Gülhane Medical Journal

ISSN: 1302-0471
E-ISSN: 2146-8052

Gulhane Med J December 2024 Volume 66 Issue 4

www.gulhanemedj.org

66/4

Gülhane
Medical Journal
Gülhane Tıp Dergisi

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk.
No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (530) 177 30 97 / +90 (539) 307 32 03
E-mail: info@galenos.com.tr • yayin@galenos.com.tr
Web: www.galenos.com.tr

Publisher Certificate Number: 14521
Publication Date: December 2024
E-ISSN: 2146-8052
International scientific journal published quarterly.

GÜLHANE MEDICAL JOURNAL
Gülhane Tıp Dergisi

GÜLHANE MEDICAL JOURNAL
Gülhane Tıp Dergisi

Gülhane
Medical Journal
Gülhane Tıp Dergisi

Please refer to the journal's webpage **(https://gulhanemedj.org/)** for "Aims and Scope", "Ethical Policy", "Instructions to Authors" and "Peer Review Process".

The editorial and publication process of Gülhane Medical Journal are shaped in accordance with the guidelines of the ICMJE, WAME, CSE, COPE, EASE, and NISO. The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing. Gülhane Medical Journal is indexed in **Scopus, British Library, DOAJ, Ulakbim TR Index, CABI, Ebsco HOST, OCLC Worldcat, Embase, Türk Medline, Türkiye Atıf Dizini, J-Gate** and **Gale**.

The journal is published online.

Owner: Gülhane Faculty of Medicine, University of Health Sciences Türkiye

Responsible Manager: Mehmet Ali Gülçelik

Gülhane
Medical Journal
Gülhane Tıp Dergisi

Contents

ORIGINAL ARTICLES

CASE REPORT

INDEX

GÜLHANE MEDICAL JOURNAL
Gülhane Tıp Dergisi

DOI: 10.4274/gulhane.galenos.2024.72681
Gulhane Med J 2024;66(4):158-166

Investigation of viral load in patients with Coronavirus disease-2019 with and without comorbidities

Clarisa Josevine[1], Andriani Andriani[2], Mahyarudin Mahyarudin[3]

[1]*Universitas Tanjungpura Faculty of Medicine, Medical Degree Program, West Kalimantan, Indonesia*

[2]*Universitas Tanjungpura Faculty of Medicine, Department of Biochemistry and Biomolecular Sciences, West Kalimantan, Indonesia*

[3]*Universitas Tanjungpura, University of Tanjungpura Faculty of Medicine, Department of Microbiology, West Kalimantan, Indonesia*

Cite this article as: Josevine C, Andriani A, Mahyarudin M. Investigation of viral load in patients with Coronavirus disease-2019 with and without comorbidities. Gulhane Med J. 2024;66(4):158-166

Date submitted:
26.08.2023

Date accepted:
03.06.2024

Online publication date:
22.11.2024

Corresponding Author:
Mahyarudin Mahyarudin, Universitas Tanjungpura Faculty of Medicine, Department of Microbiology, West Kalimantan, Indonesia
mahyarudin@medical.untan.ac.id

ORCID:
orcid.org/0000-0002-0184-3435

Keywords: COVID-19, comorbidity, viral load

ABSTRACT

Aims: Many fatal cases of Coronavirus disease-2019 (COVID-19) involved patients with comorbidities, in which a higher cellular viral copy was frequently reported. This study aimed to compare viral load (VL) among patients with COVID-19 with and without comorbidities.

Methods: This retrospective case-control study included COVID-19 patients with and without comorbidities who were hospitalized in two COVID-19 referral hospitals, Tanjungpura University Hospital and Doctor Soedarso General Hospital, in West Kalimantan, Indonesia. Demographic findings, clinical symptoms, comorbidities, and real-time polymerase chain reaction VL data were collected from medical records.

Results: This study included 136 patients (53% female and 47% male) divided equally into 68 patients with [median age interquartile range (IQR): 47 (40-58.5) years] and without comorbidities [median age (IQR): 25 (22-35.8) years]. A total of 48.5% of patients with comorbidities experienced severe COVID-19, whereas 61.8% of patients without comorbidities merely exhibited mild symptoms. The presence of comorbidities demonstrated a significant association with age and COVID-19 severity ($p<0.001$), with a trend of elevated VL among individuals with comorbidities ($p=0.023$, median: log10 1.86 vs 1.34 VL). Significant differences in VL were found between age groups ($p=0.019$) and COVID-19 severities ($p=0.001$), showing higher VL among older, moderately ill, and critically ill patients. Critical condition was experienced by COVID-19 patients with hypertension, cardiovascular disease, diabetes, and chronic obstructive pulmonary disease. Severe COVID-19 was observed in nearly all comorbid conditions, except in pregnant women and those with malignancies.

Conclusions: VL differed significantly between patients with and without comorbidities, age groups, and degrees of COVID-19 presentation. VL holds promise in monitoring disease progression in patients with COVID-19, particularly those with comorbidities.

Introduction

Coronavirus disease-2019 (COVID-19) was first reported in Indonesia on March 2nd, 2020. As of January 1st, 2021, 751.270 cases of COVID-19 were confirmed in Indonesia, with a mortality rate of 3.0% (1). Most COVID-19 fatal cases involved elderly patients and those with comorbidities. Mortality rates were reported to be higher in patients with comorbidities, such as cardiovascular disease (CVD) (10.5%), diabetes (7.3%),

chronic respiratory disease (6.3%), hypertension (6.0%), and malignancy (5.6%) (2).

As the gold standard for COVID-19 diagnosis, real-time polymerase chain reaction (RT-PCR) examination allowed the calculation of viral load (VL), a numerical description of the amount of virus copy in a fluid volume (3), which was associated with an increased risk of transmission and disease severity in other conditions (4). VL was directly linked to severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) viral binding ability on angiotensin-converting enzyme 2 (ACE2), a receptor for SARS-CoV-2 entry into the host; with VL having been suggested to be a useful marker for assessing COVID-19 severity and its prognosis (5). ACE2 expression was found to be elevated in COVID-19 patients with comorbidities, such as chronic obstructive pulmonary disease (COPD), human immunodeficiency virus (HIV) infection, type 2 diabetes mellitus, CVD, hypertension, liver diseases, and kidney diseases (6,7). Increased ACE2 levels allow more viruses to invade the cell, as observed in COVID-19 patients, in whom severely ill patients tend to have higher VL and longer viral shedding periods (8,9). Later on, increased VL was found to be associated with increased mortality rates among hospitalized patients (10), as survival analysis revealed a notable discrepancy in survival rates between individuals with high and low VL (4).

SARS-CoV-2 VL and its dynamics in different subsets of patients are still underexplored, and no study has compared VL among COVID-19 patients with and without comorbidities; though the presence of comorbidities, such as hypertension, coronary artery disease (CAD), congestive heart failure (CHF), COPD, and malignancy, was previously associated with poor prognosis in COVID-19 patients (11). This study aimed to compare VL in COVID-19 patients with and without comorbidities to observe the relationships between demographic status, clinical symptoms, comorbidities, and patients' VL; consequently, the potential application of VL as a quantitative determinant of disease progression in patients with COVID-19.

Methods

Study design and participants

This observational, retrospective, case-control design was conducted at Tanjungpura University Hospital and Doctor Soedarso General Hospital. Both settings are regional referral hospitals for COVID-19 patients in West Kalimantan, Indonesia. The study participants were the individuals who tested positive for COVID-19 between November 2020 and January 2021. The inclusion criteria were as follows: comprehensive written medical records detailing comorbidities, symptoms upon hospital admission, pertinent demographic information, and PCR analysis using identical examination kits. The exclusion criteria for the study were applied to COVID-19 patients lacking complete medical records for the specified parameters. Naso-oropharyngeal swabs were obtained from patients with suspected COVID-19, and then RNA extraction and ORF1ab (RdRp) gene amplification were performed using RT-PCR examination. Medical records of individuals with positive COVID-19 tests were compiled and analyzed.

The sample size was determined using the *OpenEpi* application (www.OpenEpi.com) using the *Sample Size: Mean Difference* formula, resulting in a minimum sample requirement of 65 cases and 65 control samples. During the study period, 262 patients tested positive for COVID-19. Among this group, 151 patients met the research criteria. Of these 151 patients 68 patients admitted with other comorbidities alongside COVID-19. Of the remaining 83 patients without comorbidities, 68 were randomly selected to be included in the "no comorbidity" group. The final analysis included 68 COVID-19 patients with comorbidities (case group) and 68 patients without comorbidities (control group).

Data collection

The study variables included demographic characteristics such as sex and age, pre-existing comorbidities, COVID-19 related symptoms upon admission along with their severity classification, and SARS-CoV-2 VL and corresponding cycle threshold (Ct) values. Patient age was categorized according to the Indonesian COVID-19 Task Force's classification (0-5, 6-18, 19-30, 31-45, 46-59, and ≥60 years old) as well as their comorbid conditions [hypertension, CVD, diabetes, COPD, chronic kidney disease (CKD), malignancy, cerebrovascular accident (CVA) or stroke, pregnancy, liver diseases, immunodeficiency, and other related conditions]. After dividing the participants into case and control groups, those with preexisting comorbidities were further stratified into subgroups based on the presence of either single or multiple comorbidities.

The severity of COVID-19 was categorized according to the China-China Centers for Disease Control and Prevention classification (12). Asymptomatic COVID-19 was defined as a positive COVID-19 nucleic acid test result without any clinical symptoms and normal chest imaging. In mild cases, the patient presented with symptoms of acute upper respiratory tract infection (fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing) or digestive symptoms (nausea, vomiting, abdominal pain, diarrhea). In moderate cases, pneumonia manifested as frequent fever and cough, but there was no evident hypoxemia or detectable lesions on chest computed tomography. In severe cases, pneumonia patients presented with SpO_2 <92% (hypoxemia). During the critical phase, patients presented with acute respiratory distress syndrome (ARDS).

Ethics

This study was conducted in accordance with the principles of the 2013 revised Declaration of Helsinki. Research permit and ethical approval were obtained from the Ethics Committee of the Faculty of Medicine of Tanjungpura University (no: 2904/UN22.9/TA/2021; research permit no: 2931/UN22.20/TU/2021, date: 08.04.2021) and the Health Research Ethics Committee of Doctor Soedarso General Hospital (no: 03.04/RSDS/KEPK/2021; research permit: no: 070/2431/RSDS/PGB-b/2021, date: 08.04.2021).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 26.0 software (IBM Corp., Armonk, NY: USA). The variables analyzed include patients' demographic characteristics, comorbidity status, the severity of COVID-19 symptoms upon admission, and log10-transformed VL and their respective Ct values. Descriptive analyses of frequencies and proportions for categorical variables and medians and interquartile ranges for continuous variables were performed. The normality of the distribution of the numerical data was tested using the Kolmogorov-Smirnov test. Subsequently, the groups were compared using statistical methods appropriate for the data type. Categorical variables were analyzed using the Pearson Chi-square test or Fisher's exact test, whereas numerical data were assessed using the independent sample t-test or analysis of variance if normally distributed. Alternatively, non-normally distributed numerical data were analyzed using the Mann-Whitney U test or the Kruskal-Wallis test.

Results

Demographic characteristics and clinical presentation

A total of 136 patients were grouped according to their demographic findings: 47.1% were male (30 males with comorbidities and 34 without) and 52.9% were female (38 females with comorbidities and 34 without), with a median age of 47 (IQR, 40-58.5) and 25 (IQR, 22-35.8) years in those with and without comorbidities, respectively. Approximately half (48.5%) of the patients with comorbidities were admitted with severe COVID-19, whereas only two (2.9%) patients without comorbidities were admitted with severe COVID-19. The majority of those without comorbidities had either mild COVID-19 symptoms (61.8%) or were asymptomatic (30.9%). Among the comorbid groups, excluding those with severe COVID-19, approximately 29.4% of patients had mild COVID-19 symptoms and 11.8% were asymptomatic. Notably, there was a significantly higher proportion of moderately ill patients (22% vs 2.9%) and critically ill patients (11.8% vs 1.8%) in the comorbid group than in the non-comorbid group. Individuals with comorbidities were observed to be notably older than those without ($p<0.001$). A statistically significant relationship was identified between the presence of comorbidities and both patient age ($p<0.001$) and COVID-19 severity ($p<0.001$). The presence of comorbidities showed no correlation with patients' gender ($p=0.492$). Similarly, sex did not correlate with the severity of COVID-19 illness ($p=0.056$), although a higher proportion of male patients was observed among those with moderate to critical illness compared in both the case (86.7% vs 47.4%) and control (14.7% vs 0%) groups (Table 1).

Comparison of viral load distribution among patients with COVID-19

Patients' VL were categorized based on their log10-transformed values and the corresponding Ct ranges; though the normality test showed that the numerical data were not normally distributed ($p<0.001$), even after log transformation. Most of the participants (71.3%) exhibited low VL, as indicated by their higher Ct values (>30). A greater proportion of patients with comorbidities demonstrated high VL (Ct ≤25) compared with those without comorbidities (19.1% vs 7.4%). There was no statistically significant difference observed in the median VL [log10 1.9 (1.2-3.1) vs 1.3 (1.1-2.3); $p=0.230$] or Ct values [32.5 (35.1-27.6) vs 34.6 (36.1-30.9); $p=0.123$] between the groups (Table 1). The VL distribution varied significantly among individuals with and without comorbidities ($p=0.023$) (Figure 1C), across different age groups ($p=0.019$) (Figure 1B), and among different levels of COVID-19 severity ($p=0.001$) (Figure 1D). The median VL was highest among moderately ill patients and lowest among asymptomatic patients (Figure 1D). There was no significant difference in VL between male and female COVID-19 patients ($p=0.195$) (Figure 1A).

Distribution of comorbidities in patients with COVID-19

As previously defined, the participants' comorbidity status was categorized into three groups: no comorbidity, single comorbidities, and multiple comorbidities. Of the 68 patients with comorbidities, 63.2% had a single comorbidity and 36.8% had multiple comorbidities (Table 2). The majority of COVID-19 patients with comorbidities suffered from hypertension (42.6%), either as a single comorbidity or in conjunction with other comorbid conditions; followed by diabetes (23.8%), CVD (13.9%), pregnancy (9.9%), malignancy (4.0%), and CVA (3.0%). COPD and CKD were each identified in one patient, with both conditions occurring alongside other comorbidities, such as diabetes, hypertension, and CVD. HIV was observed in a patient with tuberculosis co-infection. Further analysis showed an association between patients' COVID-19 severity levels and comorbid conditions ($p<0.001$). COVID-19 patients with hypertension, CVD, diabetes, and COPD were found to experience critical conditions. Severe symptoms were observed in nearly all comorbid conditions, except in pregnant women and patients with malignancies. Asymptomatic COVID-19 was observed only in pregnant women (Figure 2).

Table 1. Demographic characteristics and viral load in patients with and without comorbidities

Characteristics	Total (n=136)	With comorbidities (n=68)		Without comorbidities (n=68)		p value*
Gender, Female, n (%)	72 (52.9)	38 (52.8)		34 (47.2)		0.492
Age, median (IQR)		47 (40-58.5)		25 (22-35.8)		**<0.001**
0-5, n (%)	4 (2.9)	-		4 (100)		
6-18, n (%)	6 (4.4)	-		6 (100)		
19-30, n (%)	41 (30.1)	8 (19.5)		33 (80.5)		
31-45, n (%)	41 (30.1)	22 (53.7)		19 (46.3)		
46-59, n (%)	28 (20.6)	22 (78.6)		6 (21.4)		
≥60, n (%)	16 (11.8)	16 (100)		-		
Disease severity, n (%)		**Male**	**Female**	**Male**	**Female**	
Asymptomatic	29 (18.4)	4 (13.8)	4 (13.8)	9 (31)	12 (41.8)	
Mild	62 (45.6)	4 (6.5)	16 (25.8)	20 (32.6)	22 (35.5)	
Moderate	17 (12.5)	10 (58.8)	5 (29.4)	2 (11.8)	-	0.056
Severe	25 (18.4)	12 (48)	11 (44)	2 (8)	-	
Critical	7 (5.1)	4 (57.1)	2 (28.6)	1 (14.3)	-	
Cycle threshold, n (%)						
>30	97 (71.3)	44 (45.4)		53 (54.6)		
25-30	21 (15.4)	11 (52.4)		10 (47.6)		0.129
≤25	18 (13.2)	13 (72.2)		5 (27.8)		
Median (IQR)		32.45 (35.1-27.6)		34.58 (36.1-30.9)		0.123
Viral Load, Log10, median (IQR)		1.86 (1.20-3.12)		1.34 (1.09-2.28)		0.230

IQR: Interquartile range

The VL distribution across different comorbid conditions is depicted in Figure 3A, which shows that patients with CVD, hypertension, diabetes, and COPD had the highest median VL [log10 values of 2.13 (1.66-2.03), 1.98 (1.31-3.14), 1.86 (1.24-3.51), and 1.84, respectively]. However, no significant differences were observed in the VL distribution (p=0.457) or median (p=0.632) among the comorbid conditions (Figure 3A). VL distribution according to the degree of comorbidity is displayed in Figure 3B. Patients with multiple comorbidities exhibited a notably higher median VL compared with those with single comorbidity or no comorbidity [log10 values of 1.88 (1.24-2.65), 1.72 (1.11-3.16), and 1.34 (1.09-2.28), respectively], although this finding did not reach statistical significance (p=0.232). There was a marginally significant difference in VL between patients without comorbidities and those with either single or multiple comorbidities (p=0.072).

Discussion

Comorbidity status and characteristics of patients with COVID-19

Advanced age is associated with an increased risk of developing COVID-19. Apart from being an independent risk factor for COVID-19, older age was also associated with comorbidities (13), as evidenced in this study, which showed COVID-19 patients with comorbidities were significantly older than those without comorbidities. Most COVID-19 patients without comorbidities were younger, ranging from 19 to 30 years of age, whereas patients with comorbidities ranged from 31 to 59 years of age. A prior study from the United Kingdom indicated that individuals aged 40-64 years face the highest risk of COVID-19 infection, followed by those aged 65-74 years and individuals aged 75 years and older (14). As of July 2021, data from the Indonesian Government COVID-19 Task Force showed that the highest positivity rate for COVID-19 was in those aged between 31 and 45 years, with the mortality rate being dominated by patients aged 60 years and above (50%) (1).

Not only age but also comorbidity status was associated with disease severity. Nearly half of the patients with comorbidities suffered severe COVID-19 in this study, whereas the majority of patients without comorbidities had mild disease or were asymptomatic. Comorbidities, such as hypertension and CVD, have been reported to increase the risk of COVID-19 infection, resulting in severe clinical presentation (15), particularly among elderly individuals. A meta-analysis also reported that the presence of one or a combination of comorbidities significantly increased the severity of COVID-19 (16).

