and postpartum, signs of the onset of labor, straining and breathing exercises, elements that can be teratogenous for the fetus and family planning[7].

In the literature related to antenatal classes, it has been determined that pregnant women who receive this education have lower rates of CS, participate more actively in the birth, have a lower incidence of postpartum depression, bond with the infant more rapidly, and higher rates of feeding with breast milk only in the first six months[8, 9].

This study aimed to examine antenatal classes' effect on cesarean section delivery rates, the duration of labor, and rates of births with intervention.

Material and Method

This retrospective study included 200 nulliparity pregnant women who presented at the Obstetrics and Gynaecology Clinic of Adana City Hospital for routine check-ups from 2017-2021. The study participants included those who fully participated in the antenatal classes run by the hospital and those who received no education about pregnancy. Approval for the study was granted by the Ethics Committee of Adana City Hospital (decision no: 2020/935).

The pregnant women included were nulliparity, literate, gave birth to a single live, full-term infant, and did not require CS for any maternal or fetal reason.

The duration of labor was recorded as the time from the onset of active labor (4-6cm cervical dilatation as recommended by the American College of Obstetricians and Gynecologists -ACOG) until the infant's birth. The sociodemographic and pregnancy-related data were collected and recorded on a data collection form created by the researchers.

Statistical Analysis

Data obtained in the study were analyzed statistically using IBM SPSS vn—23 software. The conformity of the data to normal distribution was examined with the Kolmogorov-Smirnov test. Data not showing normal distribution according to education status were compared using the Mann-Whitney U-test. The Chi-square test was applied in the comparisons of categorical data. In the comparisons of APGAR scores that did not show normal distribution according to paired times, the Wilcoxon test was used. Continuous data were stated as mean ± standard deviation (SD) and median (minimum-maximum) values, and categorical data as number (n) and percentage (%). A value of $p<0.05$ was accepted as statistically significant.

Results

The evaluation was made of 200 nulliparity pregnant women, 99 attending antenatal education classes designed by the Turkish Ministry of Health, and 101 not participating in antenatal educational courses. No significant difference was determined between the groups regarding the type of birth or the rates of need for intervention in the birth (Table 1). A statistically significant difference was determined between the groups who attended or did not attend antenatal classes regarding indications for CS (p=0.007). This difference originated from the differences between the groups in respect of macrosomia and non-progression of labor. Macrosomia was determined in 1% of those who had not attended antenatal classes and 8.1% of those who had participated in antenatal classes. Non-progression of labor was determined in 27.7% of those who had not attended antenatal classes and 10% of those who had participated in antenatal classes. No statistically significant difference was determined between the groups in respect of the other variables (p>0.050) (Table 1).

Table 1. Comparisons of the groups according to antenatal education

	No antenatal classes (n=101)	Antenatal classes (n=99)	Total (n=200)	Test statistic	*p*
Type of birth					
Vaginal	55 (54.5)	59 (59.6)	114 (57)	χ^2=0.539	0.463
Cesarean	46 (45.5)	40 (40.4)	86 (43)		
Episiotomy					
Yes	57 (57)	68 (68.7)	125 (62.8)		
No	43 (43)	31 (31.3)	74 (37.2)	χ^2=2.909	0.088
Cesarean indication					
Non-	55 (54.5)	59 (59.6)	114 (57)		
Progression of labor	28 (27.7)[a]	10 (10.1)[b]	38 (19)		
CPD	5 (5)	3 (3)	8 (4)		
AFD	13 (12.9)	16 (16.2)	29 (14.5)		
Severe pre-eclampsia	4 (4)	1 (1)	5 (2.5)	χ^2=17.694	0.007
Macrosomia	1 (1)[a]	8 (8.1)[b]	9 (4.5)		
Presentation anomaly	2 (2)	2 (2)	4 (2)		

χ^2: Chi-square test statistic. [a-b]: No difference between groups with the same letter

The median TFA value was 3380 in the antenatal classes group, statistically significantly higher than the 3200 in the group that did not attend antenatal classes (p=0.005).

The age of the mothers was statistically significantly different according to the antenatal classes groups (p<0.001). The median age was 23 years in the group that received no education and 25 years in the group that attended antenatal classes. No statistically significant difference was determined between the groups regarding the other parameters (p>0.050). The groups were evaluated in relation to the 1 and 5-min APGAR scores according to whether or not they had attended antenatal classes (p=0.588, p=0.347, respectively) (Table 3). The duration of labor was statistically significantly shorter in the group that attended antenatal classes than in the group that did not (p<0.001).