This study showed no statistically significant association between comorbidity status and patients' sex. Nevertheless,

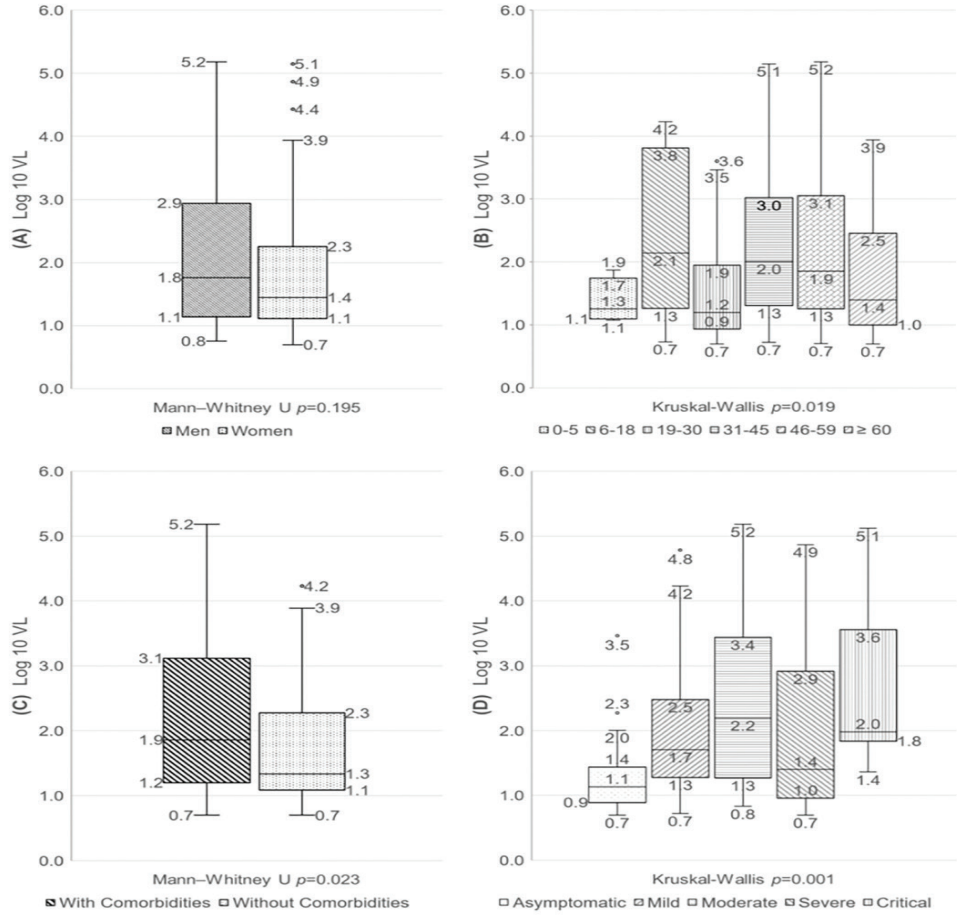

Figure 1. Distribution of log10 VL values in subsets of patients with COVID-19 according to (A) sex, (B) age range, (C) comorbidity status, and (D) disease severity

COVID-19: Coronavirus disease-2019, VL: Viral load

Table 2. Viral load distribution by comorbidity characteristics

Variables	**Total (n=136)**	**With comorbidities**	**Without comorbidities**	
Degree of comorbidity, n (%)		**Log10 VL, median (IQR)**	**Log10 VL, median (IQR)**	
Single	43 (31.6)	1.72 (1.11-3.16)	-	
Multiple	25 (18.4)	1.88 (1.24-2.65)	-	
Without	68 (50)	-	1.34 (1.09-2.28)	
Comorbidities, n (%)	**Total (n=101)**	**As single comorbidity**	**Among multiple comorbidities**	**Log10 VL, median (IQR)**
Hypertension	43 (42.6)	20 (46.5)	23 (53.5)	1.98 (1.31-3.14)
Cardiovascular disease	14 (13.9)	3 (21.4)	11 (78.6)	2.13 (1.66-2.03)
Diabetes	24 (23.8)	8 (33.3)	16 (66.7)	1.86 (1.24-3.51)
Chronic obstructive pulmonary disease	1 (1.0)	-	1 (100)	1.84
Chronic kidney disease	1 (1.0)	-	1 (100)	1.14
Malignancy	4 (4.0)	3 (75)	1 (25)	1.37 (1.05-2.02)
Cerebrovascular accident	3 (3.0)	1 (33.3)	2 (66.7)	1.26
Pregnancy	10 (9.9)	7 (70)	3 (30)	1.35 (1.05-2.01)
Immunological disorders	1 (1.0)	1 (100)	-	1.11

IQR: Interquartile range

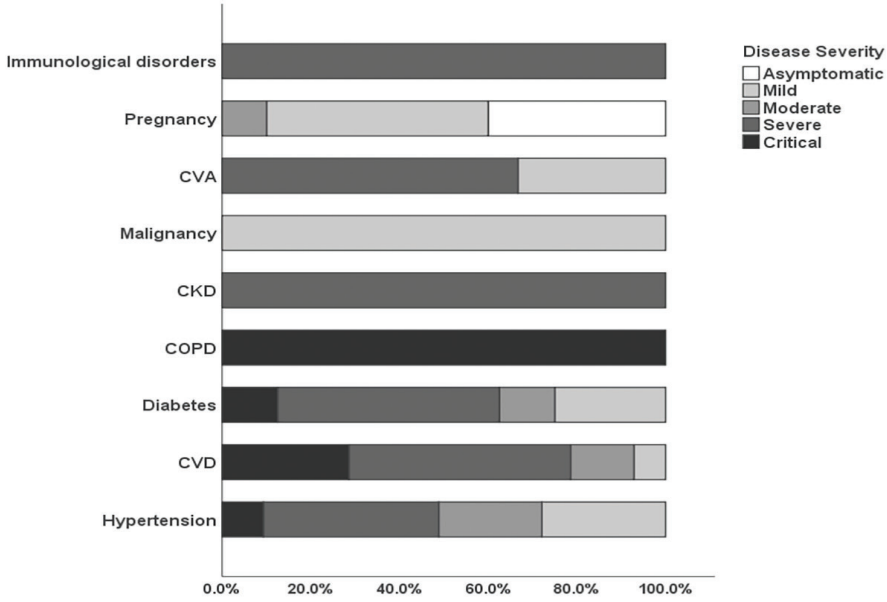

Figure 2. Distribution of COVID-19 severity according to patients' comorbidity type showed an association between various comorbidities and stages COVID-19 presentation (p<0.001)

CVA: Cerebrovascular accident, CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, CVD: Cardiovascular disease

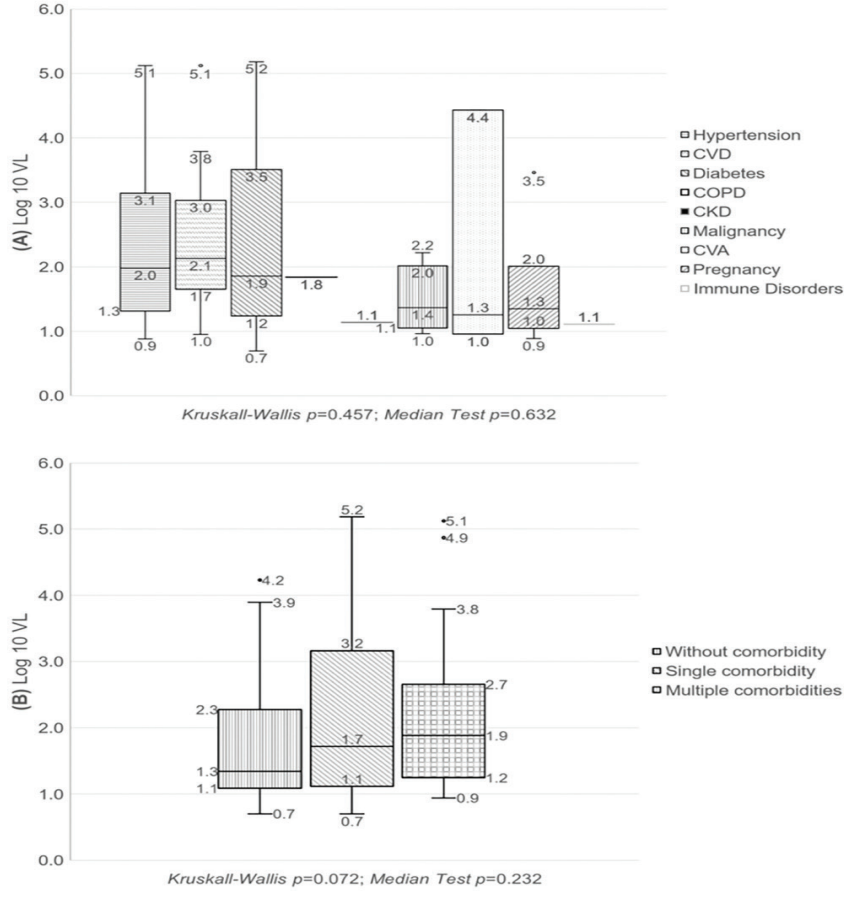

Figure 3. Distribution of log10 VL values in COVID-19 patients with comorbidities according to their (A) comorbidity type/condition and (B) comorbidity classification (single or multiple)

CVD: Cardiovascular disease, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, CVA: Cerebrovascular accident, VL: Viral load, COVID-19: Coronavirus disease-2019

male patients constituted significantly higher proportions of patients with moderate to critical illness compared with female patients, regardless of comorbidity status, consistent with findings reported in a systematic review and meta-analysis examining sex differences in COVID-19 (17). This finding could be attributed to higher immune responses to viral infections in women due to sex hormones, leading to cytokine and chemokine production (18). After viral exposure, antigen expression, recognition, and initiation of adaptive immune responses were reported to be higher in women than in men (19).

Viral load in patients with COVID-19

Significant differences in VL distribution were observed among patients with and without comorbidities, across different age groups, and at different COVID-19 severity levels. Patients with comorbidities tend to have higher VLs than patients without comorbidities, which could be attributed to the increased expression of ACE2 receptors in several comorbid conditions, such as hypertension, CVD, diabetes, kidney disease, COPD, and HIV (6,7). Elevated ACE2 receptor levels in these patients might manifest as a higher VL and a higher rate of cell damage (20). Further VL increase in comorbid individuals can contribute to hyper-inflammation, alveolar apoptosis, and ultimately ARDS and multi-organ failure (21).

The distribution of VL varied significantly across different age groups, which contrasted with a previous study that described VL in patients with COVID-19 as comparable across age groups and suggested that age is not a strong predictor of disease outcome (22). Older age was previously associated with higher VL peaks in nasopharyngeal samples, which was associated with a weaker immune system and a higher prevalence of comorbidities in elderly patients (23). Comparisons of VL between adult and pediatric patients may also be influenced by external factors, such as lower swab volumes due to smaller pediatric swab sizes and the frequently missed symptoms of mild COVID-19 in children (24). A study examining infectiousness during SARS-CoV-2 infection indicated that differences in VL were minor and insufficient to affect SARS-CoV-2 transmission dynamics significantly. Children were found to have slightly lower VL than adults, but the difference was not clinically significant (25).

All symptomatic patients had higher VL than asymptomatic patients, which was in line with a study on the relationship between VL and secondary transmission in COVID-19 (26). Higher VL was observed in mild COVID-19 rather than severe COVID-19 because it reflected the onset of infection (27). This observation was consistent with the progression of VL among patients in this study: VL levels began relatively lower in asymptomatic patients, increased in patients with mild symptoms, reached a peak in those with moderate symptoms, decreased in patients with severe COVID-19, and showed a slight increase in critically ill patients. This finding is consistent with a study investigating viral dynamics in mild and severe COVID-19 cases, showing lower VL levels in mild cases and stable or increasing VL levels in severe COVID-19 cases (5). Another study that investigated whether higher SARS-CoV-2 VL was associated with death also reported that baseline VL in patients with moderate COVID-19 was significantly higher than those with mild or severe COVID-19 (10).

Comorbidities in patients with COVID-19

Previous reports have indicated positive correlations between VL and conditions such as malignancies and diseases other than hypertension (27). Patients with CAD, CHF, cerebrovascular disease, hypertension, COPD, CKD, and active malignancy have been consistently noted to be at increased risk of high VL (11). The present study found that comorbidity status was associated with COVID-19 severity. Upon admission, critically ill presentations of ARDS were observed among patients with comorbidities such as hypertension, CVD, diabetes, and COPD. Severe COVID-19 was also noted across nearly all comorbid conditions, except in pregnant women and those with malignancies, who predominantly exhibited mild symptoms. Hypertension, diabetes, and CVD such as CHF and CAD dominated the comorbid conditions in this study, all of which have been reported to increase the risk of severe COVID-19 infection, poor prognosis, and increased mortality (28,29). Among patients with COVID-19 and CVD, increased ACE2 expression in pericytes and cardiomyocytes predisposes them to a higher risk of severe cardiac complications (30).

Comorbidities with lower prevalence in this study included pregnancy, malignancies such as osteosarcoma and colorectal cancer, and ischemic and hemorrhagic CVA. Pregnant women face an elevated risk of COVID-19 infection and related mortality due to physiological changes associated with pregnancy, such as increased oxygen demand, reduced lung capacity, and increased susceptibility to thromboembolic events (31). Patients with malignancy were also at greater risk of SARS-CoV-2 infection due to immunosuppressive therapy and repeated hospital visits, resulting in worse clinical outcomes than those without malignancy (32). COVID-19 patients with pre-existing cerebrovascular disease also exhibited worse outcomes (33). Dysregulation of ACE2 resulting from SARS-CoV-2 binding reduces perfusion in ischemic areas and contributes to increased infarct volume in patients with ischemic stroke (34). Similar dysfunctions also play a role in cerebrovascular endothelial disorders, which contribute to the pathogenesis of intracerebral hemorrhage in patients with COVID-19 (34).

COPD and CKD were each identified in one patient, with both conditions previously reported to be associated with a twofold increased risk of severe COVID-19 and death (35). ACE2 receptor expression was reported to increase in COPD, contributing to lung structural damage, weakened immunity,

and excessive mucus production (7). CKD was reported to be an independent risk factor for the development of acute kidney injury in COVID-19 patients (13). SARS-CoV-2 infection could impact the disease progression of patients with HIV and HIV-tuberculosis coinfection, particularly in individuals with suboptimal treatment adherence. Previous reports have highlighted untreated *Mycobacterium tuberculosis* as an independent risk factor associated with poorer prognosis in COVID-19 patients co-infected with tuberculosis (36). However, the prognosis was reportedly better for patients on anti-retroviral therapy (37).

Swab samples were collected upon admission rather than prospectively based on infection onset or symptoms. As a result, patients at different disease stages displayed highly varied VL results in this study. Additionally, the range of comorbidities studied during the study period was relatively limited to a select few conditions. Consequently, data on the impact of comorbidities with smaller sample sizes on VL outcomes are limited.

Conclusion

The VL in patients with COVID-19 exhibited significant variability based on comorbidity status, age, and clinical presentation severity. VL is a tool for monitoring disease progression in patients with COVID-19, particularly those with comorbidities. However, additional studies are required to validate the utility of this approach in clinical practice.

Ethics

Ethics Committee Approval: Research permit and ethical approval were obtained from the Ethics Committee of the Faculty of Medicine of Tanjungpura University (no: 2904/UN22.9/TA/2021; research permit no: 2931/UN22.20/TU/2021, date: 08.04.2021) and the Health Research Ethics Committee of Doctor Soedarso General Hospital (no: 03.04/RSDS/KEPK/2021; research permit: no: 070/2431/RSDS/PGB-b/2021, date: 08.04.2021).

Informed Consent: Retrospective case-control study.

Footnotes

Authorship Contributions

Concept: C.J., A.A., M.M., Design: C.J., A.A., M.M., Data Collection or Processing: C.J., A.A., M.M., Analysis or Interpretation: C.J., A.A., M.M., Literature Search: C.J., Writing: C.J., A.A., M.M.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Satgas Penanganan COVID-19. Peta Sebaran COVID-19. Last Accessed Date: 15.01.2022. Available from: https://covid19.go.id/peta-sebaran
2. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239-1242.
3. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581:465-469.
4. Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. Lancet Respir Med. 2020;8:e70.
5. Liu Y, Yan L-M, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis. 2020;20:656-657.
6. Zamorano Cuervo N, Grandvaux N. ACE2: Evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities. Elife. 2020;9:e61390.
7. Ejaz H, Alsrhani A, Zafar A, et al. COVID-19 and comorbidities: Deleterious impact on infected patients. J Infect Public Health. 2020;13:1833-1839.
8. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11:1620.
9. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63:364-374.
10. Faíco-Filho KS, Passarelli VC, Bellei N. Is Higher Viral Load in SARS-CoV-2 Associated with Death? Am J Trop Med Hyg. 2020;103:2019-2021.
11. Magleby R, Westblade LF, Trzebucki A, et al. Impact of Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients With Coronavirus Disease 2019. Clin Infect Dis. 2021;73:e4197-e4205.
12. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol. 2020;215:108427.
13. World Health Organization. Public Health Surveillance for COVID-19: Interim guidance. Last Accessed Date: 28.11.2020. Available from: https://www.who.int/publications/i/item/who-2019-nCoV-surveillanceguidance-2020.7
14. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis. 2020;20:1034-1042.
15. Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. 2020;94:91-95.
16. Barek MA, Aziz MA, Islam MS. Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: A meta-analysis with 55 studies and 10014 cases. Heliyon. 2020;6:e05684.

17. Abate BB, Kassie AM, Kassaw MW, Aragie TG, Masresha SA. Sex difference in coronavirus disease (COVID-19): a systematic review and meta-analysis. BMJ Open. 2020;10:e040129.

18. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16:626-638.

19. Habas K, Nganwuchu C, Shahzad F, et al. Resolution of coronavirus disease 2019 (COVID-19). Expert Rev Anti Infect Ther. 2020;18:1201-1211.

20. Gan R, Rosoman NP, Henshaw DJE, Noble EP, Georgius P, Sommerfeld N. COVID-19 as a viral functional ACE2 deficiency disorder with ACE2 related multi-organ disease. Med Hypotheses. 2020;144:110024.

21. Loganathan S, Kuppusamy M, Wankhar W, et al. Angiotensin-converting enzyme 2 (ACE2): COVID 19 gate way to multiple organ failure syndromes. Respir Physiol Neurobiol. 2021;283:103548.

22. Jacot D, Greub G, Jaton K, Opota O. Viral load of SARS-CoV-2 across patients and compared to other respiratory viruses. Microbes Infect. 2020;22:617-621.

23. Vellas C, Delobel P, de Souto Barreto P, Izopet J. COVID-19, Virology and Geroscience: A Perspective. J Nutr Health Aging. 2020;24:685-691.

24. Han MS, Choi EH, Chang SH, et al. Clinical Characteristics and Viral RNA Detection in Children With Coronavirus Disease 2019 in the Republic of Korea. JAMA Pediatr. 2021;175:73-80.

25. Jones TC, Biele G, Mühlemann B, et al. Estimating infectiousness throughout SARS-CoV-2 infection course. Science. 2021;373:eabi5273.

26. Kawasuji H, Takegoshi Y, Kaneda M, et al. Transmissibility of COVID-19 depends on the viral load around onset in adult and symptomatic patients. PLoS One. 2020;15:e0243597.

27. Argyropoulos K V, Serrano A, Hu J, et al. Association of Initial Viral Load in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Patients with Outcome and Symptoms. Am J Pathol. 2020;190:1881-1887.

28. Aggarwal G, Cheruiyot I, Aggarwal S, et al. Association of Cardiovascular Disease With Coronavirus Disease 2019 (COVID-19) Severity: A Meta-Analysis. Curr Probl Cardiol. 2020;45:100617.

29. Pranata R, Lim MA, Huang I, Raharjo SB, Lukito AA. Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: A systematic review, meta-analysis and meta-regression. J Renin Angiotensin Aldosterone Syst. 2020;21:1470320320926899.

30. Renu K, Prasanna PL, Valsala Gopalakrishnan A. Coronaviruses pathogenesis, comorbidities and multi-organ damage - A review. Life Sci. 2020;255:117839.

31. Zambrano LD, Ellington S, Strid P, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020;69:1641-1647.

32. Ofori-Asenso R, Ogundipe O, Adom Agyeman A, et al. Cancer is associated with severe disease in COVID-19 patients: a systematic review and meta-analysis. Ecancermedicalscience. 2020;14:1047.

33. Patel U, Malik P, Shah D, Patel A, Dhamoon M, Jani V. Pre-existing cerebrovascular disease and poor outcomes of COVID-19 hospitalized patients: a meta-analysis. J Neurol. 2021;268:240-247.

34. Tsivgoulis G, Palaiodimou L, Zand R, et al. COVID-19 and cerebrovascular diseases: a comprehensive overview. Ther Adv Neurol Disord. 2020;13:1756286420978004.

35. Izcovich A, Ragusa MA, Tortosa F, et al. Prognostic factors for severity and mortality in patients infected with COVID-19: A systematic review. PLoS One. 2020;15:e0241955.

36. He G, Wu J, Shi J, et al. COVID-19 in tuberculosis patients: A report of three cases. J Med Virol. 2020;92:1802-1806.

37. Udoakang AJ, Djomkam Zune AL, Tapela K, et al. The COVID-19, tuberculosis and HIV/AIDS: Ménage à Trois. Front Immunol. 2023;14:1104828.