Table 2. Comparisons of the groups according to antenatal education

	No antenatal classes (n=101)	Antenatal classes (n=99)	*p*
	median(min-max)	median(min-max)	
AFI	81 (50 - 190)	86 (50 - 190)	0.551
First dilatation finding (cm)	2 (0 - 10)	2 (0 - 10)	0.161
Duration of labor (mins)	810 (80 - 1520)	310 (10 - 970)	<0.001
Weight on ultrasound (gr)	3250 (2500 - 4300)	3335 (2550 - 4350)	0.189
EFW	3200 (2500 - 4600)	3380 (2500 - 41000)	0.005
Maternal age (years)	23 (18 - 41)	25 (18 - 42)	<0.001
Gestational age (weeks)	39 (37 - 41)	39 (37 - 41)	0.853
BMI	28 (21.3 - 43.6)	28.4 (20.9 - 38.5)	0.264

Mann Whitney U test statistic

Table 3. Comparisons of the groups according to antenatal education

		No antenatal classes (n=99)		Antenatal classes (n=101)		Total (n=200)		Test statistic	p
		mean ±Sd	median(min-max)	mean ±Sd	median(min-max)	mean ±Sd	median(min-max)		
1-min score	APGAR	8.4 ± 0.7	8 (7 - 9)	8.4 ± 0.8	9 (6 - 9)	8.4 ± 0.7	9 (6 - 9)	U=5199.5	0.588
5-min score	APGAR	9.4 ± 1.1	10 (0 - 10)	9.5 ± 0.6	10 (8 - 10)	9.5 ± 0.9	10 (0 - 10)	U=5337	0.347

Mann Whitney U test statistic

Discussion

There is currently an increasing trend for cesarean section (CS) births worldwide[10]. Similarly, in Turkey, the CS rate in 2013 was 50.4%, which was the highest rate among OECD countries[11]. The birth statistics in the Obstetrics and Gynaecology Clinic of Adana City Hospital for 2021 showed 39.5% vaginal birth rates and 60.5% CS birth. The reasons for this increase in Turkey are thought to be due to physicians and midwives being exposed to high rates of court cases, problems in the healthcare system such as low wages for long working hours, and insufficient information given to pregnant women about active participation in the birth and postpartum, thereby creating a fear of giving birth.

Therefore, in this context, the pregnancies and births followed up in this study included women who attended the antenatal classes program, which was started in 2014. The study results demonstrated no difference in the CS birth rates between primigravida pregnant women who had received no education about pregnancy and those who had fully completed the Ministry of Health antenatal classes program (p=0.463). However, the conversion rates to CS with the indication of non-progression of labor were higher, and the duration of labor was longer in the group who had not attended antenatal classes.

In a 2019 study by Möller et al., antenatal classes were similarly seen not to affect the type of birth[12]. Waldenstorm et al. (2006) determined that increasing CS rates were not associated with the fear of childbirth[13]. A similar study by Bıyık et al. in 2020 reported significantly lower CS rates in pregnant women who had attended antenatal classes. However, unlike the current study, that study included both primigravida and multigravida women[14]. In two studies conducted in Italy in 2002 and 2008, the rates of CS were determined to be lower in patients who had attended antenatal classes[15,16]. Although there is no clarity in studies related to the effect on CS rates, another study by Cantone et al. (2017) showed that antenatal classes had a moderate impact on reducing CS rates, and the reduction was reported to be approximately 10%[17]. The reason for the lack of difference between the groups in the current study in respect of the type of birth was thought to be due to the inclusion of only primigravida pregnant women and that our hospital is a tertiary level center to which women with high-risk pregnancies are referred. It is also thought that obstetricians are more liberal in their decisions for CS, especially in the follow-up of high-risk pregnancies, to avoid court cases for high compensation.

However, when the indications for CS were examined, it was seen that the rates for CS associated with the indication of non-progression of labor were higher in the group that had not attended antenatal classes. There is thought to be a positive contribution of antenatal classes in respect

of factors such as correct breathing techniques, training techniques, and increased adaptation to the second stage of birth. Although the rates of fear of giving birth were not investigated in the current study groups, it was thought that this benefit could have increased adaptation to the birth process and made it easier for the mother. It is known from the literature that patients who have received antenatal education have less fear of childbirth. A study conducted in Warsaw University in 2019 determined that antenatal classes reduced the fear of giving birth[18]. Brixval et al. (2016) also reported that women who had attended antenatal classes felt more confident that they could manage the birth process[19]. In a systematic review and meta-analysis in 2018, Moghaddam et al. reported that antenatal classes decreased the fear of birth[20].

When the duration of labor was examined in the current study, it was seen to be shorter in the group who had attended antenatal classes. In a study conducted in Spain in 2018, although the duration of labor was not directly measured, it was determined that antenatal courses positively affected the second stage of birth, consistent with the current study findings [21]. In the results, although statistical analysis of fetal birthweights of the groups showed a significant difference, median values were in the normal weighted fetus range; therefore, this finding would not change our clinic practice.

Another point examined in the process of vaginal route delivery was whether or not episiotomy was necessary. No significant difference was determined between the current study groups regarding the rates of episiotomy performed. As stated above, it was also reported in a study by Soriano that antenatal classes provided active participation at this stage, and a more significant number of patients who had received the classes did not want to have an episiotomy[21]. The results of a review by Jiang et al. in the 2017 Cochrane database showed no definitive evidence that routine episiotomy reduced perineal/vaginal trauma. It was also reported that there is a need for more advanced studies on whether selective episiotomy is beneficial in patients who require interventional birth.

Similarly, no clear data have been reached related to the long-term results of selective episiotomy on the Health of the mother and infant[22]. As no significant result was obtained in the current study, it is thought that old habits continue about the need for episiotomy as there is no clarity in the literature on this subject. Physicians wish to avoid medicolegal sanctions in the event of complications that could develop. In light of these data, it is thought that pregnant women attending antenatal classes should be more informed about episiotomy, which provides active participation in this process. In addition, providing education to the resident doctors in our clinic, which is in a tertiary-level center, and increasing in-service training for midwives who actively attend births should decrease episiotomy rates.

This study had some limitations, primarily that it was retrospective in design. As it was retrospective, no clear data could be obtained about the education level of the patients. Thus, the birth processes could not be compared according to groups of education level. Previous studies have shown that a higher education level is associated with higher participation rates in education programs[23-26]. Nevertheless, strong aspects of the study can be said to be that only nulliparity pregnant women were included, and standardization was provided in the antenatal classes as a single healthcare professional delivered them.

Conclusion

In conclusion, the literature related to antenatal classes is not homogenous. This may be due to social and cultural structural differences between societies, the education models are not standard, or the differences in medicolegal regulations. For a more effective study of antenatal classes, the education must be disseminated to a broader mass, that education is standardized, and that lawmakers create a robust legal basis for midwives and physicians to contribute to the scientific management of the birth process.

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Are preoperative monocytes and HDL values an early predictor of recurrence in the surgical ablation treatment of atrial fibrillation?

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Research Article

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ABSTRACT

Background: Monocyte HDL (high-density lipoprotein) cholesterol ratio has been accepted as a newly emerging cardiovascular prognostic marker. This study aims to investigate the determinants of monocyte and HDL cholesterol values in the early recurrence of atrial fibrillation (AF) treated with cryoablation and radiofrequency ablation.
Methods: This retrospective study was conducted between September 2006 and July 2014 in the Department of Cardiovascular Surgery, Adana City Hospital, Health Sciences University, including 100 patients who underwent surgical AF ablation with open heart surgery. Logistic regression analysis was used to determine monocytes and HDL cholesterol values, which are among the factors affecting recurrence in the first three months postoperatively.
Results: 100 patients who underwent surgical ablation together with open heart surgery were evaluated for early postoperative recurrence. According to the logistic regression analysis, the most effective features and measurements for early recurrence were diabetes mellitus (DM), AF duration, left atrial diameter, low HDL cholesterol, and high monocyte values before the procedure.
Conclusion: Preoperative low HDL and high monocyte values can be considered a determining factor for early recurrence in surgical ablation treatment of AF.

Keywords: Monocytes, ablation, atrial fibrillation

Preoperatif monosit ve HDL değerleri atriyal fibrilasyonun cerrahi ablasyon tedavisinde rekürrensin erken belirleyicisi midir?

Süreç

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ÖZ

Amaç: Monosit HDL(high-density lipoprotein) kolesterol oranı yeni çıkan bir kardiyovasküler prognostik marker olarak kabul edilmiştir. Bu çalışmanın amacı kriyoablasyon ve radyofrekans ablasyon uygulanan atriyal fibrilasyon (AF) tedavisinde erken dönem rekürrensinde monosit ve HDL kolesterol değerlerinin belirleyiciliğini araştırmaktır.
Yöntemler: Bu retrospektif çalışmamız Eylül 2006 ile Temmuz 2014 tarihleri arasında, Sağlık Bilimleri Üniversitesi Adana Şehir Hastanesi Kalp ve Damar Cerrahisi kliniğinde açık kalp cerrahisi ile birlikte cerrahi AF ablasyonu uygulanan 100 hasta dahil edilerek yürütüldü. Postoperatif ilk 3 ay içinde rekürrensi etkileyen faktörlerden monosit ve HDL kolesterol değerlerinin belirleyiciliği için logistik regresyon analizi kullanıldı.
Bulgular: Açık kalp cerrahisi ile birikte cerrahi ablasyon uygulanan 100 hasta postoperatif erken rekürrens açısından değerlendirildi. Logistik regresyon analizine göre erken rekürrensin görülmesine en etkili özellik ve ölçümler diabetes mellitus (DM) , AF süresi, sol atriyum çapı ve işlem öncesi düşük HDL kolesterol ve yüksek monosit değerleri bulunmuştur.
Sonuç: Preoperatif düşük HDL ve yüksek monosit değerleri AF'nin cerrahi ablasyon tedavisinde erken rekürrensi belirleyici bir faktör olarak düşünülebilir.