DOI: 10.4274/gulhane.galenos.2024.99705
Gulhane Med J 2024;66(4):167-175

Relationship between sleep quality, chronotype, and depression in youth: A cross-sectional study

Necla İpar

Koç University Faculty of Medicine, Department of Pediatrics, İstanbul, Türkiye

Cite this article as: İpar N. Relationship between sleep quality, chronotype, and depression in youth: A cross-sectional study. Gulhane Med J. 2024;66(4):167-175

Date submitted:
20.03.2024
Date accepted:
03.06.2024
Online publication date:
22.11.2024

Corresponding Author:
Necla İpar, M.D., Koç University Faculty of Medicine, Department of Pediatrics, İstanbul, Türkiye

ORCID:
orcid.org/0000-0002-2614-0832

Keywords: Chronotype, depression, eveningness, sleep quality, mental health, youth

ABSTRACT

Aims: In today's electronic age, the frequency of both poor sleep quality and the evening chronotype are increasing and are associated with numerous adverse physical and mental health outcomes. This study was conducted on a group of healthy Turkish youth aged 18-24 years to determine whether there are any potential associations between sleep quality, chronotype, and depression.

Methods: In this cross-sectional study involving healthy youth, we utilized the Pittsburgh Sleep Quality Index (PSQI), the Morningness-Eveningness Questionnaire (MEQ), and the Beck Depression Inventory (BDI) to evaluate sleep quality, chronotype, and depression.

Results: The study analyzed 165 youths with a mean age of 21.9±1.6 years (77.6% were females). Overall, 84% of the study participants (PSQI mean score: 7.4±3.0) exceeded accepted thresholds for poor sleep quality, whereas 35.7% (BDI mean score: 16.3±11) exceeded thresholds for depression. The participants were categorized into the following chronotypes: morning (n=19, 11.5%), intermediate (n=109, 66.1%), and evening (n=37, 22.4%) types. Poor sleep quality and eveningness were positively associated with depression (p=0.033 and p=0.012, respectively). Mediation analysis showed that sleep quality acted as a mediator in the relationship between chronotype and depression (β=-0.003, p=0.011).

Conclusions: The results of this study demonstrated a significant association between poor sleep quality, evening chronotype, and an increased likelihood of depression among Turkish youth.

Introduction

Sleep is a fundamental element of both human biology and life, and its impact is crucial for human well-being. It is associated with many physiological processes in the brain and other organs (1,2). The American Academy of Sleep Medicine (AASM) recommends that adults aged 18 to 60 aim for a consistent sleep duration of 7 or more hours per night to promote optimal health (3). Nevertheless, lack of sleep is increasingly becoming a more common health issue in modern societies and, as such, is becoming a greater focus for health professionals (2,4). An analysis of epidemiologic studies showed that approximately one-third of the general population experiences insomnia symptoms (difficulty falling asleep and/or staying asleep) and between 4% and 26% experience excessive sleepiness (5). Although sleep disorders are common in the general population, they are reported to be underrecognized, and less than 20% of insomniacs receive an accurate diagnosis and treatment (5). It has been demonstrated that there is an association between poor sleep and excessive daytime sleepiness, depression, and poor concentration (6), as well as long-term health consequences, such as chronic diseases, including obesity, metabolic syndrome, and cardiovascular problems (4). Consequently, inadequate sleep and untreated sleep disorders can adversely affect the health and safety of a community (7).

The biological and rhythmic activity observed in psychological, cognitive, and physiological variables in humans that affect sleep-wake cycles, mood, endocrine functions, cognition, and body temperature is known as the “circadian rhythm” (8). The biological rhythmicity observed in human metabolism over a period of 24 h is responsive to the light-dark cycle (8,9). Three chronotypes represent individual circadian preferences: morning, evening, and intermediate chronotypes (10-12). A morning person, often called the lark, goes to bed and gets up early. They prefer doing their major jobs and activities in the morning. An evening person, often called the owl, goes to bed and gets up late. They prefer doing their major jobs and activities in the evening. Alongside this, a third category, the intermediate type, has no leaning toward morning or evening (10). A study on people aged 25 to 74 years in Finland over 10 years found an increased prevalence of insufficient sleep, shorter sleep duration, and increased eveningness, with eveningness being more prevalent among young adults across all working years (13). Individuals of the evening type experience more sleep disturbances than those of morning and intermediate types. This condition is characterized by a higher frequency of nightmares, shorter sleep duration, reduced sleep quality, and a longer time taken to fall asleep than morning or intermediate types (14). Recent research suggests that individuals with an evening chronotype are at increased risk of depression (10,15). A meta-analysis showed a significant relationship between evening type and depression in both longitudinal and cross-sectional studies (16). Nevertheless, the link between chronotype and depression remains unclear and warrants further investigation (16).

Maintaining a stable circadian rhythm is critical for human health (10), but university students are known to be more susceptible to sleep disturbances and psychological distress due to the rigorous study, work, and extracurricular activities (17). Improving sleep quality has also been reported to contribute to mental health (18). It is possible that understanding these patterns and establishing a consistent daily routine could help reduce the incidence of the aforementioned complications (10).

Studies on sleep health, including chronotype, sleep disorders, and depression in youth, are limited, with inadequate sample sizes and inconsistent findings (10,15). The AASM recommends more sleep and circadian rhythm research to highlight their importance for public health and the negative consequences of insufficient sleep (7). Given the increased incidence of sleep disturbances, eveningness, and susceptibility to depressive symptoms among youth, the purpose of this study was to explore the association between sleep quality and chronotype and depression in university students.

Methods

Study design and participants

This cross-sectional study enrolled university students aged 18-24 using a non-probability sampling method. The study was conducted in İstanbul between April and June 2023. Approval for this research was granted by the Koç University Institutional Review Board (decision no: 2022.290.IRB3.125, date: 16.09.2022). The research was conducted according to the tenets of the Declaration of Helsinki.

The eligibility criteria included being a healthy youth aged 18-24 years, being a university student, and having no previous history of chronic disease or current regular medication use.

For the study, university students were contacted directly or via e-mail. The participants were asked to answer four questionnaires covering sociodemographics, sleep quality, chronotype, and depression. Questionnaires were made available via Qualtrics. Before the start of the surveys, each participant read a brief explanation of the study and provided online consent. All surveys were conducted anonymously.

Measures

The sociodemographics form was used to collect data on age, gender, weight, height, electronic device usage (television, computer, phone), tea and caffeine consumption, university department attendance, and smoking status.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI), initially developed by Buysse et al. (19) and subsequently adapted into Turkish by Agargun et al. (20), is a self-report scale comprising 19 items. The instrument evaluates standards of sleep and disruptions in one month past. It contains 24 questions, with 19 being self-report queries and 5 requiring responses from a spouse or roommate. The 18 questions scored within the scale encompass seven key areas: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disorder, sleep medication use, and daytime dysfunction. These components are assessed on a scale of 0-3. The total score across the seven components gives the total scale score ranging from 0 to 21. A total score greater than 5 indicates “poor sleep quality”.

Beck Depression Inventory

Depression was evaluated with the Beck Depression Inventory (BDI). The BDI was developed by Beck et al. (21) and was later adapted for Turkish use by Hisli et al. (22,23). The scale comprises 21 items, each scored between 0 and 3. The highest score was 63, indicating more significant depression. The participants were further categorized as having minimal depression (BDI score <10) or clinically significant depression (moderate to severe) (BDI score >18). A Cronbach’s alpha coefficient of BDI was reported to be 0.80 (23). The Cronbach’s

alpha coefficient for the measurement instrument used in this study was 0.910, indicating high internal consistency among the items.

The Morningness-Eveningness Questionnaire

Chronotype was evaluated using the 19-item Morningness-Eveningness Questionnaire (MEQ), which was developed by Horne and Ostberg (11) in 1976. A Turkish reliability study was conducted in 2005 by Pündük et al. (24). This self-reporting scale is designed to assess individual differences in the degree to which a respondent is active and alert at certain times of the day (e.g., in the morning and the evening). According to the total score obtained at the end of the questionnaire, chronotype characteristics are determined. The final questionnaire scores ranged from 16 to 86, with higher scores indicating a preference for the morning and lower scores indicating a preference in the evening. Scores between 16 and 41, 42 and 58, and 59 and 86 indicate "evening type", "intermediate type" and "morning type", respectively (24). The Cronbach's alpha coefficient for the measurement instrument used in this study was 0.798, indicating high internal consistency among the items.

Statistical Analysis

The data analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM Corp., Armonk, NY., USA)

The Shapiro-Wilk test was used to test the assumption of normality. The Mann-Whitney U test was used to compare variables that were non-normally distributed. Chi-square tests were performed to compare categorical variables. The association between PSQI, MEQ, faculty type, smoking status, and electronic device use and depression was examined using logistic regression. Mediation models were analyzed using the partial least squares (PLS) structural equation modeling algorithm in SmartPLS4. A comprehensive examination of the mediated effects for each model was conducted using a total of 10,000 bootstrapped samples to ensure statistical rigor.

Results

Sociodemographic results

A total of 455 individuals were contacted, and 276 (60.6%) completed the questionnaires. One hundred and eleven participants were excluded from the analyses because of chronic illness (n=59), regular medication usage (n=26), and unconfirmed current university engagement (n=26). Finally, the analyses included 165 youths with a mean age of 21.9±1.6 years (77.6% were females). The characteristics of the participants are presented in Table 1.

The mean PSQI score was 7.4±3.0, ranging from 1 to 16. The mean MEQ score was 47.7±9.0, ranging from 28 to 72. The mean BDI score was 16.3±11, ranging between 0 and 63.

BDI analyses

Increased depression scores above the BDI cut-off (≥19) were detected in 59 (35.7%) participants. There were no differences in age, gender, body mass index (BMI), electronic device usage, or daily tea and coffee consumption between participants with and without depression, as determined by the BDI score. The prevalence of depression was lower among students in medical faculty than among students in other faculties [29.7% (n=35) and 51.1% (n=24), p=0.012].

Table 1. Characteristics of the study participants (n=165)

Age, years, mean±SD	21.9±1.6
Gender, female, n (%)	128 (77.6)
BMI, (kg/m²), mean±SD	22.9±4.2
Duration of electronic device use (TV, computer, phone); n (%)	
<3 h/day	22 (13.3)
≥3 h/day	143 (86.7)
Faculty type, n (%)	
Medical faculty	118 (71.5)
Other university departments	47 (28.5)
Smoking current, n (%)	23 (13.9)
Daily tea consumption, cups, mean±SD	2.0±2.0
Daily coffee consumption, cups, mean±SD	1.4±1.0

SD: Standard deviation, BMI: Body mass index

Chronotypes and depression

Participants were found to be morning, intermediate, and evening type by 11.5% (n=19), 66.1% (n=109), and 22.4% (n=37), respectively. There were no differences in age, gender, faculty type, BMI, daily tea and coffee consumption, electronic device usage, smoking status, or sleep quality across the three chronotypes. Depression, as defined by the BDI score, was more prevalent among evening-type individuals (p=0.012). Comparisons of gender, faculty type, duration of electronic device use, depression, and sleep quality between morning, intermediate, and evening are presented in Table 2.

Sleep quality and depression

According to the PSQI, 16% of participants had good sleep quality and 84% had poor sleep quality. In addition, 33.9% of the participants slept less than seven hours, and 63% slept after midnight. There were no differences in age, BMI, or daily tea and coffee consumption between participants with a good vs. poor sleep quality. Poor sleep quality was significantly associated with depression, as defined by the BDI score (p=0.033). Comparisons of gender, faculty type, duration of electronic device use, and depression between sleep quality classes are presented in Table 3.

Correlations analyses

Higher MEQ scores were associated with morningness. BDI was inversely correlated with MEQ (r=-0.238, p<0.001) and positively correlated with PSQI (r=0.522, p<0.001).

Regression analyses

The multivariable logistic regression model included the following explanatory variables: PSQI score, chronotype, faculty type, and other factors that may be associated with depression, such as smoking status and duration of electronic device use; depression was the outcome variable. Poor sleep quality [odds ratio (OR): 1.514, 95% confidence interval (CI): 1.293-1.772] and non-medical faculty program (OR: 2.710, 95% CI: 1.147-6.402) were independently associated with depression diagnosis. The overall model significance was confirmed by the Omnibus test (χ^2=57.944, p<0.001), with evidence of no multicollinearity (VIF values< 5). The Hosmer-Lemeshow test indicated good model fit (χ^2=6.524, p=0.589). The Nagelkerke R^2 value of 0.411 (Table 4).

Mediation analyses

Mediation analysis was conducted to investigate the role of sleep quality in the influence of chronotype on depression. Mediation analysis showed that sleep quality acted as a mediator in the relationship between chronotype and depression (β=-0.003, p=0.011). The outcomes of the mediation analyses are presented in Figure 1. In addition, in the mediation analysis conducted to examine the effect of electronic device use time on chronotype, sleep quality, and depression, we found that electronic device use had no mediating effect on any relationship between sleep, chronotype, and depression.

Table 2. Association between youth chronotype and several factors (n=165)

	Morningness (n=19)	Intermediate (n=109)	Eveningness (n=37)	p*
Gender, n (%)				
Female	14 (10.9)	86 (67.2)	28 (21.9)	0.839
Faculty type, n (%)				
Medical faculty	15 (12.7)	76 (64.4)	27 (22.9)	0.696
Other university departments	4 (8.5)	33 (70.2)	10 (21.3)	
Duration of electronic device use, n (%)				
<3 h/day	4 (18.1)	11 (50.0)	7 (31.9)	0.144
≥3 h/day	15 (10.5)	98 (68.5)	30 (21.0)	
Smoking, n (%)		20 (87.0)	3 (13)	0.053
Depression level according to BDI score, n (%)				
Minimal (BDI score <10)	11 (20.4)	34 (63.0)	9 (16.7)	**0.012**
Mild (BDI score 10-18)	5 (9.6)	39 (75.0)	8 (15.4)	
Moderate (BDI score 19-29)	3 (7.3)	27 (65.9)	11 (26.8)	
Severe (BDI score 30-63)	0 (0.0)	9 (50.0)	9 (50.0)	
Sleep quality level according to PSQI score, n (%)				
Good sleep quality	5 (19.2)	16 (61.5)	5 (19.2)	0.414
Poor sleep quality	14 (10.2)	91 (66.4)	32 (23.4)	

*Chi-square tests.
BDI: Beck Depression Inventory, PSQI: Pittsburgh Sleep Quality Index

Discussion

The current study examined sleep quality, chronotype, and its association with depression among university students aged 18-24 years. In terms of sleep quality, 84% of participants had poor sleep quality according to the PSQI, and 66.1% had an intermediate chronotype according to chronotype. In terms of depression levels, 35.7% of the participants were depressed according to the BDI (≥19). It was also found that evening chronotype was associated with poor sleep quality and depression. There were also no differences in age, gender, coffee and tea consumption, chronotype, sleep quality, and depression between the participants. In the literature, while there are studies on chronotype, sleep disorders, and depression (10,15,25), this area is not well known, and there is little public awareness of the importance of this disorder and its health problems in our country.

Chronotype and mental health

Stable circadian rhythm is a critical element of human well-being, and unstable circadian rhythms have been associated with physical and psychiatric disorders (10). In recent years, there has been a growth in the interest in studies examining any relationship between sleep, chronotype, and depression (10,15,25). Our findings showed that the majority of young people had an intermediate chronotype (66.1%), with a smaller proportion having an evening chronotype (22.4%) and the smallest proportion having a morning chronotype (11.5%). In addition, no gender or age differences were observed between chronotypes. In contrast, a national study completed in Finland estimated that evening types made up 11-13% of the general adult population, with the eveningness characteristic being slightly more prevalent in women than in men (26). In a Norwegian study of 59,554 students, 10% were morning, 60% were intermediate, and 30% were evening types (27) whereas in a Canadian study of 3160 young adults, 9% were morning, 55% were intermediate, and 36% were evening types (14) and in a Turkish study of 339 university students, 18% were morning, 55.7% were intermediate, and 26.2% were evening types (28).

Table 3. Youth sleep quality level according to several factors

	Good sleep quality (n=31)	Poor sleep quality (n=183)	p*
Gender, female, n (%)	5 (13.5)	32 (86.5)	0.645
Faculty type			
Medical faculty	18 (15.3)	100 (84.7)	0.694
Other university departments	8 (17.8)	37 (82.2)	
Duration of electronic device use, n (%)			
<3 h/day	4 (18.1)	18 (81.8)	0.622
≥3 h/day	22 (15.6)	119 (84.4)	
Smoking, n (%)	4 (17.4)	19 (82.6)	0.839
Depression level according to BDI score, n (%)			
Minimal (BDI score <10)	14 (26.4)	39 (73.6)	**0.033**
Mild (BDI score, 10-18)	8 (15.4)	44 (84.6)	
Moderate (BDI score 19-29)	4 (9.8)	37 (90.2)	
Severe (BDI score 30-63)	-	17 (100.0)	

*Chi-square tests, BDI: Beck Depression Inventory, PSQI: Pittsburgh Sleep Quality Index.

Table 4. The association of PSQI, MEQ, faculty type and smoking status with depression as defined by the BDI in multivariable logistic regression analysis

Variables	β	Standard error	Wald	df	β	OR	95% confidence interval	
							Lower	Upper
PSQI score	0.414	0.080	26.523	1	**<0.001**	1.514	1.293	1.772
MEQ score	-0.016	0.046	0.117	1	0.732	0.984	0.900	1.077
Faculty type (reference: medical faculty)	0.997	0.439	5.168	1	**0.023**	2.710	1.147	6.402
Smoking status (reference: non-users)	0.813	0.556	2.133	1	0.144	2.254	0.757	6.707
Duration of electronic device use (reference: <3 h/day)	-0.261	0.622	0.177	1	0.674	0.770	0.228	2.604

Independent variable: Depression as defined by the BDI score (≥19); Hosmer-Lemeshow test p=0.589; β: regression coefficient of the variable. OR: Odds ratio; BDI: Beck Depression Inventory, MEQ: Morningness Eveningness Questionnaire, PSQI: Pittsburgh Sleep Quality Index

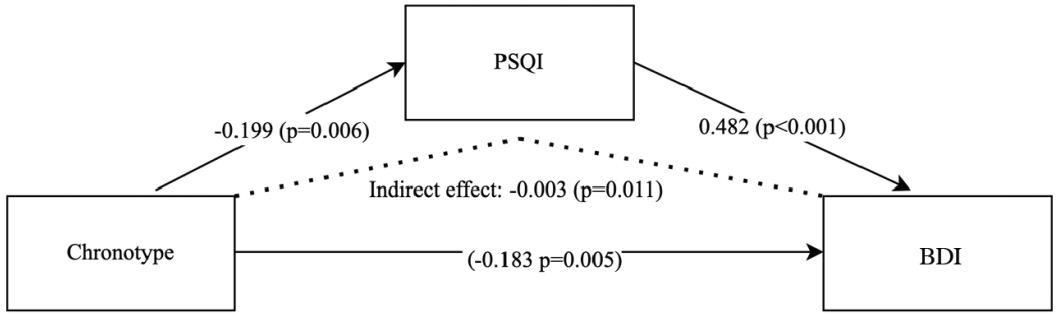

Figure 1. Mediation of the association between chronotype and depression by sleep quality

PSQI: Pittsburgh Sleep Quality Index, BDI: Beck Depression Inventory

Our study also showed that eveningness is associated with a higher depression rate. Moreover, a higher PSQI score, indicating poor sleep, was correlated with a lower chronotype score, indicating eveningness. Our results are consistent with a meta-analysis of 43 studies involving 27,996 participants, which found that the evening chronotype was associated with depression (15). Moreover, one review found that evening preference was related to an increased risk of depressive symptoms, whereas morning chronotype was considered a protective factor (10). Meanwhile, a study of medical students in Türkiye found no association between chronotype and depression (29), and a further study conducted in Türkiye with 339 participants observed no direct relationship between evening chronotype and depression (28).

Similar studies have found a connection between eveningness and depression and anxiety symptoms, alcohol and tobacco use, and a high suicide risk. If we analyze our study alongside other recent studies (10,15,25,30), it can be seen that chronotype indeed plays an important role in depressive disorders and that people with depression or mental health problems should be considered for evaluation of chronotype and sleep disorders. In addition, young people should be educated about the importance of regular routines and sleep patterns as part of preventive health care.

Alongside this, it is also believed that several other factors may influence the associations between chronotypes and depression. It has been proposed that functional relationships between regulation of mood and the biological clock might exist which control day preference, and that evening preference may heighten vulnerability to mood complaints (30). In a study of 913 Polish adults, high neuroticism and low conscientiousness were found to be associated with depression in individuals with evening chronotypes (31). In a study of adults between the ages of 18 and 35, while eveningness was found to be related to depression, it was also found that this effect could be mitigated by high briskness and low emotional reactivity (32). Hence, taking the above into consideration it is hypothesized that various factors, such as personality and lifestyle, might influence correlations between circadian preference and depression. Further studies are required to better understand this relationship and to adopt preventive measures.