Anahtar sözcükler: Monosit, ablasyon, atriyal fibrilasyon

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Introduction

Atrial fibrillation (AF) is a supraventricular tachyarrhythmia that develops as a result of the irregular electrical activity of the atria and is characterized by a loss of atrial mechanical function. The prevalence of AF is around 1.5-2% in adults, and it is known to increase with age. One of the most critical and devastating clinical conditions of AF is stroke. In addition, AF is a strong and independent risk factor for mortality and morbidity [1]. AF is particularly important for patients undergoing cardiac surgery because 30-84% of patients admitted for mitral valve surgery have chronic AF. This rate is 5% for coronary artery patients [2]. Although AF is so common, medical treatment has been tried for a long time, but the desired level of success has not been achieved. Different surgical techniques have been developed for this purpose. The Maze III procedure, which was developed and subsequently modified twice by James Cox et al., has become the gold standard. However, radiofrequency and cryoablation methods are widely used today because this procedure takes a lot of time, has a high risk of complications, and requires a lot of experience. However, recurrence of AF is common and frequently occurs in the first three months after ablation [3].

Inflammation and oxidative stress have been shown to contribute significantly to the structural formation of AF. It has been shown that CRP, IL-1, IL-6, IL-8, and TNF are closely related to thrombogenesis and electrical and structural formation in AF. Recent studies have supported that the monocyte/HDL cholesterol (M/H) ratio is an inflammation and oxidative stress factor and can be a cardiovascular prognostic biomarker. Canpolat et al. evaluated the M/H value in late recurrence after catheter ablation in patients with non-valvular AF [4]. Adil et al. In another study conducted by Maze, they showed the prognostic M/H value that determines the recurrence in the early period after the procedure [5]. Our study investigated the determinants of preoperative monocytes and HDL cholesterol values in the early recurrence of AF after surgical ablation.

MATERIALS AND METHODS

Patients

In this study, 100 patients who underwent surgical AF with open heart surgery at the Department of Cardiovascular Surgery of Adana City Hospital, Health Sciences University, between September 2006 and July 2014 were retrospectively analyzed. Radiofrequency ablation methods were applied to the patients as surgical ablation. He was examined for early recurrence after the surgical procedure. The study was conducted according to the recommendations set by the Declaration of Helsinki for Human Subjects Biomedical Research, and the institutional ethics committee approved the protocol.

Determination of Clinical Information and Risk Factors

Demographic data (age, gender, DM, HT, hyperlipidemia (HPL), smoking, and family history of coronary artery disease) of the patients included in the study were recorded. Diabetic patients using oral antidiabetic and/or insulin or fasting blood glucose levels above 126 mg/dL in at least two measurements were recorded as diabetic patients defined. The presence of HPL was defined in patients with total cholesterol of 200 mg/dL or a low-density lipoprotein (LDL) value of 100 mg/dL according to the 'Adult Treatment Panel III' guideline or in patients receiving lipid-lowering drug therapy.

Standard transthoracic echocardiography (TTE) examinations were performed with the EPIQ 7 TTE device. TTE examinations were performed with the patient in the supine position or lying on the left side using appropriate echocardiographic windows using Mmod, two-dimensional, color Doppler, and pulse wave Doppler echocardiography methods. Left ventricular and end-systolic dimensions, ejection fraction (EF), posterior wall thicknesses, interventricular septum thicknesses, and left atrium dimensions were measured.

Surgical Ablation Methods and Follow-up

After the median sternotomy, bicaval cannulation was performed in all patients, and cardiopulmonary bypass (CPB) was performed. Antegrade and retrograde isothermal blood cardioplegia were used for myocardial protection. Medtronic Cardioblate BP ablation system (Cardioblate, Medtronic Inc, Minnesota, USA) was used for bipolar radiofrequency ablation. Thrombectomy was performed first in cases with thrombosis. Left pulmonary veins were explored and freed. Bipolar ablation was applied epicardially, and both pulmonary veins were isolated as islands. Then, the lesion was created by applying epicardial ablation to the left atrial appendage. A lesion was created around both right pulmonary veins by means of a bipolar catheter, and the left atriotomy incision was combined.

Amiadoron loading infusion (5 mg/kg/hour) was applied at the end of the CPB, and amiodarone maintenance (5 micrograms/kg/minute) treatment was applied in the intensive care unit. At discharge, the patients were given amiadoron therapy at a dose of 400 mg/day for 60 days. Electrocardiographs of the patients were evaluated for the first three months.