Sleep and mental health

Most of the participants in this study met the criteria for poor sleep quality (84%), which was associated with depression. A study of 559 medical students in Greece found that 52.4% had poor sleep quality, 22.6% had moderate symptoms of depression, and 13.9% had moderately severe symptoms of depression. In addition, changes in sleep parameters were associated with moderate to severe deterioration in mental health (33). A systematic review of 16-25 year olds a significant association between poor sleep quality and mental health (34). A previous study found that people with sleep problems were 10 and 17 times more likely to have clinically significant depression than people without sleep problems (35). A meta-analysis of 21 longitudinal studies found that people with sleep problems at baseline were twice as likely to develop depression at follow-up as those without sleep problems (36). A meta-analysis of randomized controlled trials found that sleep improvement had a beneficial effect on mental health disorders, regardless of the severity of mental disorders or the presence of comorbid health problems (18). Taken together, existing studies suggest that improving sleep quality is a preventive parameter for depression and has a positive effect on recovery from mental illness.

In our study, the rate of depression among university students was 2.7 times lower in the medical faculty group than in the non-medical faculty group. In contrast to our study, a systematic review of students in the USA and Canada found a high prevalence of depression among medical students, with levels of overall psychological distress being consistently higher than those in the general population and their peers in later years of training (37). Another study from Portugal found that anxiety symptoms were significantly more common among

medical students than among non-medical students (38). The results obtained in our study may be attributable to the large number of medical students. Moreover, the fact that medical students are more disciplined due to intensive programs than in other departments may also be a factor. However, our results are inconsistent with the limited existing literature. Further research is required to clarify this situation.

Duration of electronic device use, sleep, and mental health

In recent years, as electronic devices have become lighter and more portable, their use has increased significantly, and people have started to use them more frequently in daily life and in bed (39). In our study, most of the participants (86.7%) used electronic devices for 3 hours or more per day; there was no association between electronic device use and chronotype, sleep quality, or depression, and mediation analysis also showed that electronic devices were not a mediator in the associations between chronotype, sleep quality, and depression, which may be due to overuse by the overall majority. A systematic review of 16-25 years olds found that extended time spent on social media was associated with poor sleep quality and issues with mental wellbeing (34). In another study, a significant number of students met the criteria for low sleep, Internet addiction, and depression. In addition, Internet addiction and sleep quality were found to mediate a substantial portion of the indirect effect on depression (40). A study of 319 university students in Türkiye found that the quality of sleep and symptoms of depression were associated with excessive smartphone use (41). To fully elucidate the relationship between the use of electronic devices, circadian rhythms, sleep, and depression, more detailed studies should be conducted by examining the type, duration, location, and reason for the use of such devices. It should also be noted that young people with depression and sleep disorders should be screened for screen addiction.

This research has a number of limitations. First, the cross-sectional study design makes it difficult to infer causal relationships between sleep-related variables and depression. In addition, because non-probability sampling was used and the study was voluntary, it is possible that people with depression might not have participated, which could have affected the results. Second, participants' socio-cultural and economic status, family structure, and relationship status, which may affect sleep quality and depression, were not analyzed. Third, sleep quality and chronotype may vary seasonally and during the day between weekdays and weekends, but these differences were not examined. Finally, factors that may influence the association between chronotype and depression, such as temperament, personality traits, and lifestyle, were not examined and should be considered in future studies.

Despite these limitations, the results of the current study are believed to contribute to the literature by drawing attention to the increase in depression and mental disorders in young people, especially after Coronavirus disease-2019, and factors such as sleep disturbance and chronotype that may contribute to this increase.

Conclusion

This study revealed a significant relationship between eveningness, poor sleep quality, and an increased likelihood of depression among Turkish youths. Given that poor sleep quality, eveningness, and depression, which are common among young people, affect many areas of life, such as work, social and family life, and also increase the risk of accidents on the road, at work or at home, it is clear that these conditions are a significant public health problem that deserves more attention from health authorities. Moreover, promoting a more routine lifestyle alongside healthier sleep habits among youths could make a significant contribution to preventing sleep and mental health conditions, such as depression.

Ethics

Ethics Committee Approval: Approval for this research was granted by the Koç University Institutional Review Board (decision no: 2022.290.IRB3.125, date: 16.09.2022).

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

Acknowledgments

I would like to thank Prof. Dr. Sibel Sakarya for her valuable feedback and guidance in the preparation of this article.

Footnotes

Financial Disclosure: The author declared that this study received no financial support.

References

1. Grandner MA, Fernandez FX. The translational neuroscience of sleep: A contextual framework. Science. 2021;374:568-573.
2. Chattu VK, Manzar MD, Kumary S, Burman D, Spence DW, Pandi-Perumal SR. The Global Problem of Insufficient Sleep and Its Serious Public Health Implications. Healthcare (Basel). 2018;7:1.
3. Panel CC, Watson NF, Badr MS, et al. Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society. Sleep. 2015;38:843-844.
4. Institute of Medicine (US) Committee on Sleep Medicine and Research. Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Colten HR, Altevogt BM, editors. Washington (DC): National Academies Press (US); 2006.

5. Ohayon MM. Epidemiological overview of sleep disorders in the general population. Sleep Medicine Research. 2011;2:1-9.

6. Goel N, Rao H, Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. Semin Neurol. 2009;29:320-339.

7. Ramar K, Malhotra RK, Carden KA, et al. Sleep is essential to health: an American Academy of Sleep Medicine position statement. J Clin Sleep Med. 2021;17:2115-2119.

8. Reddy S, Reddy V, Sharma S. Physiology, circadian rhythm. StatPearls [Internet]: StatPearls Publishing; 2022.

9. Eid B, Bou Saleh M, Melki I, et al. Evaluation of Chronotype Among Children and Associations With BMI, Sleep, Anxiety, and Depression. Front Neurol. 2020;11:416.

10. Zou H, Zhou H, Yan R, Yao Z, Lu Q. Chronotype, circadian rhythm, and psychiatric disorders: Recent evidence and potential mechanisms. Front Neurosci. 2022;16:811771.

11. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4:97-110.

12. İpar N. The effect of circadian preference and sleep disturbances on depression in children 6 to 12 years of age. Chronobiol Int. 2023;40:1375-1386.

13. Merikanto I, Partonen T. Increase in eveningness and insufficient sleep among adults in population-based cross-sections from 2007 to 2017. Sleep Med. 2020;75:368-379.

14. Walsh NA, Repa LM, Garland SN. Mindful larks and lonely owls: The relationship between chronotype, mental health, sleep quality, and social support in young adults. J Sleep Res. 2022;31:e13442.

15. Norbury R. Diurnal preference and depressive symptomatology: a meta-analysis. Sci Rep. 2021;11:12003.

16. Au J, Reece J. The relationship between chronotype and depressive symptoms: A meta-analysis. J Affect Disord. 2017;218:93-104.

17. Wang J, Liu S, Guo J, et al. Chronotypes, Sleep and Mental Distress Among Chinese College Students: A Cross-Sectional Study. Front Psychiatry. 2022;13:883484.

18. Scott AJ, Webb TL, Martyn-St James M, Rowse G, Weich S. Improving sleep quality leads to better mental health: A meta-analysis of randomised controlled trials. Sleep Med Rev. 2021;60:101556.

19. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193-213.

20. Agargun M, Kara H, Anlar O. Pittsburgh Uyku Kalitesi İndeksi'nin Geçerliği ve Güvenirliği. Turk Psikiyatri Derg. 1996;7:107-111.

21. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-571.

22. Hisli N. Beck Depresyon Envanterinin Gecerligi Uzerine bir Calisma. Journal of Psychology. 1988;6:118-122.

23. Hisli N. The validity and reliability of Beck Depression Inventory for university students. Psikoloji dergisi. 1989;7:3-13.

24. Pündük Z, Gür H, Ercan I. Sabahçil-Akşamcil Anketi Türkçe Uyarlamasinda Güvenilirlik Calişmasi [A reliability study of the Turkish version of the mornings-evenings questionnaire]. Turk Psikiyatri Derg. 2005;16:40-45.

25. Merikanto I, Kronholm E, Peltonen M, Laatikainen T, Vartiainen E, Partonen T. Circadian preference links to depression in general adult population. J Affect Disord. 2015;188:143-148.

26. Merikanto I, Kronholm E, Peltonen M, Laatikainen T, Lahti T, Partonen T. Relation of chronotype to sleep complaints in the general Finnish population. Chronobiol Int. 2012;29:311-317.

27. Kjørstad K, Vedaa Ø, Pallesen S, Saxvig IW, Hysing M, Sivertsen B. Circadian preference in young adults: Associations with sleep and mental health outcomes from a national survey of Norwegian university students. Chronobiol Int. 2022;39:1465-1474.

28. Üzer A, Yücens B. Chronotype and depressive symptoms in healthy subjects: the mediating role of hopelessness and subjective sleep quality. Chronobiol Int. 2020;37:1173-1180.

29. Cim EFA, Kiran SG, Kurhan F, Atli A. Relationship between chronotype and depression schemas and sensitivity to depression in medical students. Annals of Medical Research. 2021;28:767.

30. Kitamura S, Hida A, Watanabe M, et al. Evening preference is related to the incidence of depressive states independent of sleep-wake conditions. Chronobiol Int. 2010;27:1797-1812.

31. Gorgol J, Waleriańczyk W, Stolarski M. The moderating role of personality traits in the relationship between chronotype and depressive symptoms. Chronobiol Int. 2022;39:106-116.

32. Gorgol J, Waleriańczyk W, Stolarski M, Cyniak-Cieciura M. Temperament moderates the association between chronotype and depressive symptoms: A Regulative Theory of Temperament approach. Personality and Individual Differences. 2022;185:111304:1-6.

33. Eleftheriou A, Rokou A, Arvaniti A, Nena E, Steiropoulos P. Sleep Quality and Mental Health of Medical Students in Greece During the COVID-19 Pandemic. Front Public Health. 2021;9:775374.

34. Alonzo R, Hussain J, Stranges S, Anderson KK. Interplay between social media use, sleep quality, and mental health in youth: A systematic review. Sleep Med Rev. 2021;56:101414.

35. Taylor DJ, Lichstein KL, Durrence HH, Reidel BW, Bush AJ. Epidemiology of insomnia, depression, and anxiety. Sleep. 2005;28:1457-1464.

36. Baglioni C, Battagliese G, Feige B, et al. Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. J Affect Disord. 2011;135:10-19.

37. Dyrbye LN, Thomas MR, Shanafelt TD. Dyrbye LN, Thomas MR, Shanafelt TD. Systematic review of depression, anxiety,

and other indicators of psychological distress among U.S. and Canadian medical students. Acad Med. 2006;81:354-373.

38. Moreira de Sousa J, Moreira CA, Telles-Correia D. Anxiety, Depression and Academic Performance: A Study Amongst Portuguese Medical Students Versus Non-Medical Students. Acta Med Port. 2018;31:454-462.

39. Fossum IN, Nordnes LT, Storemark SS, Bjorvatn B, Pallesen S. The association between use of electronic media in bed before going to sleep and insomnia symptoms, daytime sleepiness, morningness, and chronotype. Behav Sleep Med. 2014;12:343-357.

40. Bhandari PM, Neupane D, Rijal S, Thapa K, Mishra SR, Poudyal AK. Sleep quality, internet addiction and depressive symptoms among undergraduate students in Nepal. BMC Psychiatry. 2017;17:106.

41. Demirci K, Akgönül M, Akpinar A. Relationship of smartphone use severity with sleep quality, depression, and anxiety in university students. J Behav Addict. 2015;4:85-92.

DOI: 10.4274/gulhane.galenos.2024.05900
Gulhane Med J 2024;66(4):176-184

Effects of fiber or probiotic yogurt supplementation on intestinal barrier integrity in constipation-predominant irritable bowel syndrome

Nuket Emine Ünsal[1], Gamze Akbulut[2], Mustafa Gülşen[3]

[1]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Nutrition and Dietetics, Ankara, Türkiye
[2]İstanbul Kent University Faculty of Health Sciences, Department of Nutrition and Dietetics, İstanbul, Türkiye
[3]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Gastroenterology, Ankara, Türkiye

Cite this article as: Ünsal NE, Akbulut G, Gülşen M. Effects of fiber or probiotic yogurt supplementation on intestinal barrier integrity in constipation-predominant irritable bowel syndrome. Gulhane Med J. 2024;66(4):176-184

Date submitted:
06.10.2023
Date accepted:
11.06.2024
Online publication date:
22.11.2024

Corresponding Author:
Nuket Emine Ünsal, M.D., University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Nutrition and Dietetics, Ankara, Türkiye
nuketbuyukgenc@hotmail.com

ORCID:
orcid.org/0000-0002-1491-7093

Keywords: Irritable bowel syndrome, soluble fiber, probiotic, intestinal barrier integrity, zonulin

ABSTRACT

Aims: This study aimed to evaluate the effects of different dietary treatments on intestinal integrity in female subjects aged 19-50 years with a previous diagnosis of constipation-predominant irritable bowel syndrome (IBS).

Methods: This randomized controlled trial was conducted at the Gastroenterology Clinic of Gülhane Training and Research Hospital, Ankara, Türkiye. Individuals with IBS were randomly assigned to three groups. Group 1 received a regular constipation diet (n=21), group 2 received a constipation diet rich in soluble fiber (n=17), and group 3 received a constipation diet supplemented with probiotic yogurt (n=22). All participants were followed up for 8 weeks. Intestinal integrity was assessed using plasma zonulin levels before and after treatment.

Results: The study included 60 patients (age, mean±SD 38.3±8.1 years). Following the intervention, zonulin levels showed non-significant increases from 24.41±25.10 to 28.59±24.05' (p=0.434) in group 1 and 25.91±25.10 to 28.59 (p=0.758) in group 2. It showed a non-significant decrease from 26.37±24.22 to 24.44±22.22 (p=0.393) in group 3. Fasting blood glucose, C-reactive protein, total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels also showed no significant differences between the groups at the beginning and end of the study. There was no significant relationship between zonulin levels and nutrient levels in group 1 and group 3 at the 8th-week measurements. In group 2, zonulin level was inversely and moderately correlated with fat percentage, monounsaturated fatty acid content, and vitamin E content (p<0.05). There was a linear, moderate relationship between zonulin levels and omega 6/omega 3 ratio (r=0.582; p=0.015).

Conclusions: The serum zonulin levels did not change significantly after consumption of fiber or probiotic yogurt (NCT06421922).

Introduction

Irritable bowel syndrome (IBS) is an intestinal disease characterized by abdominal pain, constipation, and/or diarrhea. IBS is a frequent disease with a 5-20% worldwide prevalence. In Western countries, its prevalence is 8-23%, of which 60-70% are women (1). In Türkiye, 10-14.9% of adults were found to have IBS, which was more frequent among women between the ages of 20-40 years (2).

Factors such as heredity, environment, diet, gastrointestinal microbiota, and inflammation in the gastrointestinal tract play a role in the pathogenesis of IBS (3). Hypersensitivity to certain nutrients may also contribute to pathogenesis by causing low grade intestinal inflammation and increased epithelial barrier permeability (4).

The epithelial cells in the intestinal mucosa are held together by tight bands tight junction (TJ) (5). TJ areas close the spaces between cells and form an intestinal barrier. In dysbiosis, bacterial toxins and lipopolysaccharides damage the intestinal mucosa and disrupt the function of the intestinal microbiota. With these stimuli, zonulin release from TJ points increases the permeability in the intestines (5). Increased intestinal permeability is thought to be an early stimulus leading to low-grade inflammation in the intestinal mucosa (6).

Serum zonulin levels increase in patients diagnosed with IBS (7). It has also been shown that zonulin may be a useful biomarker for altered intestinal permeability in patients with IBS (8).

In recent years, more attention has been paid to the role of diet in IBS (9). Dietary changes and nutritional habits differ among individuals, which significantly affect strategies for improving health and preventing diseases. To prevent IBS attacks, approaches such as increasing soluble fiber intake, eliminating foods thought to cause symptoms, and using probiotics/prebiotics are recommended in medical nutritional therapy (10).

Probiotics stabilize the intestinal microbiota and maintain its balance. Moreover, they increase mucosal integrity and improve the intestinal barrier (11). A meta-analysis concluded that the use of probiotics can reduce IBS symptoms (12). Soluble fiber also dissolves in water and forms consistency in the small intestine, showing little laxative effects because of its rapid fermentation (13). A meta-analysis of fourteen randomized controlled trials concluded that soluble fibers such as psyllium may favorably affect IBS courses (14). Therefore, this study evaluated the effects of different dietary treatments on zonulin levels in female subjects aged 19-50 years with a previous diagnosis of constipation-predominant IBS.

Methods

Study design and participants

This non-pharmacological randomized controlled study was conducted in the Gastroenterology Outpatient Clinic of Gülhane Training and Research Hospital, Ankara, Türkiye, between June 2019 and March 2020. The participants were women aged 19-50 years who were diagnosed with IBS. The inclusion criteria diagnosis with IBS according to the Rome 4 criteria 2017 (15), no metabolic disease history (e.g., diabetes mellitus, cardiovascular disease), no history of chronic disease such as cancer and autoimmune diseases, no use of probiotics, and no use of nutritional supplements (vitamins, minerals) in the last 6 months. The main exclusion criterion was pregnancy. This study followed the Helsinki Declaration guidelines and was registered at www.clinicaltrials.gov (NCT06421922).

Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Non-Interventional Research Ethics Committee (ethics approval code: 46418926, project/decision no: 18/253, evaluation date: 21.11.2018). All participants signed a voluntary consent form, and participation in the study was voluntary.

Dietary randomization

The participants were randomly assigned to three groups using random allocation software for parallel group randomized trials (16). Group 1 received a regular constipation diet; group 2 received a constipation diet rich in soluble fiber, and group 3 received a constipation diet supplemented with probiotic yogurt. The constipation diet included 2 L of water, 2 portions of vegetables, and 3 portions of fruits and legumes 2 times a week. Soluble fiber (resistant starch) (5 g/day) was added to the constipation diet in group 2 as 1 sachet/day (5 g/day) during the initial 4 weeks and 2 sachets/day (10 g/day) during the subsequent 4 weeks. "*Bifidobacterium infantis 35624 (B. infantis 35624)*" strain, specific to IBS, was added to yogurt in group 3 and consumed before lunch. The follow-up period was 8 weeks.

All data were collected via face-to-face surveys. In the first visit, we assessed sociodemographic characteristics, 3-day food consumption, serum zonulin level, and biochemical tests [fasting blood glucose, cholesterol, blood triglyceride, low density lipoprotein (LDL) cholesterol, and C-reactive protein (CRP)]. Serum zonulin levels were measured using a "BT Lab Human zonulin ELISA Kit" (China, E1117). Three-day food consumption was recorded for 2 consecutive days on weekdays and one day on weekends. The daily energy and nutrients intake were analyzed using the Nutrition Information System 8 (BeBis 8) computer package program (17). Biochemical tests and serum zonulin levels were measured at the beginning of the study and at the end of the 8th week.

Power and sample size

The sample size was calculated using G*Power (G*Power Ver. 3.1.9.7, Franz Faul, Universität Kiel, Germany). With an estimated 90% power, α=0.05 type 1 error, β=0.10 type 2 error, and f=0.25 effect size, the required total sample size was 54, comprising 18 participants in each group. To compensate for the exclusions, 10% more patients were enrolled, resulting in 60 participants. A total of 100 patients were initially invited, but 10 were excluded because they did not fulfill the inclusion criteria. Finally, 31 volunteers were assigned to groups 1, 30 to groups 2, and 29 to groups 3. A total of 30 volunteers (10 in group 1, 13 in group 2, and 7 in group 3) were excluded from the study because they did not participate in the control visits.

Primary and secondary outcomes

The primary outcome was the change in blood zonulin levels after the addition of probiotic yogurt to a regular constipation diet instead of soluble fiber. The secondary outcomes were changes in fasting blood glucose, cholesterol, blood triglyceride, LDL cholesterol, and CRP levels after adding probiotic yogurt to a regular constipation diet instead of soluble fiber.