Statistical analysis

IBM SPSS 20.0 statistical program was used to analyze the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements as mean and standard deviation (median and minimum-maximum where appropriate). When comparing categorical measures across treatments, a Chi-square test statistic was used. T-test was used in independent groups to compare

numerical measurements between treatments. Logistic regression analysis was used to determine the most effective features and measures in determining the presence of recurrence. Statistical significance level was taken as 0.05 in all tests.

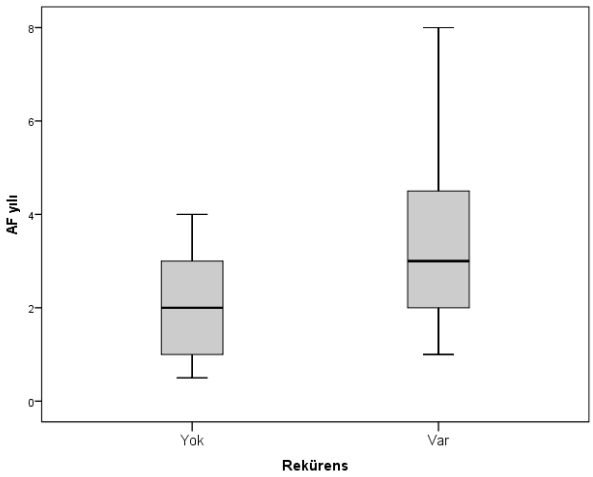
Results

One hundred patients who underwent surgical AF ablation were included in our study. All patients were followed up after surgery. The patients' clinical, echocardiographic, and laboratory parameters were compared in both groups with and without early recurrence (Table 1). Smoking was found to be significant in those with recurrence (P: 0.022). In the analysis of categorical and numerical measurements, the increase in AF year, left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVSd), and left atrial diameter (LA diameter) was statistically significant (Table 2). According to the logistic regression analysis, recurrence was determined by the presence of DM in patients (P: 0.04), AF durations (every 1-year increase) (P: 0.03), and left atrial diameter (each 1 unit increase) (LA) (P: 0). ,02) values. Every 0.1 unit increase in monocytes before surgical ablation (P: 0.03) and a decrease of 1 unit in preoperative HDL values (P: 0.009) were statistically significant (Table 3).

Table 1: Analysis of categorical measures to detect early postoperative recurrence

	recurrence		P	
	No	**Yes**		**Odds Ratio (%95 GA)**
Age, average±SS	56,2±13,9	61,4±15,9	0,116	--
Sex, average (%)			0,225	--
Male	37 (%51)	10 (%37)		
Female	36 (%49)	17 (%63)		
BMI, average±SS	25,5±3,9	26,2±3,5	0,369	--
Cigarette, number (%) *			0,022	2,85 (1,15-7,12)
Yes	53 (%80)	13 (%20)		
No	20 (%59)	14 (%41)		

BMI, body mass index; SS, standard deviation



Recurrence

Graph 1: AF year in determining early recurrence in patients undergoing surgical ablation AF year

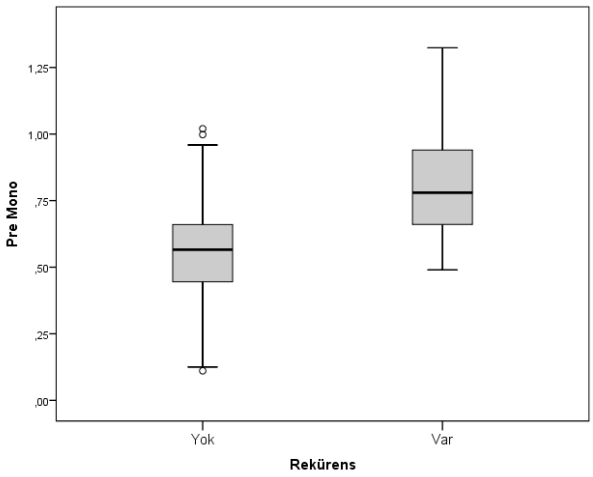
Table 2: Analysis of risk factors in early recurrence detection

	recurrence		P	Odds Ratio
	No	Yes		(%95 GA)
AF year, average±SS	2,2±1,0	3,4±1,9	0,001	1,99 (1,35-2,95)
median (min-max)	2 (1-4)	3 (1-8)		
EF, average±SS	52,3±7,4	48,9±12,2	0,183	--
LVDd, average±SS	4,8±0,4	5,1±0,4	0,007	4,76 (1,45-15,61)
LVSd, average±SS	3,7±0,4	4,1±0,5	<0,001	6,10 (2,13-17,48)
LA diameter, average±SS	4,9±0,5	5,4±0,5	<0,001	6,73 (2,63-17,25)