Statistical Analysis

The obtained data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 22.00 (IBM Corp., Armonk, NY, USA). The normality of continuous variables (age, zonulin level, fasting blood sugar, CRP, total and LDL cholesterol, triglycerides) was assessed using the Shapiro Wilk test. To make continuous variables more understandable and ensure consistency with findings from other studies, they are presented as mean±standard deviation (SD). Correlations between continuous variables are displayed using Spearman rank correlation coefficients. Inter-group comparisons were made by analysis of variance or Kruskal-Wallis test, as appropriate. Baseline to 8th-week within-group comparisons were performed using the Wilcoxon signed-rank test. The significance level was set at $p<0.05$.

Results

The study included 60 patients with a mean age of 38.3±8.1 years. Groups 1, 2, and 3 consisted of 21, 17, and 22 patients, respectively.

Biochemical findings

Compared with the baseline, there was no change in zonulin level at 8th week in any group, despite some increases of approximately 3 ng/mL in groups 1 and 2, and a decrease of approximately 2 ng/mL in group 3 (Table 1). There were also no intergroup differences in zonulin levels between baseline and follow-up.

At baseline and 8th week, biochemical parameters including fasting blood glucose, total cholesterol, LDL cholesterol, triglyceride, and CRP were similar in the three groups (Table 1). Similarly, there was no change from baseline to the 8th week in the levels of these biochemical parameters (Table 1).

Energy and nutrient intake

As shown in Table 2, dietary energy intake decreased in groups 1 and 2 and remained unchanged in group 3 by the 8th week. In all three groups, proteins comprised approximately 16-17% of the energy intake at baseline and 8th week. While the percentage of energy intake from carbohydrates was approximately 40% at baseline, it decreased to 37-38% at the 8th week. The percentage of energy intake from fat increased significantly in group 1 during the study period ($p=0.021$), whereas the increase in the other groups was not statistically significant ($p>0.05$) (Table 2).

Table 1. Biochemical findings of individuals in different dietary treatment groups

	Group 1 (n=21)			Group 2 (n=17)			Group 3 (n=22)			Inter-groups		
	Baseline	8th week		Baseline	8th week		Baseline	8th week		Baseline	8th week
Biochemical findings	x̄±SD	x̄±SD	p^{1**}	x̄±SD	x̄±SD	p^{2**}	x̄±SD	x̄±SD	p^{3**}	p^{4*}	p^{5*}
Fasting blood glucose (mg/dL)	91.33±8.92	89.67±13.04	0.596	90.88±24.68	91.18±12.95	0.933	93.39±18.84	91.64±18.96	0.574	0.898	0.911
Cholesterol (mg/dL)	181.00±25.74	174.85±26.14	0.352	184.76±34.26	199.76±48.43	0.434	178.00±39.35	179.45±37.92	0.821	0.824	0.115
Triglyceride (mg/dL)	105.48±75.30	101.14±64.4	0.362	154.65±141.68	137.59±98.82	0.469	88.55±31.60	90.05±29.62	0.884	0.073	0.126
LDL-C (mg/dL)	108.00±18.54	101.86±17.92	0.054	111.71±27.48	126.88±42.88	0.163	113.18±40.01	110.36±30.45	0.626	0.731	0.173
CRP (mg/L)	3.33±5.31	4.81±10.12	0.274	1.75±1.85	2.11±3.07	0.535	1.82±2.39	2.18±2.63	0.715	0.449	0.809
Zonulin (ng/mL)	25.41±25.10	28.59±24.05	0.434	25.91±25.10	28.59±24.05	0.758	26.37±24.22	24.44±22.22	0.393	0.923	0.893

*Wilcoxon test for within-group comparisons or analysis of variance for repeated measures.
**Mann-Whitney U test for intergroup comparisons or analysis of variance for repeated measurements, $p<0.05$. p^{1}: comparison of the before and after values of the 1st group, p^{2}: comparison of the before and after values of the 2nd group, p^{3}: comparison of the before and after values of the 3rd group, p^{4}: comparison of the initial values of the groups, p^{5}: comparison of the 8th week values of the groups.
SD: Standard deviation, LDL-C: Low-density lipoprotein-cholesterol, CRP: C-reactive protein

Table 2. Energy and nutrient intake of individuals in different dietary treatment groups

	Group 1			Group 2			Group 3			Overall	
	Baseline	8th week		Baseline	8th week		Baseline	8th week		Baseline	8th week
Energy and nutrients	**x̄±SD**	**x̄±SD**	**p[1]**	**x̄±SD**	**x̄±SD**	**p[2]**	**x̄±SD**	**x̄±SD**	**p[3]**	**p[4]**	**p[5]**
Energy (kcal/day)	1546.36±681.64	1262.94±232.41	**0.021**	1274.04±439.97	1099.01±152.52	0.192	1445.49±526.28	1456.16±476.84	0.927	0.340	**0.006**[c]
Carbohydrate (g/day)	149.66±67.5	115.56±30.68	**0.014**	125.56±49.72	100.53±18.66	0.072	141.15±48.38	138.07±61.0	0.798	0.422	**0.026**[c]
Carbohydrate (%)	40.05±6.87	37.10±5.05	0.125	40.12±5.78	37.59±7.62	0.231	40.5±5.73	38.09±5.04	0.195	0.967	0.858
Simple carbohydrates (%)	22.72±16.61	17.05±4.38	**0.011**	7.89±11.72	4.46±6.54	0.598	23.5±34.52	25.0±30.0	0.836	0.068	**0.001**[a]
Protein (g/day)	64.68±27.6	51.28±9.97	**0.025**	50.39±12.87	47.78±10.72	0.674	60.84±26.27	59.48±22.89	0.800	0.180	0.071
Protein (%)	17.52±3.8	16.76±3.37	0.474	16.76±3.21	17.82±3.13	0.366	16.86±2.85	16.59±2.68	0.789	0.733	0.423
Plant-based protein (g/day)	21.77±10.66	18.56±4.44	0.163	18.06±6.76	16.21±3.53	0.398	18.52±7.25	20.19±7.78	0.371	0.326	0.107
Animal protein (g/day)	42.91±24.34	32.72±9.19	**0.048**	32.33±10.64	31.56±11.76	0.887	42.32±19.72	39.29±16.9	0.514	0.195	0.138
Fat (g/day)	74.81±38.32	65.51±13.8	0.199	62.63±25.0	55.5±12.61	0.362	69.75±28.65	72.49±19.05	0.687	0.500	**0.005**[c]
Fat (%)	42.19±5.23	46.05±4.38	**0.021**	43.12±5.95	44.41±6.25	0.454	42.64±5.08	45.27±4.94	0.086	0.870	0.626
SFA (g/day)	25.37±13.54	20.42±5.03	0.064	18.25±6.02	17.91±3.34	0.945	24.15±12.08	23.59±9.54	0.821	0.136	**0.038**[c]
SFA (%)	14.63±3.50	14.53±1.70	0.982	13.11±2.83	14.68±2.25	0.276	14.63±3.50	14.53±1.70	0.922	0.230	0.900
MUFA (g/day)	23.88±11.96	21.17±3.13	0.236	20.84±9.3	18.17±2.91	0.291	22.75±9.89	15.89±5.18	0.793	0.674	**0.021**[c]
MUFA (%)	13.68±1.99	15.14±1.55	0.055	14.46±3.38	14.77±1.98	0.862	13.68±1.99	15.14±1.55	0.364	0.630	0.440
PUFA (g/day)	20.67±13.58	19.11±5.97	0.574	19.59±12.05	15.82±5.89	0.224	17.95±7.29	20.73±4.89	0.304	0.726	**0.028**[c]
PUFA (%)	11.69±3.91	13.65±3.60	0.687	13.32±3.70	12.68±3.79	0.383	11.69±3.91	13.65±3.60	0.118	0.200	0.670
Omega-6/omega-3	0.08±0.05	0.06±0.02	0.129	0.05±0.02	0.06±0.03	0.480	0.08±0.06	0.06±0.03	**0.027**	0.083	0.936
Cholesterol (mg/day)	263.66±190.20	253.39±83.32	0.765	197.6±90.17	222.72±84.21	0.515	286.55±157.21	305.26±149.41	0.573	0.202	0.075
Fiber (g/day)	15.69±6.45	18.35±1.54	0.582	15.12±4.88	25.78±1.66	0.827	14.59±5.05	15.89±2.06	0.264	0.808	0.474
Soluble fiber (g/day)	5.15±2.22	5.13±1.84	0.963	4.51±2.19	14.52±2.0	0.918	4.29±1.98	4.74±3.50	0.319	0.393	0.503
Insoluble fiber (g/day)	9.5±3.96	9.95±7.45	0.534	8.38±2.68	8.80±4.04	0.595	8.54±2.65	10.12±7.45	0.251	0.483	0.259
Vitamin A (mcg/day)	1097.25±1135.35	740.64±198.38	0.212	834.29±471.21	677.55±238.53	0.624	793.03±451.37	1145.71±1649.59	0.202	0.388	0.284

Table 2. Continued

	Group 1			Group 2			Group 3			Overall	
	Baseline	8th week		Baseline	8th week		Baseline	8th week		Baseline	8th week
Energy and nutrients	**x̄±SD**	**x̄±SD**	**p[1]**	**x̄±SD**	**x̄±SD**	**p[2]**	**x̄±SD**	**x̄±SD**	**p[3]**	**p[4]**	**p[5]**
Vitamin C (mg/day)	62.19±39.60	71.95±33.60	0.255	65.4±33.92	68.7±27.78	0.941	64.51±30.84	63.92±38.82	0.943	0.817	0.741
Vitamin E (mg/day)	19.42±12.66	18.63±5.61	0.776	19.89±12.19	16.26±5.85	0.223	16.33±6.80	20.86±5.34	0.085	0.517	**0.045[c]**
Vitamin K (mcg/day)	308.3±172.63	307.23±61.79	0.977	282.91±116.93	265.22±59.16	0.631	249.89±130.85	292.6±121.29	0.188	0.414	0.344
Thiamine (mg/day)	0.63±0.24	0.56±0.09	0.148	0.54±0.15	0.55±0.12	0.842	0.59±0.19	0.62±0.20	0.491	0.390	0.243
Riboflavin (mg/day)	1.08±0.50	0.89±0.12	0.099	0.90±0.22	0.86±0.14	0.766	1.08±0.43	1.17±0.53	0.407	0.294	**0.008[c]**
Niacin (mg/day)	21.50±9.24	16.62±3.96	**0.021**	16.39±4.89	15.8±4.40	0.792	19.54±8.0	20.48±8.20	0.633	0.138	**0.034[c]**
Vitamin B_6 (mg/day)	1.07±0.48	0.83±0.10	**0.012**	0.85±0.23	0.78±0.13	0.475	0.99±0.37	0.95±0.39	0.617	0.234	0.114
Folic acid (mcg/day)	205.44±85.02	211.03±38.15	0.746	203.92±64.02	198.69±34.09	0.784	217.49±84.53	235.11±81.20	0.279	0.836	0.133
Vitamin B_{12} (µg/day)	4.15±2.79	3.10±0.64	0.315	2.83±1.25	2.86±0.81	0.985	4.57±3.88	4.94±6.01	0.719	0.183	0.151
Iron (mg/day)	9.25±3.95	8.23±1.47	0.178	8.05±2.21	7.50±1.12	0.532	8.82±3.41	8.82±2.79	0.995	0.543	0.131
Magnesium (mg/day)	204.45±84.43	186.37±32.44	0.310	168.69±40.99	174.34±42.67	0.771	189.44±82.95	203.43±64.98	0.416	0.343	0.188
Zinc (mg/day)	8.47±3.75	7.29±1.15	0.122	6.59±1.48	6.70±1.34	0.894	8.34±3.82	8.18±2.83	0.817	0.168	0.070
Calcium (mg/day)	563.82±284.52	489.16±100.55	0.191	466.25±114.22	453.84±100.71	0.847	522.80±259.74	597.39±254.25	0.18	0.460	**0.030[c]**
Potassium (mg/day)	1710.66±742.91	1506.17±201.93	0.155	1480.87±395.43	1403.92±245.76	0.638	1658.41±599.48	1682.77±663.95	0.861	0.492	0.140
Phosphorus (mg/day)	921.85±372.58	805.5±122.32	0.136	752.81±161.95	750.98±116.82	0.986	895.13±378.21	940.96±307.16	0.543	0.258	**0.017[c]**
Cupper (mg/day)	1.17±0.49	1.03±0.14	0.148	1.06±0.32	0.95±0.14	0.342	1.13±0.39	1.20±0.46	0.458	0.702	**0.034[c]**

The fiber content in the second group: 5 g in the first 4 weeks and 10 g in the second 4 weeks.
*Analysis of variance in group comparisons and repeated measurements. **Analysis of variance in group comparisons, repeated measurements, Bonferroni test in pairwise comparisons, p<0.005. p[1]: before and after the first group, p[2]: before and after the second group, p[3]: before and after the third group, p[4]: comparison of the initial values of the groups, p[5]: comparison of the 8th week values of the groups. [a]: difference between 1st and 2nd groups, [b]: difference between 1st and 3rd groups, [c]: a difference between 2nd and 3rd groups.
SD: Standard deviation, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

Relationship between serum zonulin levels and nutrient intake

At baseline, plant-based protein (r=-0.565; p=0.008) and soluble fiber (r=-0.626; p=0.002) were inversely correlated with zonulin levels in group 1. There was also a moderate linear relationship between cholesterol and zonulin levels (r=0.440; p=0.046). In group 2, there was a linear correlation between zonulin levels and protein intake (r=0.485; p=0.049). In group 3, there was an inverse correlation between the levels of zonulin and Monounsaturated fatty acid (MUFA) (r=-0.501; p=0.018).

There was no significant correlation between zonulin levels and nutrient intake in groups 1 and group 3 by the 8th week. In group 2, the zonulin level was inversely correlated with the percentage of fat (r=-0.549; p=0.022), MUFA (r=-0.547; p=0.023) and Vitamin E (r=-0.525; p=0.031). There was a positive correlation between levels of zonulin and omega 6/ omega 3 ratio (r=0.582; p=0.015) (Table 3).

Discussion

This study was planned and conducted to evaluate the effects of different dietary treatments on several biochemical parameters [fasting blood glucose, CRP, cholesterol (total and LDL), triglyceride] and zonulin levels in female subjects aged 19-50 years with a previous diagnosis of IBS.

The diagnosis of IBS is a "symptom-based" disease. Elevated CRP level is also an important symptom of IBS (18). Although considered a functional disorder, intestinal inflammation is an element of the pathophysiology of IBS. Therefore, plasma high-sensitivity CRP, a marker of micro-inflammation, may be elevated in IBS (19).

Dietary fiber has anti-inflammatory effects by reducing lipid oxidation (20). Conversely, a low-fiber diet increases the levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-18, and tumor necrosis factor-alpha (21). An epidemiological study showed that increased dietary fiber intake was significantly associated with lower CRP levels (22). Several authors have also reported reduced serum IL-6, CRP, C-peptide, and insulin levels following higher consumption of whole grain products (23). In mouse models of colorectal cancer, consumption of resistant starch increases the production of short-chain fatty acids and reduces inflammation and cell proliferation (24).

Probiotic supplementation increases immunity, reduces inflammation by stimulating cytokines that prevent inflammation, and prevents the growth of pathogens (25). In addition, probiotics affect immune cells and stimulate the production and secretion of anti-inflammatory cytokines (26). In a double-blind, placebo controlled study by Hod et al. (19), after 8 weeks of probiotic supplementation in individuals with diarrhea-predominant IBS, CRP levels did not significantly change compared with baseline. In another study, total and LDL cholesterol levels were reduced following supplementation with probiotics among individuals with obesity (27). However, at the end of the study, the observed difference was not statistically significant despite the positive effects of probiotics on lipid parameters (27).

No difference was observed in the levels of biochemical parameters at baseline or at the end of our study. The lack of a decrease in CRP levels after 8 weeks in the soluble fiber and probiotic supplement groups may be related to factors such as stress since CRP is an indicator of acute inflammation. The lack of a decrease in biochemical parameters in the Infantis 35624 supplement group may be due to the higher saturated fat consumption of individuals in that group.

Dietary fiber has a positive effect on both inflammation and intestinal permeability. With high fiber intake, the number of bacteria that produce short-chain fatty acids in the intestine increases. Short-chain fatty acids help reduce inflammation by promoting intestinal tissue repair and increasing mucus secretion (28). This study showed that both constipation predominant and diarrhea-predominant IBS zonulin levels were higher than in the control group. Zonulin may be a useful simple biomarker for altered intestinal permeability in patients with IBS (8). In another study, supplementation with kefir, a local product rich in probiotics, for 3 weeks improved serum zonulin levels compared with milk supplementation among overweight subjects (29). Obese individuals who received frozen green leafy vegetables during the first or last four weeks of a 12-week trial had increased serum zonulin levels with no effect on fecal zonulin levels (30). Significant reductions in serum zonulin levels were also observed in IBS patients who received probiotic therapy for 12 weeks, but not in those treated for 8 weeks (31).

In the present study, serum zonulin levels did not increase in the intervention groups. The reason supplementation with fiber or probiotics did not affect zonulin levels may be related to the higher percentage of dietary fat intake than the recommended value by TÜBER as 2015 recommendations include 25-30% of energy from fat sources (32). Animal studies have shown that a high-fat diet increases intestinal permeability and decreases the expression of TJ proteins such as zonulin and occludin in intestinal epithelial cells, thereby accelerating the passage of bacterial endotoxins into the blood (20). In humans, data are sparse because serum zonulin levels are correlated with fat intake only in several studies (33-36). However, there are notable differences between the published studies regarding participant characteristics and study design.

We observed that the serum zonulin levels of individuals in the first group were negatively correlated with the amount of dietary plant-based protein and soluble fiber and positively correlated with cholesterol. This finding may be due to the anti-inflammatory and intestinal barrier-strengthening effects of butyrate, an end-product of the fermentation of soluble fiber (37). The positive correlation between zonulin levels and cholesterol levels in group 1 may be due to high-fat consumption

Table 3. Relationship between pre- and posttreatment zonulin levels and energy and nutrient intake levels among individuals receiving different dietary treatments

Energy and nutrients	Zonulin level (ng/mL) (baseline)						Zonulin level (ng/mL) (8th week)					
	Group 1 (n=21)		Group 2 (n=17)		Group 3 (n=22)		Group 1 (n=21)		Group 2 (n=17)		Group 3 (n=22)	
	r	p	r	p	r	p	r	p	r	p	r	p
Energy (kcal/day)	-0.312	0.169	-0.223	0.390	-0.322	0.143	0.248	0.278	-0.087	0.740	0.065	0.774
Carbohydrate (g/day)	-0.373	0.096	-0.265	0.305	-0.322	0.143	0.113	0.626	0.418	0.095	0.033	0.883
Carbohydrate (%)	-0.317	0.161	0.009	0.974	0.210	0.348	0.134	0.564	0.417	0.096	-0.041	0.855
Protein (g/day)	-0.047	0.841	-0.110	0.673	-0.302	0.172	0.099	0.670	-0.092	0.726	-0.054	0.813
Protein (%)	0.289	0.204	0.485	**0.049**	-0.145	0.519	-0.073	0.754	0.088	0.736	-0.083	0.714
Plant-based protein (g/day)	-0.565	**0.008**	-0.203	0.434	-0.351	0.110	-0.021	0.929	0.210	0.419	0.028	0.903
Animal-based protein (g/day)	0.082	0.724	0.174	0.504	-0.330	0.133	0.144	0.533	-0.085	0.745	-0.015	0.946
Fat (g/day)	-0.149	0.518	-0.272	0.291	-0.261	0.240	0.182	0.430	-0.460	0.063	0.091	0.687
Fat (%)	0.199	0.387	-0.251	0.331	-0.206	0.357	0.088	0.706	-0.549	**0.022**	0.066	0.769
Saturated fatty acid (g/day)	0.430	0.052	0.235	0.363	-0.322	0.143	-0.008	0.973	-0.131	0.616	-0.234	0.294
MUFA (g/day)	0.312	0.169	-0.250	0.333	-0.501	**0.018**	0.277	0.225	-0.547	**0.023**	-0.014	0.950
PUFA (g/day)	-0.384	0.085	-0.208	0.422	-0.072	0.751	0.061	0.793	-0.635	0.006	0.178	0.428
Omega-6/omega-3	0.340	0.131	0.012	0.963	-0.411	0.058	-0.173	0.454	0.582	**0.015**	0.128	0.570
Cholesterol (mg/day)	0.440	**0.046**	-0.211	0.417	-0.119	0.597	0.218	0.342	-0.088	0.736	0.086	0.702
Fiber (g/day)	-0.410	0.065	-0.147	0.573	-0.407	0.060	-0.090	0.699	-0.012	0.963	-0.179	0.425
Soluble fiber (g/day)	-0.626	**0.002**	-0.150	0.567	-0.341	0.120	-0.117	0.614	0.098	0.708	-0.380	0.081
Insoluble fiber (g/day)	-0.426	0.054	-0.007	0.978	-0.324	0.142	-0.110	0.634	0.115	0.659	-0.083	0.713
Vitamin A (mcg/day)	0.210	0.360	-0.145	0.580	0.001	0.998	-0.036	0.876	-0.056	0.830	0.084	0.710
Vitamin C (mg/day)	-0.023	0.920	-0.473	0.055	0.126	0.577	-0.034	0.884	-0.395	0.117	0.190	0.396
Vitamin E (mg/day)	-0.423	0.056	-0.301	0.240	0.057	0.801	0.042	0.858	-0.525	**0.031**	0.156	0.487
Vitamin K (mcg/day)	-0.174	0.451	-0.368	0.147	-0.235	0.291	-0.208	0.366	-0.298	0.245	0.077	0.732
Niacin (mg/day)	0.000	1.000	0.022	0.933	-0.319	0.148	0.131	0.571	-0.092	0.725	0.042	0.852
Folic acid (mcg/day)	-0.287	0.208	-0.229	0.376	-0.391	0.072	-0.051	0.827	0.071	0.786	-0.020	0.930
Vitamin B_{12} (µg/day)	-0.342	0.130	-0.225	0.384	-0.182	0.417	0.032	0.889	-0.170	0.513	-0.121	0.590
Iron (mg/day)	0.282	0.216	0.001	0.996	-0.068	0.763	0.130	0.575	0.002	0.993	0.040	0.859
Magnesium (mg/day)	-0.279	0.220	-0.262	0.309	-0.331	0.132	-0.161	0.486	0.077	0.768	0.158	0.484
Zinc (mg/day)	-0.294	0.197	-0.042	0.874	-0.418	0.053	-0.148	0.522	-0.244	0.345	-0.092	0.684
Calcium (mg/day)	-0.184	0.423	0.094	0.719	-0.267	0.230	-0.095	0.683	0.056	0.830	0.010	0.966
Potassium (mg/day)	0.039	0.867	0.051	0.844	-0.153	0.497	0.114	0.622	-0.168	0.519	-0.061	0.787
Sodium (mg/day)	-0.206	0.369	-0.238	0.358	-0.348	0.112	0.071	0.758	0.023	0.929	-0.167	0.459
Phosphorus (mg/day)	-0.109	0.638	-0.115	0.660	-0.252	0.257	0.123	0.594	-0.195	0.453	0.063	0.782
Cupper (mg/day)	-0.094	0.687	-0.105	0.687	-0.240	0.282	0.197	0.391	-0.078	0.765	-0.043	0.848

r: Spearman rank correlation coefficient, $p<0.05$.
MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

in this group, as a high-fat meal can cause inflammation and the formation of advanced glycation end products associated with increased oxidative stress and inflammation (38).

Blood samples were not collected from certain patients at the conclusion of the investigation because their follow-up appointments occurred during the COVID-19 pandemic. This resulted in a smaller sample size than anticipated for the study.

Conclusion

The serum zonulin level did not change after fiber or probiotic yogurt supplementation. Future randomized controlled trials with larger sample sizes are needed to evaluate the effects of fiber and probiotic yogurt on serum zonulin levels in individuals with IBS.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Non-Interventional Research Ethics Committee (ethics approval code: 46418926, project/decision no: 18/253, evaluation date: 21.11.2018).

Informed Consent: All participants signed a voluntary consent form.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.G., N.E.Ü., Concept: M.G., G.A., N.E.Ü., Design: G.A., N.E.Ü., Data Collection or Processing: N.E.Ü., Analysis or Interpretation: G.A., N.E.Ü., Literature Search: N.E.Ü., Writing: N.E.Ü.

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

Financial Disclosure: The authors thank the Clinical Enteral Parenteral Nutrition Association (KEPAN) for financial support for this project (2019).

References

1. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10:712-721.
2. Yilmaz S, Dursun M, Ertem M, Canoruc F, Turhanoğlu A. The epidemiological aspects of irritable bowel syndrome in Southeastern Anatolia: a stratified randomised community-based study. Int J Clin Pract. 2005;59:361-369.
3. El-Salhy M. Irritable bowel syndrome: diagnosis and pathogenesis. World J Gastroenterol. 2012;18:5151-5163.
4. Oświęcimska J, Szymlak A, Roczniak W, Girczys-Połedniok K, Kwiecień J. New insights into the pathogenesis and treatment of irritable bowel syndrome. Adv Med Sci. 2017;62:17-30.
5. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. Immunol Rev. 2017;279:70-89.
6. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. Nat Rev Dis Primers. 2016;2:16014.
7. Barbaro MR, Cremon C, Caio G, et al. Zonulin serum levels are increased in non-celiac gluten sensitivity and irritable bowel syndrome with diarrhea. Gastroenterology. 2015;148:S-56.
8. Singh P, Silvester J, Chen X, et al. Serum zonulin is elevated in IBS and correlates with stool frequency in IBS-D. United European Gastroenterol J. 2019;7:709-715.
9. Yılmaz B, Akbulut G. Current outlook on irritable bowel syndrome. Journal of Nutrition and Diet. 2018;46:276-284.
10. Chey WD, Maneerattaporn M, Saad R. Pharmacologic and complementary and alternative medicine therapies for irritable bowel syndrome. Gut Liver. 2011;5:253-266.
11. Ford AC, Moayyedi P, Lacy BE, et al. American College of Gastroenterology monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. Am J Gastroenterol. 2014;109(Suppl 1):S2-26.
12. Dale HF, Rasmussen SH, Asiller ÖÖ, Lied GA. Probiotics in Irritable Bowel Syndrome: An Up-to-Date Systematic Review. Nutrients. 2019;11:2048.
13. Moayyedi P, Quigley EM, Lacy BE, et al. The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. Am J Gastroenterol. 2014;109:1367-1374.
14. Kaya N, Turan N. Reliability and validity of the constipation severity scale. Turkiye Clinics Journal of Medical Sciences. 2011;31:1491-1501.
15. Schmulson MJ, Drossman DA. What Is New in Rome IV. J Neurogastroenterol Motil. 2017;23:151-163.
16. Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol. 2004;4:26.
17. BEBİS (version 8.0). Ebispro for windows. Germany: Turkish version nutrition information systems.
18. Ünal S, Doğan İ. Irritable bowel syndrome. Turkiye Klinikleri J Gastroenterohepatol-Special Topics. 2011;4:1-7.
19. Hod K, Sperber AD, Ron Y, et al. A double-blind, placebo-controlled study to assess the effect of a probiotic mixture on symptoms and inflammatory markers in women with diarrhea-predominant IBS. Neurogastroenterol Motil. 2017;29.
20. Ma Y, Hébert JR, Li W, et al. Association between dietary fiber and markers of systemic inflammation in the Women's Health Initiative Observational Study. Nutrition. 2008;24:941-949.
21. Yalçın T, Rakıcıoğlu N. Dietary factors, type 2 diabetes and ınflammation. Sakarya Medical Journal. 2018;8:686-694.
22. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. J Nutr. 2004;134:1181-1185.

23. Ampatzoglou A, Williams CL, Atwal KK, et al. Effects of increased wholegrain consumption on immune and inflammatory markers in healthy low habitual wholegrain consumers. Eur J Nutr. 2016;55:183-195.

24. Hu Y, Le Leu RK, Christophersen CT, et al. Manipulation of the gut microbiota using resistant starch is associated with protection against colitis-associated colorectal cancer in rats. Carcinogenesis. 2016;37:366-375.

25. Whorwell PJ. Do probiotics improve symptoms in patients with irritable bowel syndrome? Therap Adv Gastroenterol. 2009;2:37-44.

26. Orel R, Kamhi Trop T. Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. World J Gastroenterol. 2014;20:11505-11524.

27. Bulut S. Obez bireylerde probiyotik takviyesinin ağırlık kaybı ve kan lipit düzeyleri üzerindeki etkisinin değerlendirilmesi (Master's thesis, Eastern Mediterranean University EMU-Doğu Akdeniz Üniversitesi (DAÜ). 2017.

28. Thorburn AN, Macia L, Mackay CR. Diet, metabolites, and "western-lifestyle" inflammatory diseases. Immunity. 2014;40:833-842.

29. Pražnikar ZJ, Kenig S, Vardjan T, Bizjak MČ, Petelin A. Effects of kefir or milk supplementation on zonulin in overweight subjects. J Dairy Sci. 2020;103:3961-3970.

30. Riviere AJ, Smith KS, Schaberg MN, Greene MW, Frugé AD. Plasma and fecal zonulin are not altered by a high green leafy vegetable dietary intervention: secondary analysis of a randomized control crossover trial. BMC Gastroenterol. 2022;22:184.

31. Caviglia GP, Tucci A, Pellicano R, et al. Clinical Response and Changes of Cytokines and Zonulin Levels in Patients with Diarrhoea-Predominant Irritable Bowel Syndrome Treated with Bifidobacterium Longum ES1 for 8 or 12 Weeks: A Preliminary Report. J Clin Med. 2020;9:2353.

32. Pekcan EG, Şanlıer N, Baş M, Başoğlu S, Acar Tek N. Türkiye Beslenme Rehberi 2015 (TÜBER). Ankara: Sağlık Bakanlığı. 2016;1-288.

33. Mörkl S, Lackner S, Meinitzer A, et al. Gut microbiota, dietary intakes and intestinal permeability reflected by serum zonulin in women. Eur J Nutr. 2018;57:2985-2997.

34. Çelik E. Evaluation of the relationship between plasma zonulin level and ınsulin resistance, depression and nutritional status in women with polycystic ovary syndrome, Master Thesis, Gazi University Institute of Health Sciences, Ankara. 2018;39-59.

35. de Punder K, Pruimboom L. The dietary intake of wheat and other cereal grains and their role in inflammation. Nutrients. 2013;5:771-787.

36. Ohlsson B, Roth B, Larsson E, Höglund P. Calprotectin in serum and zonulin in serum and feces are elevated after introduction of a diet with lower carbohydrate content and higher fiber, fat and protein contents. Biomed Rep. 2017;6:411-422.

37. Geinart A, Calatayud M, Grootaert C, et al. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci Rep. 2017;7:11450.

38. Calder PC, Ahluwalia N, Brouns F, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106(Suppl 3):5-78.

DOI: 10.4274/gulhane.galenos.2024.57984
Gulhane Med J 2024;66(4):185-189

Salivary mucin 4 levels in subjects with oral potentially malignant disorders and oral squamous cell carcinoma

Vaibhav Pandita[1], Vidya Ajila[1], Subhas Babu[1], Suchetha N Kumari[2], Shruthi Hegde[1], Mohamed Faizal Asan[1]

[1]Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Department of Oral Medicine and Radiology, Mangalore, India

[2]Nitte (Deemed to be University), Justice K.S. Hegde Medical Academy, Department of Biochemistry, Mangalore, India

Cite this article as: Pandita V, Ajila V, Babu S, Kumari SN, Hegde S, Asan MF. Salivary mucin 4 levels in subjects with oral potentially malignant disorders and oral squamous cell carcinoma. Gulhane Med J. 2024;66(4):185-189

Date submitted:
23.02.2024
Date accepted:
11.07.2024
Online publication date:
22.11.2024

Corresponding Author:
Vidya Ajila, MDS, Prof., Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Department of Oral Medicine and Radiology, Mangalore, India
ajila_v@yahoo.com

ORCID:
orcid.org/0000-0002-5744-9322

Keywords: Mucin 4, saliva, biomarker, oral cancer

ABSTRACT

Aims: Oral cancer remains a substantial global health burden. Oral squamous cell carcinoma (OSCC) is a multi-step process characterized by invasive cancer and metastasis. Mucin 4 (MUC4) has been studied for its differential expression in cancer. The current study aimed to evaluate salivary MUC4 levels in subjects with oral potentially malignant disorders (OPMD) and OSCC.

Methods: This case-control, cross-sectional study evaluated salivary MUC4 levels in healthy subjects (Group 1), OPMD patients (Group 2), and OSCC patients (Group 3). Saliva was collected from the subjects 1 h before food consumption using the spit method, and MUC4 levels were analyzed using an Enzyme-linked immunosorbent assay.

Results: The study included 26 controls (group 1, age, mean±SD: 42.9±7.2, males: 50%), 26 subjects with OPMD (group 2, age, mean±SD: 46.2±7.5, males: 73.1%), and 26 subjects with OSCC (group 3, age, mean±SD: 57.2±6.2, males: 65.4%). MUC4 levels were significantly higher in patients with OPMD (6.20±3.07 ng/dL) and OSCC (7.87±4.30 ng/dL) than in controls (4.22±2.05 ng/dL) (p=0.001). Group 3 had significantly higher salivary MUC4 levels than group 1. OSCC with TNM stage 4a had higher salivary MUC4 levels (8.53±4.15 ng/dL), followed by TNM stage 3 (7.49±4.93 ng/dL) and TNM stage 2 (6.33±2.89 ng/dL).

Conclusions: Salivary MUC4 levels were significantly higher in patients with OPMD and OSCC. This study showed that MUC4 may play a role in the diagnosis of OPMD and OSCC.

Introduction

Oral cancer is a non-homogeneous group of cancers of the head and neck region, including neoplasms affecting the oral cavity (1). In South Asia, approximately 90% of oral cancers develop from pre-existing oral potentially malignant disorders (OPMDs), including leukoplakia, oral submucous fibrosis (OSMF), and erythroplakia (2). Although the oral cavity can be easily examined, oral squamous cell carcinoma (OSCC) is routinely diagnosed in advanced stages. Common reasons for this include ignorance and neglect of the changes occurring in the oral mucosa (3). Saliva is a potential source of biomarkers because of its diverse composition. Because it is in close contact with oral lesions like carcinoma, locally expressed molecules may reflect signs of tumorigenesis and malignant transformation

(4). Saliva collection is a non-invasive procedure that is cost-effective compared with other investigations and is therefore an ideal non-invasive medium for diagnosing OSCC (4).

Salivary mucins play an important role in innate immunity by promoting bacterial colony aggregation and clearance in the oral cavity. There are approximately 22 types of clinically recognized mucins, of which mucin 1 and mucin 4 (MUC4) are associated with OSCC (5). Mucins are classified based on their transmembrane domain, which directly influences their attachment to the plasma membrane. MUC4 is a membrane-bound mucin that has been studied extensively in several types of cancer and many systemic diseases (5). Various authors have demonstrated the expression of MUC4 in normal epithelia like cornea and conjunctiva, lacrimal gland, salivary gland secretory epithelium, upper aerodigestive tract, gastrointestinal tract, breast, endocervix, and vagina. MUC4 is also found in several body fluids such as blood, saliva, tears, and breast milk. Abnormal expression of MUC4 has been noted in several human cancers, including breast, lung, pancreas, salivary gland, oral mucosa, esophagus, and cervix (5,6). To date, there has been limited research into the relationship between MUC4 expression in OPMD and OSCC. The current study examined salivary MUC4 levels in subjects with OPMD and OSCC using enzyme-linked immunosorbent assay (ELISA).

Methods

Study population and recruitment

A total of 78 subjects aged >30 years who were reporting to the department of oral medicine and radiology between November 2019 and February 2021 were included in the study after obtaining informed consent. The subjects were categorized into three groups of 26 each: Group 1 consisted of randomly selected healthy controls, Group 2 consisted of subjects diagnosed clinically with OPMD, and Group 3 consisted of subjects diagnosed clinically and histopathologically with OSCC. Clinical TNM staging was performed for OSCC cases. Subjects with a history of any systemic complications, suffering from any major illness or malignancy other than OSCC, and those under treatment for OPMD or OSCC were excluded.

Saliva samples were obtained from the subjects one hour before food consumption using the spit technique. Salivary samples included oral fluid from major and minor salivary glands. Samples were subjected to centrifugation at 3000 rpm for 20 min and stored at -20 °C. Salivary MUC4 levels were estimated using an ELISA (Human MUC4 ELISA Kit, Immunoconcept India Pvt Ltd).

Statistical Analysis

The data obtained were computed, and statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 24.00 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test the normality of the data distribution. Categorical variables are presented as frequencies and percentages, and continuous variables are presented as means and standard deviations. Because the data followed a normal distribution, data analysis was performed using one-way ANOVA. The mean difference between the groups was calculated, and the association was assessed using Tukey's post-hoc multiple comparison test. P value <0.05 was considered statistically significant.

Results

Demographic characteristics

In the control group, subjects were aged between 30 and 70 years, with a mean age of 42.9±7.2 years and 50% were men. In the OPMD group, the age range was from 30 to 70 years, with a mean age of 46.2±7.5 years and 73.1% were men (Table 1). Among the OPMDs, 13 cases were OSMF (50%), 10 cases were leukoplakia (38%), and 3 cases were erosive lichen planus (12%). In the OSCC group, the age range was 30 to 70 years, with a mean of 57.2±6.2 years and 65.4% were men.

Salivary MUC4 levels in groups

The mean salivary MUC4 levels in the control, OPMD, and OSCC groups were 4.22±2.05 ng/dL, 6.20±3.07 ng/dL, and 7.87±4.30 ng/dL, respectively. The OSCC group had higher salivary MUC4 levels, followed by the OPMD and control groups. There was a significant difference (p=0.001) in salivary MUC4 levels between the three groups using ANOVA. Post-hoc results revealed that the OSCC group had significantly higher (p<0.001) salivary MUC4 (7.87±4.30 ng/dL) than the controls (4.22±2.05 ng/dL). There were no significant differences in salivary MUC4 between the OPMD group and the control and OSCC groups (p=0.083 and p=0.176, respectively) (Table 2).

Salivary MUC4 levels according to OSCC stages

In the OSCC group, 12 (46.2%) patients presented with stage 4a, 11 (42.3%) with stage 3, and 3 (11.5%) with stage 2 OSCC. Stage 4a had higher salivary MUC4 levels (8.53±4.15 ng/dL), followed by stage 3 (7.49±4.93 ng/dL) and stage 2 (6.33±2.89 ng/dL). The difference in salivary MUC4 levels among the three OSCC stages was not significant (p=0.706).

Table 1. Age and gender distribution in the groups

	Age, years, mean±SD	Men, n (%)	Women, n (%)
Control group	42.9±7.2	13 (50.0)	13 (50.0)
OPMD group	46.2±7.5	19 (73.1)	7 (26.9)
OSCC group	57.2±6.2	17 (65.4)	9 (34.6)

SD: Standard deviation, n: Number, OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma

Table 2. Intergroup comparison using Tukey's post-hoc test

	Control vs. OPMD	OPMD and OSCC	Controls vs. OSCC
Mean difference	-1.97	-1.63	-3.61
P value	0.083	0.176	**<0.001***

*p<0.05 was considered as statistically significant.
OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma

Discussion

Oral carcinogenesis is multifactorial and occurs when the squamous epithelium is affected by various genetic alterations. Various cellular, inflammatory, biochemical, and hematological changes occur during carcinogenesis and have been well documented in the literature (3). Numerous tumor markers can be used to identify malignant transformation (7). Mucins are expressed by epithelial cells (8). High molecular weight proteins are responsible for various biological functions, such as growth, differentiation, and cell signaling (5,9). Various clinically significant mucins are documented in the literature, including mucin 1, 5AC, 5B, 6, 7, 16, and MUC4 (10).

The normal and pathophysiological functions of MUC4 have been extensively studied. MUC4 is commonly expressed in the respiratory epithelium and body fluids like saliva, tears, and breast milk. It has also been identified in carcinomas like OSCC, laryngeal carcinoma, adenocarcinoma of the lungs, and pancreatic carcinoma (5,11). Cancer cells utilize mucin for proliferation, survival, invasion, and the avoidance of innate immunity (11). Immunohistochemical studies have revealed that aberrant MUC4 expression is associated with increased tumor aggressiveness and poor outcomes in many carcinomas. However, in OSCC, the role of MUC4 is not yet clear (12). Saliva sampling is a noninvasive, readily available method with high compliance in all populations, and it is increasingly being used as an alternative to blood sampling (4). The current study assessed salivary MUC4 levels in patients with OPMD and OSCC.