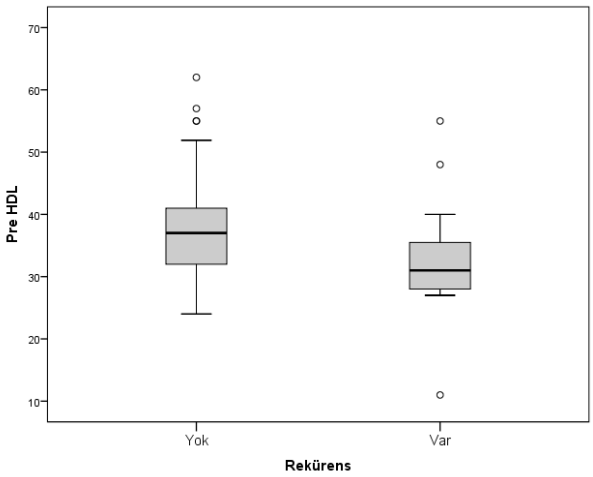
EF, ejection fraction; LVDd, left ventricular end-diastolic diameter; LVSd, left ventricular end-systolic diameter; LA, left atrium

Table 3: The most effective features and measures in determining recurrence according to logistic regression analysis HDL, high-density lipoprotein

	P	Odds Ratio	OR için %95 Güven aralığı
DM existance	0,041	6,72	1,08-41-75
AF period (every one year increase)	0,035	1,87	1,05-3,36
LA diameter (every 1 unit increment)	0,021	5,70	1,30-25,02
Preoperative monocytes (every 0.1 unit increase)	0,034	1,55	1,03-2,33
Preoperative HDL (every 1 unit decrease)	0,009	1,17	1,04-1,31



Graph 2: The effect of preoperative monocytes and HDL values on recurrence



Graph 3: PreHDL Levels and Recurrence

Discussion

Inflammation and oxidative stress are essential in the pathogenesis of atrial fibrillation and cardiovascular diseases. Inflammatory mediators are closely related to the electrical and structural new formation of the atria 6. Experimental and clinical studies have shown that oxidative stress is closely related to AF 7. Monocytes are important sources of proinflammatory and pro-oxidant cytokines. It plays a key role in initiating, maintaining, and recurrence of AF triggered by inflammatory events. Monocytes and macrophage infiltration have been detected in the atria of patients with AF 8.

Dyslipidemia, especially high triglyceride (TG), high LDL cholesterol, and low HDL cholesterol levels, are risk factors for cardiovascular disease 9. HDL cholesterol has antioxidant, anti-inflammatory, and antithrombotic effects such as reducing macrophage accumulation, inhibiting the transmigration of monocytes, increasing nitric oxide synthase expression in endothelial tissues, and protecting endothelial cells 10. However, the relationship between lipid profile and AF recurrence is inconsistent. Increased AF recurrence was demonstrated in patients with low blood HDL cholesterol levels and high TG levels. Still, no correlation was observed between total cholesterol or LDL cholesterol levels and AF 11.

Smoking; the relationship between systemic inflammatory response, vascular endothelial damage, and atherosclerosis has been defined by studies 12. In a study by Köylü et al., erythrocyte, hemoglobin, hematocrit, monocytes, HDL cholesterol, and monocyte/HDL averages were found to be significantly higher in non-smokers 13.

In this retrospective study, we analyzed the factors affecting early recurrence as an inflammatory parameter in 100 patients with AF who underwent surgical ablation with open heart surgery. Our study found that many factors, such as AF year, LVDd, LVSd, LA diameter, and the presence of Diabetes Mellitus (DM), significantly affect the early recurrence of AF. The relationship between inflammatory parameters, preoperative monocytes, HDL cholesterol levels, and early recurrence of AF was investigated. It was determined that every 0.1 unit increase in preoperative monocyte values and every 1 unit decrease in HDL cholesterol levels significantly affect the early recurrence of AF after surgical ablation.

In our study, early recurrence after surgical ablation was significantly higher in individuals who actively quit smoking before surgery but were ex-smokers.

Limitations of our study: Being a single-center study, insufficient measurement of serum HDL and monocyte levels at once and details of surgical procedures are the limitations of our study

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A Multiple Myeloma Case with Ascites

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Case Report

History

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ABSTRACT

Ascite is a rare complication of multiple myeloma. It is a symptom of poor prognosis and can be noticed at any stage of the disease, not just at the initial diagnosis. The peritoneal involvement of a relapsed refractory multiple myeloma patient with Ig G lambda type is described in this case report. The patient has been prescribed two cycles of lenalidomide (15 mg) and dexamethasone (20 mg). The patient, however, did not respond to treatment.

Keywords: Multiple myeloma, ascites, plasma cell

Asitli Bir Multipl Miyelom Vakası

ÖZ

Asit, multipl miyelomun nadir bir komplikasyonudur. Kötü prognoz belirtisidir ve sadece ilk tanıda değil, hastalığın herhangi bir aşamasında fark edilebilir. Bu olgu sunumunda Ig G lambda tipi ile nüksetmiş refrakter multipl miyelom hastasının peritoneal tutulumu anlatılmaktadır. Hastaya 2 kür lenalidomid (15 mg) ve deksametazon (20 mg) reçete edildi. Ancak hasta tedaviye yanıt vermedi.