The mean age of the OPMD group was 46.23±7.47 years. Similar to the results reported by Hosagadde et al. (13), who reported a mean age of 42.39 years among patients with OPMDs. In the current study, the mean age of patients diagnosed with OSCC was 57.5±6.22 years. Baykul et al. (14) conducted a study in which the mean age of patients with oral cancer was 57±19 years. In general, OSCC has been reported in individuals older than 60 years of age who have tobacco habits. Decreased age-related immunological surveillance and the cumulative effects of tobacco cause alterations at the molecular level, leading to malignant transformation (15). In recent years, there has been a greater tendency for OPMDs to occur in younger individuals, probably due to increased substance abuse (13).

In our study, OSCC and OPMD were predominantly diagnosed in male patients. Male predominance was previously reported by Hosagadde et al. (13) and Iype et al. (16), and it can be attributed to lifestyle factors or habits such as smoking and alcohol consumption, which are more prevalent in males.

In the present study, OSMF was the most common OPMD, followed by leukoplakia and erosive lichen planus. According to Hosagadde et al. (13), leukoplakia is the most frequently reported form of OPMD. However, studies in Karnataka, India, have stated that OSMF is more prevalent than leukoplakia, which could be attributed to the high betel quid consumption in the region (15,17).

The predominant stage of OSCC in our study was stage 4a, followed by stage 3 and stage 2. This is similar to the study by Oliveira et al. (18), in which 82.1% of patients were diagnosed with stage 3 and 4 OSCC. Delayed diagnosis of OSCC is a major cause of high mortality and morbidity (19). This can be due to various factors, such as inadequate access to healthcare services and poor socioeconomic status, resulting in delays in seeking medical attention (19). Inadequate awareness, poor socioeconomic status, and delayed access to healthcare facilities may be responsible for the higher OSCC stage of our study subjects.

In the current study, the mean salivary MUC4 level in the control group was 4.22±2.05 ng/dL. Similar findings were demonstrated by Lundmark et al. (20), who reported a mean salivary MUC4 level of the control group was 4.5 ng/ml. Mucins are associated with the renewal and differentiation of epithelial cells, signaling, and adhesion (5). Altered mucin levels lead to abnormal cellular function and possible malignancy (5). In our study, salivary MUC4 levels were increased compared with the normal levels in patients with OPMD. Narashiman et al. (6) evaluated MUC4 expression in leukoplakia cases and correlated it to different grades of dysplasia. They found that MUC4 expression increased steadily from mild to severe dysplasia (6). Abidullah et al. (21) reported that MUC4 expression increased from mild to severe dysplasia in cases with oral epithelial dysplasia. This increase in MUC4 expression according to dysplasia grade indicates a role in the malignant transformation of OPMD.

The mean salivary MUC4 level in the OSCC group was the highest among the three groups. MUC4 overexpression in OSCC sends continuous growth signals, causing uncontrolled cell proliferation (6). Regarding the correlation of salivary MUC4

levels in the three groups, higher levels were observed in the OSCC group, followed by the OPMD and control groups. A statistically significant intergroup difference was detected regarding the mean salivary MUC4 level. Although the control group was correlated with the OSCC group, the difference in salivary MUC4 levels was statistically significant. However, no significant difference in salivary MUC4 levels was observed between the OPMD and control and OSCC groups. Narashiman et al. (6) compared MUC4 levels between oral leukoplakia and OSCC and observed higher MUC4 expression in OSCC samples, followed by leukoplakia. In a systematic review and meta-analysis, Normando et al. (22) identified MUC4 as a protein marker of malignant oral leukoplakia. Since MUC4 levels in our study increased from OPMD to OSCC, MUC4 may help predict the conversion of OPMD to OSCC.

The current study demonstrated that salivary MUC4 levels were higher in stage 4a OSCC, followed by stage 3 and stage 2. No statistically significant association of salivary MUC4 was found among different stages of OSCC. These observations were similar to the study by Hamada et al. (12), which showed that MUC4 was expressed in patients with OSCC and was significantly correlated with greater tumor stage, nodal metastasis, advanced tumor stage, and cancer cell invasion. MUC4 expression in OSCC has been associated with increased invasion, tumor progression, nodal metastasis, and decreased survival (12). MUC4 has also been correlated with local recurrence and lymph node metastasis following treatment (12). Therefore, high MUC4 expression is considered a poor prognostic factor in patients with OSCC (12).

MUC4 may attain novel functions in malignant cells because of its aberrant expression and biochemical and cellular changes, leading to the interaction of MUC4 with cyto-architecturally segregated proteins (22). MUC4 colocalizes and interacts with human epidermal growth factor receptor 2 (HER2) in pancreatic cancer cells, leading to their activation (23). Multiple ligands can bind, causing activation of specific receptors (23). Once HER2 receptors are activated, cascades of intracellular signaling are initiated leading to cell proliferation, angiogenesis, metastasis, apoptosis inhibition, and other events leading to cancer development (23). Kohli et al. (11) and Abidullah et al. (21) found that MUC4 is overexpressed in well-differentiated OSCC, and this expression decreases in moderately and poorly differentiated OSCC. Similarly, in esophageal carcinoma, MUC4 expression decreases with the differentiation of the lesion (24). However, this parameter was not evaluated in the current study; thus, we were unable to determine any correlation.

Hamada et al. (12) reported the presence of MUC4 in premalignant and malignant epithelium but not in normal squamous epithelium. Thus, it could serve as a future diagnostic biomarker for the initial detection of malignant changes. The current study noted a significant increase in salivary MUC4 levels in patients with OSCC and OPMD. These findings indicate that MUC4 is an adjunctive salivary biomarker in OSCC and OPMD.

The current study had a few limitations, such as a smaller sample size of 26 subjects in each group. This result may have been insufficient to explore the diagnostic role of salivary MUC4 levels in OPMD and OSCC.

Conclusion

The current study showed significantly higher salivary MUC4 levels in patients with OSCC and OPMD than in healthy controls. On intergroup comparison, salivary MUC4 levels were significantly increased in the OSCC group compared with the control group. Our findings emphasize the role of salivary MUC4 in OSCC. Further studies are required to explore the diagnostic and prognostic role of salivary MUC4 levels in patients with OPMD and OSCC.

Ethics

Ethics Committee Approval: This case-control, cross-sectional study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethical Clearance Committee AB Shetty Memorial Institute of Dental Sciences (ABSMIDS) (decision no: EC/35/2019, dated: 10.10.2019).

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

Footnotes

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Salehiniya H, Raei M. Oral Cavity and Lip Cancer in the World: An Epidemiological Review. Biomed Res Ther. 2020;7:3898-3905.
2. Kumar MH, Sanjai K, Kumarswamy J, Keshavaiah R, Papaiah L, Divya S. Expression of MUC1 mucin in potentially malignant disorders, oral squamous cell carcinoma and normal oral mucosa: An immunohistochemical study. J Oral Maxillofac Pathol. 2016;20:214-218.
3. Markopoulos AK. Current aspects on oral squamous cell carcinoma. Open Dent J. 2012;6:126-130.

4. Song X, Yang X, Narayanan R, et al. Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proc Natl Acad Sci U S A. 2020;117:16167-16173.

5. Chaturvedi P, Singh AP, Batra SK. Structure, evolution, and biology of the MUC4 mucin. FASEB J. 2008;22:966-981.

6. Narashiman S, Narasimhan M, Venkatraman G. Expression of Mucin 4 in leukoplakia and oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol. 2014;18:25-31.

7. Rajguru JP, Mouneshkumar CD, Radhakrishnan IC, et al. Tumor markers in oral cancer: A review. J Family Med Prim Care. 2020;9:492-496.

8. Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA. Mucins in the mucosal barrier to infection. Mucosal Immunol. 2008;1:183-197.

9. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer. 2004;4:45-60.

10. Kufe DW. Mucins in cancer: function, prognosis and therapy. Nat Rev Cancer. 2009;9:874-885.

11. Kohli M, Shivam AK, Ahuja P, Dutta J. Mucin-4: A novel marker for oral cancer. J Oral Maxillofac Pathol. 2019;23:49-53.

12. Hamada T, Wakamatsu T, Miyahara M, et al. MUC4: a novel prognostic factor of oral squamous cell carcinoma. Int J Cancer. 2012;130:1768-1776.

13. Hosagadde S, Dabholkar J, Virmani N. A clinicopathological study of oral potentially malignant disorders. J Head Neck Physicians Surg. 2016;4:29-34.

14. Baykul T, Yilmaz HH, Aydin U, Aydin MA, Aksoy M, Yildirim D. Early diagnosis of oral cancer. J Int Med Res. 2010;38:737-749.

15. Kumar GK, Abidullah M, Elbadawi L, Dakhil S, Mawardi H. Epidemiological profile and clinical characteristics of oral potentially malignant disorders and oral squamous cell carcinoma: A pilot study in Bidar and Gulbarga Districts, Karnataka, India. J Oral Maxillofac Pathol. 2019;23:90-96.

16. Iype EM, Pandey M, Mathew A, Thomas G, Sebastian P, Nair MK. Oral cancer among patients under the age of 35 years. J Postgrad Med. 2001;47:171-176.

17. Keluskar V, Kale A. An epidemiological study for evaluation of oral precancerous lesions, conditions and oral cancer among Belgaum population with tobacco habits. Biosci Biotech Res Comm. 2010;3:50-54.

18. Oliveira ML, Wagner VP, Sant'ana Filho M, Carrard VC, Hugo FN, Martins MD. A 10-year analysis of the oral squamous cell carcinoma profile in patients from public health centers in Uruguay. Braz Oral Res. 2015;29:S1806-83242015000100270.

19. Coelho KR. Challenges of the oral cancer burden in India. J Cancer Epidemiol. 2012;2012:701932.

20. Lundmark A, Johannsen G, Eriksson K, et al. Mucin 4 and matrix metalloproteinase 7 as novel salivary biomarkers for periodontitis. J Clin Periodontol. 2017;44:247-254.

21. Abidullah M, Nahar P, Ahmed SA, Kothari H, Vakeel S. MUC4 Expression in Oral Dysplastic Epithelium and Oral Squamous Cell Carcinoma: An Immunohistochemical Study. J Int Soc Prev Community Dent. 2023;13:124-132.

22. Normando AGC, Dos Santos ES, Sá JO, et al. A meta-analysis reveals the protein profile associated with malignant transformation of oral leukoplakia. Front Oral Health. 2023;4:1088022.

23. Chaturvedi P, Singh AP, Chakraborty S, et al. MUC4 mucin interacts with and stabilizes the HER2 oncoprotein in human pancreatic cancer cells. Cancer Res. 2008;68:2065-2070.

24. Guillem P, Billeret V, Buisine MP, et al. Mucin gene expression and cell differentiation in human normal, premalignant and malignant esophagus. Int J Cancer. 2000;88:856-861.

DOI: 10.4274/gulhane.galenos.2024.44827
Gulhane Med J 2024;66(4):190-195

Comparison of sampling techniques and sterilization methods for *Bacillus anthracis*-contaminated surfaces

Mesut Ortatatlı, Batuhan Çebi, Levent Kenar

University of Health Sciences Türkiye, Department of Medical CBRN Defense, Ankara, Türkiye

Cite this article as: Ortatatlı M, Çebi B, Kenar L. Comparison of sampling techniques and sterilization methods for *Bacillus anthracis*-contaminated surfaces. Gulhane Med J. 2024;66(4):190-195

Date submitted:
07.02.2024

Date accepted:
12.07.2024

Online publication date:
22.11.2024

Corresponding Author:
Mesut Ortatatlı, M.D., Assoc. Prof., University of Health Sciences Türkiye, Department of Medical CBRN Defense, Ankara, Türkiye
mortatatli@gmail.com

ORCID:
orcid.org/0000-0002-7899-6157

Keywords: *Bacillus anthracis*, sampling, recovery, decontamination, sterilization

ABSTRACT

Aims: Sampling techniques and disinfection methods are essential for surfaces in critical areas for human health, such as food processing areas, hospital operating rooms, and laboratories. Thus, in the case of biological warfare agent use, the detection and decontamination of contamination sites have become a public health concern. From this perspective, our study aimed to evaluate the effectiveness of two distinct sampling techniques and three sterilization methods for surfaces contaminated with *B. anthracis* spores.

Methods: To collect samples from surfaces and for recovery, two methods, including a moistened technique using sterile cotton swabs and a dry cotton swab technique following the suspension of the contaminated surfaces in saline solution, were applied. To sterilize the contaminated surface, 0.5% sodium hypochlorite (NaOCl), 3% H_2O_2, and UVC-254 were applied for various durations.

Results: The suspension method yielded a recovery of 427.50 cu for *B. anthracis*, whereas the moistened method yielded 272.50 cu (p=0.003). Among the decontamination methods tested, complete sterilization was achieved within 15 min using 0.5% NaOCl and within 6 h with 3% H_2O_2, whereas UVC-254 led to a decrease in the spore count by up to 96.73% after 24 h.

Conclusions: In the evaluation of surface contamination and sterilization-disinfection, sampling with a dry cotton swab by forming a suspension on the surface increased recovery. Thus, it was concluded that the use of NaOCl is appropriate for decontaminating *B. anthracis* spores on surfaces resistant to oxidation, whereas H_2O_2 is preferable for more delicate surfaces.

Introduction

The possible use of *B. anthracis* as a biological warfare agent (BWA) has descended from bookshelves into real life and poses a significant threat. The ease of use and low production cost have contributed to the extent of this threat in terms of terrorist purposes (1). *B. anthracis*, a member of the *Bacillus cereus* group, is a gram-positive, aerobic, encapsulated, spore-forming bacterium whose resistance to environmental conditions allows it to remain viable for many years (1). In the event of BWA deployment, the undetectability of biological agents by the senses, along with incubation periods ranging from days to weeks, complicates the identification of the affected area (2). Like other BWAs, *B. anthracis* in aerosolized form can also cause contamination of various surfaces, posing a long-term risk of contact transmission (3).

A proper and accurate technique for sample collection from suspected surfaces plays a crucial role in ensuring the accuracy of both on-site and laboratory analyses (4). An effective response to a BWA attack includes not only the detection of the agent but also the decontamination and sterilization of

contaminated surfaces rapidly and effectively (4,5). Routine assessments of microbial contamination and sterilization efficacy on surfaces in laboratories, hospitals, and in contact with food are indispensable for ensuring human health (5). Heat and radiation treatment, filtration, and treatment with gases and liquid chemicals are among the primary sterilization methods currently used (6).

Although several studies have been conducted on sampling and sterilization procedures associated with human health risks using surrogates, research using real highly pathogenic live agents, resulting in complete data, is not common (7,8). Thus, it has been emphasized that further research that targets more accurate and proper findings using real live agents is required (8,9).

The present study aimed to determine an appropriate sampling technique for detecting surface contamination following an attack with *B. anthracis* spores and to identify the sterilization method and time required for effective surface decontamination.

Methods

In this prospective study, the preparation of relevant materials, application of sterilization methods, and control evaluation processes were conducted within a class 2 type B2 biosafety cabinet (BLF2000-Bilser, Ankara, Türkiye). The required sample size was 56 for the four groups based on a 95% confidence interval, 85% power value, and an effect size of 0.5 using G*Power V3.1.9 statistical analysis software.

Preparation and surface contamination of *Bacillus anthracis* spores

B. anthracis spores were obtained from stock samples from a previous study (10). Spores were inoculated onto sheep blood agar plates (RDS, Ankara, Türkiye) and incubated at 36 °C in an incubator (Nüve, EN 400, Ankara, Türkiye) for 24 h. For sporulation, the bacteria were maintained at room temperature for 24 h and then refrigerated at +4 °C for 7 days. Daily spore staining (Schaeffer-Fulton Spore Stain Kit, Sigma-Aldrich, Switzerland) confirmed the >90% sporulation rate. The bacterial spore suspensions were adjusted to a McFarland 0.5 standard, equivalent to 1×10^8/mL) using isotonic saline. From a dilution prepared at a final concentration of 1×10^4/mL, 10 µL inoculations were made onto sheep blood agar plates to verify the spore counts. A 1 mL suspension of *B. anthracis* spores at a concentration of 1×10^4/mL was placed in sterile Petri dishes with a diameter of 6 cm and left to rest for 24 h inside a biosafety cabinet with lids closed.

Swab sampling and recovery methods

Two different sampling methods were used: surface sampling and recovery. In the first method (the moistened method), swab samples were collected from previously contaminated Petri dish surfaces using sterile cotton swabs moistened with saline solution. In the second method (the suspension method), contaminated Petri dish surfaces were suspended in 0.5 mL of saline solution, followed by sampling with dry sterile cotton swabs. All collected swab samples were inoculated onto sheep blood agar plates and incubated at 36 °C for 24 h, after which colony counts were conducted.

Sterilization methods

For sterilization of 1 mL *B. anthracis* spore suspension at a concentration of 10^4/mL, which was left to dry in Petri dishes for 24 h, the following was used: 3% hydrogen peroxide (H_2O_2) (Kim-Pa Pharmaceutical Lab. Co. Ltd., İstanbul, Türkiye), a 10-fold diluted solution of 5% sodium hypochlorite (NaOCl), commonly known as bleach, and an ultraviolet C (UVC) lamp with a wavelength of 254 nm located inside a type 2 biosafety cabinet (OSAKA T8 30 W TUV; China). The doses and durations of the treatments are presented in Table 1.

Statistical Analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences 21.0 (IBM, Inc., USA). The Shapiro-Wilk test was used to investigate the normality of the variables. The differences in continuous variables among the groups were tested using the Kruskal-Wallis test, and the Bonferroni corrected Mann-Whitney U test was used for post-hoc analyses. The Chi-square test was used to compare nominal

Table 1. The sterilization methods, duration, number of positive Petri dishes, and median and interquartile range values (25-75%) of the colonies

Sterilization method	Application time	Positive Petri dishes (n)	Colony count (cfu) (median and interquartile)
0.5% NaOCl	5 min	6	101.0 (85.25-153.75)
	10 min	4	26.0 (20.25-31.0)
	15 min	0	
3% H_2O_2	1 h	8	118.0 (96.25-140.75)
	3 h	3	29.0 (26.0-33.5)
	6 h	0	
UVC	6 h	10	323.0 (277.75-407.0)
	12 h	8	42.5 (34.5-56.0)
	24 h	3	14.0 (10.0-18.0)
Control		14	427.50 (289.75-470.0)

NaOCl: Sodium hypochlorite, H_2O_2: Hydrogen peroxide, UVC: Ultraviolet C

values. Differences with a p value <0.05 were considered statistically significant.

Results

The median and interquartile range values of the recovery colony count for swab samples taken 24 h later from sterile Petri dishes contaminated with a 1 mL suspension of *B. anthracis* spores at a concentration of 10^4/mL were 272.50 cu (234.25-339.00) for the moistened method and 427.50 cu (289.75-470.00) for the suspension method (p=0.003) (Figure 1). The recovery rates for the moistened and suspension methods were 2.73% and 4.28%, respectively.

Table 1 demonstrates the treatment time for each sterilization method, the number of positive Petri dishes, and the median and interquartile range values of the colony count for *B. anthracis*. Following the application of 0.5% NaOCl for 5 and 10 min, positive results were observed in 6 and 4 Petri dishes, respectively, and no statistical significance was observed (p=0.430), but a significant difference was found between the numbers of growing colonies (p=0.011). The relationship between the number of positive Petri cells and colony counts in the control group and after NaOCl treatment for 5 and 10 min is presented in Figure 2.

The number of positive Petri dishes after sterilization with 3% H_2O_2 for 1 and 3 h was 8 and 3, respectively, and no significant difference was observed (p=0.053). Figure 3 illustrates the statistical significance found not only between the numbers of positive Petri dishes of the group after 6 h and controls, but also between the colony counts of each group.

Neither of the sterilization studies involving ultraviolet radiation for 6, 12, or 24 h provided complete sterilization, but bacterial growth was observed in 10, 8, and 3 Petri dishes, respectively. No significant difference was detected between the control and 6-h UV treatment groups concerning the number of colonies. The data related to all other variables are presented in Figure 4.