Anahtar sözcükler: Multipl miyelom, asit, plazma hücresi

Süreç

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Introduction

Multiple myeloma accounts for 10% of all hematologic malignancies. The disease is typically characterized by bone marrow infiltration as well as effects on the renal, cardiac, pulmonary, neurological, and bone structures. Ascites is a rare complication of multiple myeloma patients, and it is especially common in relapsed refractory multiple myeloma patients. There may be different mechanisms, such as hepatic infiltration of malignant plasma cells and myelomatous infiltration of the peritoneum in the pathogenesis of ascites of multiple myeloma. Multiple myeloma patients with ascites are more likely to have Ig G and Ig A monoclonal gammopathy. A case of multiple myeloma of the Ig G lambda type is presented here[1,2].

Case History

A 72-year-old patient was diagnosed with IgG lambda multiple myeloma. The patient's ECOG performance was 3. She was treated with seven cycles of melphalan-prednisolone therapy. Three cycles of bortezomib-melphalan-prednisolone were started due to recurrent disease. The patient gave up the bortezomib treatment voluntarily. However, the patient reached remission. She was unsuitable for stem cell transplantation at that time. Due to the disease's recurrence, lenalidomide and dexamethasone treatment were initiated. There was an excellent partial response. After that, the patient voluntarily gave up the medication. Then abdominal ultrasonography due to abdominal pain showed the free fluid measured 4 cm in depth between the bowel loops in the pelvic region.

Laboratory findings were: Hgb 9.86 g / dL, leucocyte 3.26x109 / L, platelet 136 x109 / L, alkaline phosphatase 80 U /L, aspartate aminotransferase 15 U / L, alanine aminotransferase 5 U / L, total bilirubin 0.55 mg/dL, direct bilirubin 0.5 mg / dL, lactic dehydrogenase 324 U / L, albumin 2.9 g / dL, total protein 10.3 g / dL, creatinine 0.86 mg / dL, calcium 9. 1 mg/ dL, CRP 0.6 mg/dL and INR [international normalized ratio]

100 cc fluid was obtained by paracentesis, and its color was yellowish. Total protein was found to be 7.81 g / dL, albumin 1.64 g / dL, LDH 1099, and glucose 73, respectively, in ascites. Flow cytometry could not be sent from the peritoneal fluid because the hospital could not work for technical reasons. After centrifugation, excentric nucleated plasma cells and atypic plasmacytoid cells are observed [Figure 2].

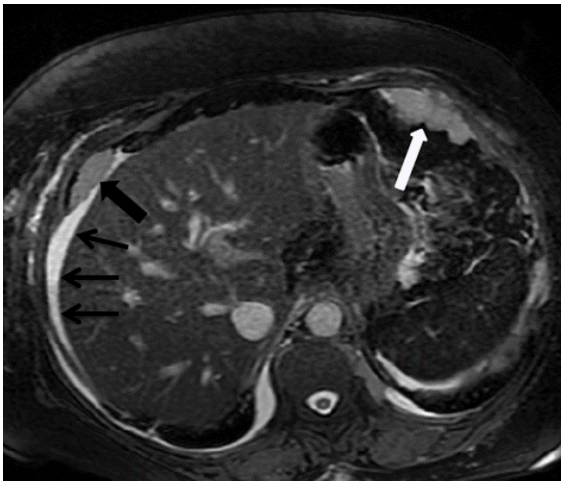

Figure 1: In T2-weighted lipid-printed axial cross-sections of the MR examination of the patient, hyperintense lesions with the largest 52x25 mm lobular contour (white arrow) are observed in the peritoneum of the epigastric region and in the right upper region of abdomen; adjacent to the free intra-abdominal fluid (thin black arrows) there is a hyperintense, 39x15 mm solid mass [solid black arrow] localized in parietal peritoneum.

A

B

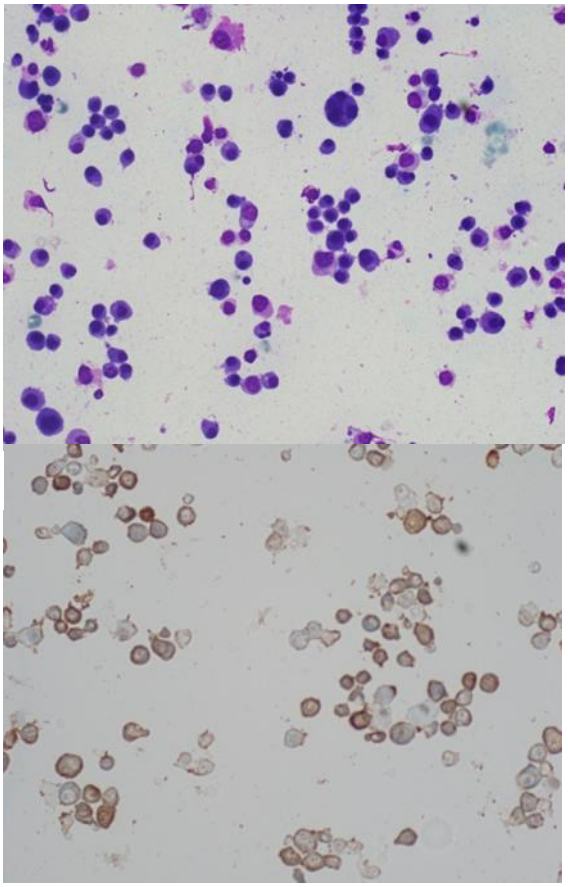

Figure 2: A) Ascites fluid cytology; May Grünwald-Giemsa [MGG] staining of atypical plasma cells (400x). B) Demonstration of plasma cells in ascites with liquid cytology CD138 (400x).

During this period, Ig G (7264 mg / dL) was found to be high; Ig A was 31 mg / dL, and Ig M was 34 mg / dL. Serum Ig G Lambda monoclonal band was also found, showing the recurrence of the disease.

Treatment with lenalidomide (15 mg) and dexamethasone (20 mg) was started. 2 cycles of therapy were given. However, the patient did not respond and was eventually exitus at the end.

Results and Discussion

Multiple myeloma can cause pleural effusion or serous cavity involvement in the peritoneum. It could be due to primary peritoneal infiltration of the disease, but it could also be due to hepatic involvement, renal amyloid accumulation, or cardiac failure [3].

In multiple myeloma, aspiration and cytological examination of ascites (with/ without biopsy) can be used for diagnosis or follow-up of patients. Malignant plasmacytic ascites is associated chiefly with implant formation in the peritoneum, and in most of these cases, liver involvement is either very small or absent [2,4]. In the secondary causes other than malignant plasmacytic ascites, the ascites is mostly severe, and the number of cells varies between 30-120 cells / mm^3. In plasmacytic ascites, plasma cells are present, and the cell number is usually between 8000 and 9000 cells/mm^3 [2].

Atypical, large-nucleated plasma cells can be seen, but so can more mature, excentric nucleated cells [4]. These cells can be distinguished from metastatic cancer cells, lymphocytes, and reactive mesothelial cells using immunohistochemistry, flow cytometry, immunofluorescence, or electron microscopy. In our case, the total cell count in ascites fluid was 11 000 cells / mm^3. Plasma cells were shown to be immunohistochemically positive for CD 138.

Hepatic infiltration was demonstrated histologically in 53% of the patients with multiple myeloma. Hepatic infiltration can be nodular or diffuse [2,5,6]. A review collected data between 1952 and 2014; the cases of multiple myeloma with ascites were examined. It was shown that only 7 of 65 cases presented with ascites at the diagnosis. 27 of the 65 cases have been identified as plasmacytic ascites [2].

No peritoneal involvement was detected in an autopsy series of 32 patients [6]. In an autopsy series consisting of 30 multiple myeloma patients with extraosseous participation, only one patient with peritoneal involvement was observed [7].

Exudative ascites was observed in 9 cases in 64 necropsy material of multiple myeloma patients, but peritoneal infiltration was not observed in these patients [4,5]. In another study, peritoneal involvement was demonstrated in 3 of 182 multiple myeloma cases with extramedullary involvement [8].

A published review showed that the mean age of myeloma patients with ascites was 60.6 (60.2 in males, 61 in females), 50% of cases had IgG, and 38% had IgA monoclonal gammopathy [2]. In our case, the patient's age at the time of diagnosis was 73 years.

Plasmacytic ascites is an indicator of increased tumor burden or widespread extramedullary involvement. The subsequent prognosis is poor, and the median survival after ascites formation is reported to be 1.5- 2 months [2,9]. In our case, the mean survival time after the ascites was approximately seven months.

Systemic chemotherapy, intraperitoneal chemotherapy, plasmapheresis, radiotherapy, and stem cell transplantation are the treatment options in cases of myelomatous ascites. Treatment options like bortezomib, melphalan, cyclophosphamide, and thalidomide have been reported [2]. One patient with multiple myeloma diagnosed with ascites was reported to have a complete response to autologous stem cell transplantation following one course of VAD chemotherapy. This response persisted over more than 14 months [10].

As a result, ascites can be seen at the diagnosis of multiple myeloma, or they can be seen at any stage of the disease. Ascites indicate that the prognosis is poor.

Conflict of Interest

The authors declared no conflict of interest.

Patient's Consent

The patient's son signed a document of informed consent.

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