Discussion

The use of aerosolized spores of *B. anthracis* as a bioweapon or bioterrorism agent remains a concern due to potential environmental contamination. Therefore, it is important to make great efforts toward detection and decontamination for public health measures (4,5). The swab sampling technique is the most common method for identifying pathological microbial contamination on environmental surfaces, including hospital locations and food processing facilities, and for various cleaning procedures (4). Typically, sticks with cotton tips made of plastic

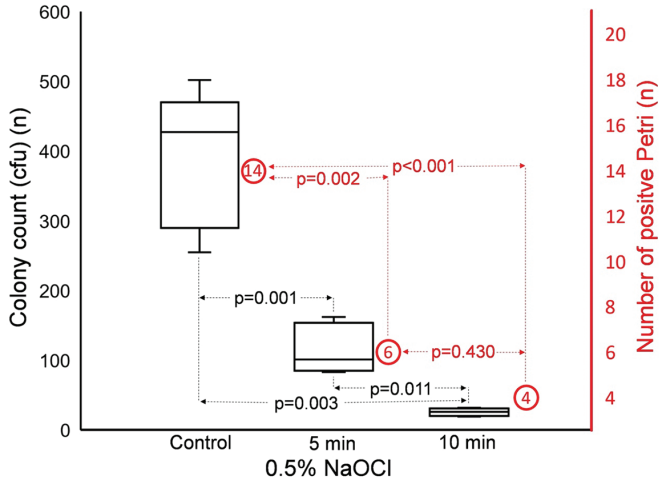

Figure 2. Box plot of median values of *B. anthracis* colony counts after the control group, 5-and 10-min incubations of 0.5% NaOCl, number of positive Petri dishes with growth (data within circles), and p values
NaOCl: Sodium hypochlorite

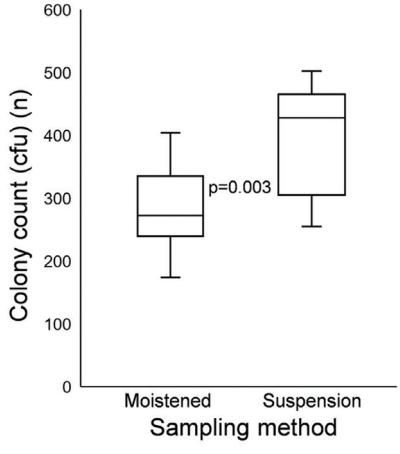

Figure 1. Box plot representing the median values of *B. anthracis* colony counts from surface specimens obtained using moistened and suspension methods and the statistical significance of the differences between these methods

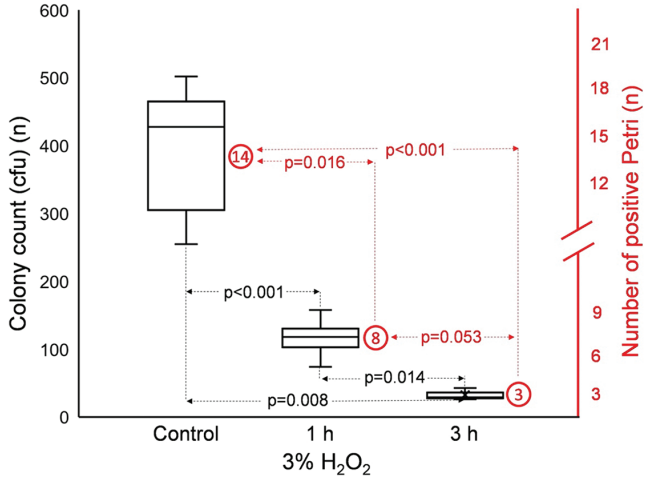

Figure 3. Box plot of median *B. anthracis* colony counts following the control group and 1 and 3 h of 3% H_2O_2 application; number of positive Petri dishes showing growth (data within circles); and p values

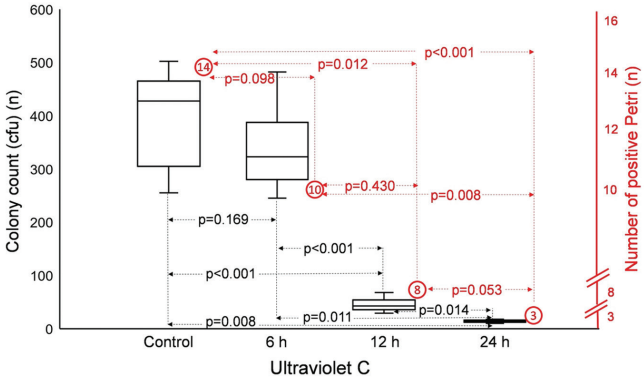

Figure 4. Box plot of median *B. anthracis* colony counts for the control group and after 6, 12, and 24 h of UV light application, number of positive Petri dishes with growth (data within circles), and p values
UV: Ultraviolet

or wood are used for this purpose. The principle involves transferring bacteria from the contact surface to the material and then to the culture medium (5). However, it has been reported that the number of bacteria grown (recovery) by the swab sampling technique is significantly lower than that on the surface (4,5). The material used in the swab sample (cotton, foam, polyester, artificial silk, etc.), the extraction method (shaking, sonication, vortexing, etc.), and the type of tip (dry or premoistened) affect the recovery rate (5). In a study of *B. anthracis* spores by Rose et al. (11), the recovery rates for dry and moistened cotton-tipped swabs were 0.5% and 4.7%, respectively, without extraction; and 7.5% and 27.5%, respectively, with extraction. In another study involving live bacteria, the average recovery rate of moistened cotton-tipped swabs was 48.5% (9). In these studies, the cotton on the tips of the swabs was generally premoistened, and the swab samples taken from the dry surface were first transferred to liquid media by various methods and subsequently inoculated in solid culture media (9,11).

In our study, samples were taken from the *B. anthracis*-contaminated surface and left to dry for 24 h using cotton-tipped swabs via two different methods, and inoculations were made directly onto sheep blood agar without pretreatment. Colony numbers recovered using the suspension method were significantly higher than those recovered using the moistened method (Figure 1). It is suggested that wetting the surface with a sterile liquid before taking the swab sample facilitates the removal of existing microorganisms from the surface and enhances their adsorption of liquid on the cotton, leading to an increase in recovery. Keeratipibul et al. (9) also supported this suggestion and found that the recovery decreased by 30-40% in samples after 1 hour of drying compared with that before the suspension was dried. Our results showed that the recovery rate decreased by 36.25% with the moistened method compared with the suspension method (4.28% and 2.73%, respectively) in samples dried on the surface for 24 h. Sterilization of surfaces and materials often involves both physical methods, including heating, filtration, ionizing radiation, and UV radiation, and chemical methods like ethylene oxide, H_2O_2, glutaraldehyde, and NaOCl, depending on the characteristics of the surface and material (12). Among the aforementioned methods, NaOCl, which we also applied, is known to affect the inner membrane structure of bacterial spores, whereas UV radiation causes DNA damage (12). Another agent, H_2O_2, acts on DNA in a vegetative form and causes damage to the core proteins of bacterial spores (13).

DeQueiroz and Day (14) demonstrated in their study that a mixture of H_2O_2 and NaOCl could achieve a more effective sporicidal effect at lower concentrations and for shorter contact times. They observed a 90% reduction in the *B. subtilis* spore count after 5 min of 2.5% NaOCl treatment, and complete sterilization was achieved after 10 min (14).

A 0.5% NaOCl solution was used in our experiment because of its applicability to intact skin. The number of *B. anthracis* spores was reduced by 76% compared with the control group after 5 min of contact time and by 94% after 10 min. Complete sterilization was achieved after 15 minutes. The reduction in colony counts and the number of positive Petri dishes was statistically significant after 5 or 10 min of treatment compared with the control group (Table 1, Figure 2). Although no complete sterilization was observed, the findings suggested that a 10-min contact time with NaOCl significantly reduced the number of *B. anthracis* spores on the surface. When high NaOCl concentrations cannot be used, extending the contact time can increase its effectiveness. This makes it useful for sensitive surfaces, such as intact human skin.

An ideal agent for sterilizing and disinfecting surfaces important for public health and safety, including those in contact with food sources, laboratories, and hospital operating rooms, should be effective and safe without producing toxic waste (14,15). One of the aforementioned agents, H_2O_2, generates free hydroxyl radicals and can be used in both liquid and gas forms. It is known for its environmentally friendly properties, as it decomposes into water and oxygen (15). Although H_2O_2 is generally recommended for use at high concentrations (6-35%) for its sporicidal effects (13,16), it has been emphasized that even lower concentrations (1-2.5%) can be effective if H_2O_2 is combined with alcohol-and chlorine-containing chemicals (17,18). According to the study of Hayrapetyan et al. (19), as the concentration of H_2O_2 decreases, the exposure time should be extended to increase the sporicidal effect.

We applied 3% H_2O_2 for 1 and 3 h, resulting in a reduction of 72.39% and 93.22% in the number of live spores compared with the control group, respectively, and achieved complete sterilization after 6 h (Table 1). Although there was no significant difference in the number of positive Petri dishes after 1 and 3 h of exposure, a significant reduction was observed in the number

of live spores (Figure 3). Our results indicate that H_2O_2, which is known to leave no toxic waste (15), can be safely used at a 3% concentration as we observed a significant reduction in the number of live *B. anthracis* spores. However, increasing the contact time of H_2O_2 should be considered for the sterilization and disinfection of surfaces in working environments.

"UV sterilization" has been referred to as the "UVC-254 method" since 254 nm is the most commonly used and selected wavelength for sterilization (16). It has been reported that while UVC-254 application rapidly reduces bacterial spore counts in the initial phases, its effectiveness gradually decreases as the exposure continues, which generally leads to incomplete sterilization (16,20). Moreover, despite an extended exposure time and increased dose, UVC might shield live spores and protect them beneath inactivated spores (16,20). In line with previous studies, our study showed a 90% reduction in *B. anthracis* spore counts after 12 h of UVC-254 treatment, with efficacy reaching only 96.73% after 24 h (Table 1, Figure 4). These findings indicate that UVC-254 treatment is a valuable tool for mitigating the risk of *B. anthracis* contamination, although this method cannot achieve complete sterilization.

Conclusion

In conclusion, it is recommended to wet the surface of the *B. anthracis* spore-contaminated environment before obtaining the swab sample because this method has shown significant recovery rates for monitoring surface contamination and sterilization-disinfection. The study also suggests using 0.5% NaOCl for 15 min to decontaminate *B. anthracis* spores and applying 3% H_2O_2 for 6 h on sensitive surfaces. However, UVC-254 did not completely remove all spores from the surface, but significantly reduced the number of viable spores. Additional sampling and decontamination studies on various types of surfaces with live agents should be conducted to determine appropriate procedures following the use of BWA.

Ethics

Ethics Committee Approval-Informed Consent: Not required for this study.

Footnotes

Authorship Contributions

Concept: M.O., Design: M.O., Data Collection or Processing: M.O., B.Ç., Analysis or Interpretation: M.O., L.K., Literature Search: B.Ç., Writing: M.O., L.K.,

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Ortatatli M. Biyolojik Silahlar. İçinde: Eryılmaz M, Dizer U, editörler. Afet Tıbbı. Ankara: Ünsal Yayınları; 2005:1207-1255.
2. Treadwell TA, Koo D, Kuker K, Khan AS. Epidemiologic clues to bioterrorism. Public Health Rep. 2003;118:92-98.
3. Stanek SA, Saunders D, Alves DA, et al. USAMRIID's medical management of biological casualties handbook. 9th ed. Fort Detrick, Maryland; 2020.
4. Jones SL, Ricke SC, Keith Roper D, Gibson KE. Swabbing the surface: critical factors in environmental monitoring and a path towards standardization and improvement. Crit Rev Food Sci Nutr. 2020;60:225-243.
5. Rawlinson S, Ciric L, Cloutman-Green E. How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. J Hosp Infect. 2019;103:363-374.
6. Dağlı G, Özyurt M. Hastane Ortamında Sterilizasyon Uygulamaları. In: Haznedaroğlu T, Özgüven V, Pekcan M, editors. Hastane İnfeksiyonları. Ankara: GATA Basımevi; 2001;68-78.
7. Spotts Whitney EA, Beatty ME, Taylor TH Jr, et al. Inactivation of Bacillus anthracis spores. Emerg Infect Dis. 2003;9:623-627.
8. Setlow P. Resistance of Bacterial Spores to Chemical Agents. Principles and practice of disinfection, preservation and sterilization. Oxford, UK: Wiley-Blackwell;2013;121-130.
9. Keeratipibul S, Laovittayanurak T, Pornruangsarp O, Chaturongkasumrit Y, Takahashi H, Techaruvichit P. Effect of swabbing techniques on the efficiency of bacterial recovery from food contact surfaces. Food Control. 2017;77:139-144.
10. Ortatatli M, Karagoz A, Percin D, Kenar L, Kilic S, Durmaz R. Antimicrobial susceptibility and molecular subtyping of 55 Turkish Bacillus anthracis strains using 25-loci multiple-locus VNTR analysis. Comp Immunol Microbiol Infect Dis. 2012;35:355-361.
11. Rose L, Jensen B, Peterson A, Banerjee SN, Srduino MJ. Swab materials and Bacillus anthracis spore recovery from nonporous surfaces. Emerg Infect Dis. 2004;10:1023-1029.
12. Cho WI, Chung MS. Bacillus spores: a review of their properties and inactivation processing technologies. Food Sci Biotechnol. 2020;29:1447-1461.
13. Setlow P, Christie G. What's new and notable in bacterial spore killing! World J Microbiol Biotechnol. 2021;37:144.
14. DeQueiroz GA, Day DF. Disinfection of Bacillus subtilis spore-contaminated surface materials with a sodium hypochlorite and a hydrogen peroxide-based sanitizer. Lett Appl Microbiol. 2008;46:176-180.
15. Lineback CB, Nkemngong CA, Wu ST, et al. Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds. Antimicrob Resist Infect Control. 2018;7:154.
16. Wood JP, Adrion AC. Review of Decontamination Techniques for the Inactivation of Bacillus anthracis and Other Spore-Forming Bacteria Associated with Building or Outdoor Materials. Environ Sci Technol. 2019;53:4045-4062.

17. Kenar L, Ortatatli M, Yaren H, Karayilanoglu T, Aydogan H. Comparative sporicidal effects of disinfectants after release of a biological agent. Mil Med. 2007;172:616-621.

18. Mickelsen RL, Wood J, Calfee MW, et al. Low-concentration hydrogen peroxide decontamination for Bacillus spore contamination in buildings. Remediation (N Y). 2019;30:47-56.

19. Hayrapetyan H, Nederhoff L, Vollebregt M, Mastwijk H, Nierop Groot M. Inactivation kinetics of Geobacillus stearothermophilus spores by a peracetic acid or hydrogen peroxide fog in comparison to the liquid form. Int J Food Microbiol. 2020;316:108418.

20. Owens MU, Deal DR, Shoemaker MO, Knudson GB, Meszaros JE, Deal JL. High-Dose Ultraviolet C Light Inactivates Spores of Bacillus Atrophaeus and Bacillus Anthracis Sterne on Nonreflective Surfaces. Appl Biosaf. 2005;10:240-247.

DOI: 10.4274/gulhane.galenos.2024.92259
Gulhane Med J 2024;66(4):196-202

Comparison of morphometric and histological features of placenta of in vitro fertilization and naturally conceived pregnancies

Jyothi N. Nayak[1], Hannahsugirthabai RajilaRajendran[1], Sunitha Mahesh[2], Suma Srinivasan[3]

[1]Chettinad Hospital and Research Institute, Chettinad Academy of Research & Education, Department of Anatomy, Chennai, India

[2]Milann Fertility & Birthing Centre, J P Nagar, Clinic of Maternal-Fetal Medicine, Bangalore, India

[3]DA Pandu Memorial RV Dental College and Hospital, Clinic of Oral Pathology, Karnataka, India

Cite this article as: Nayak JN, RajilaRajendran H, Mahesh S, Srinivasan S. Comparison of morphometric and histological features of placenta of in vitro fertilization and naturally conceived pregnancies. Gulhane Med J. 2024;66(4):196-202

Date submitted:
15.03.2024
Date accepted:
24.07.2024
Online publication date:
22.11.2024

Corresponding Author:
Hannahsugirthabai RajilaRajendran, M.D., Prof, Chettinad Hospital and Research Institute, Chettinad Academy of Research & Education, Department of Anatomy, Chennai, India
drrajianat@gmail.com

ORCID:
orcid.org/0000-0002-7708-0163

Keywords: In vitro fertilization (IVF), placenta, histological, morphological, velamentous insertion

ABSTRACT

Aims: In vitro fertilization (IVF) is associated with an increased risk of placental abnormalities and placental-related complications. This study aimed to compare the morphological and histological features of placentae of IVF pregnancy with those of natural pregnancies.

Methods: This cross-sectional study included placentae from both IVF and natural conception pregnant mothers aged between 20-45 years who delivered at Milan Fertility Center and Birthing Hospital, JP Nagar Bangalore. Pregnant women with hypertension, diabetes mellitus, anemia, multiple pregnancies, or preterm pregnancies were excluded from the study. Morphometric parameters of the placenta like weight, thickness, diameter, attachment of cord, and number of cotyledons were recorded, and histological examinations of placentae were performed.

Results: The study included a total of 100 placentae, 50 from IVF pregnancy and 50 from natural pregnancy (age, mean±standard deviation: 31.5±3.0 years, IVF pregnant mothers; 30.9±4.0 years, natural pregnant mothers; p=0.254). The IVF group had a lower gestational ages at delivery (37.0±1.3 weeks vs. 38.1±0.9 weeks; p=0.001), low birth weight (BW) (2.7±0.5 kg vs. 3.0±0.5 kg; p=0.001), and increased placental thickness (2.43±0.36 cm vs. 2.17±0.39 cm; p=0.043). There was no significant difference in placental weight, placental diameter, and the mean number. of cotyledons between the two groups (p>0.05). The IVF group had an increased rate of velamentous cord insertion (20% vs. 2%; p=0.001) and marginal cord insertion (38% vs. 8%; p=0.001), calcification (74% vs. 54%, p=0.004), syncytial knot (44% vs. 38%; p=0.014), and fibrinoid necrosis (30% vs. 12%, p=0.027). Infarction and stromal fibrosis were also increased in IVF, but the difference was statistically in significant (p=0.052, 0.542 respectively).

Conclusions: The IVF group had a higher incidence of marginal and velamentous insertion of the umbilical cord, increased placental thickness, low BW, and a higher tendency toward early calcification, infarction, and fibrosis.

Introduction

The placenta is the most vital organ of the intrauterine life of an embryo. It is derived from decidua basalis and chorion frondosum (1). The placenta is unique among all organs in that it conducts the functional activities of most fetal organs from its early beginning throughout its development. The placenta is a substitute for immature embryonic and fetal organs (2). Pathological changes in the placenta adversely influence the fetal outcome. The placenta is a valuable resource for understanding prenatal experiences (3). The placenta serves as a direct interface between the maternal and fetal circulatory systems, and abnormalities in its structure or function can impact fetal well-being. Placental examination after delivery provides an opportunity to identify and understand the underlying causes of major obstetrical complications like maternal hypertension, fetal growth restriction (FGR), premature birth, and intrauterine death of the fetus and it is also useful in the clinical management of future pregnancies.

In vitro fertilization (IVF) is a widely used assisted reproductive technology that helps couples with fertility difficulties conceive a child. In the process of IVF, embryos can be transferred fresh immediately after culturing in the incubator or frozen/thawed, which are transferred later in an upcoming cycle (4). IVF is associated with an increased risk of placental abnormalities and placental-related complications. These complications include conditions such as placenta previa, placental abruption, and intrauterine growth restriction (5). Several studies have explored the potential association between IVF and placental abnormalities or complications (5-7). Advanced maternal age is a risk factor for various pregnancy complications, including those related to the placenta. Women of advanced age who have chosen to conceive with IVF because of reduced fertility may be at higher risk (7).

In IVF technology, the use of ovulation-induction drugs, in vitro embryo culture, and embryo freezing may influence the formation and function of the placenta, lead to structural abnormalities in placental villi and vascular changes, and eventually affect pregnancy outcomes. Increased blood hormone levels might alter the timing of endometrial receptivity, potentially leading to suboptimal embryo implantation and development (8). Assisted Reproductive Technologies (ART), including IVF, can potentially influence the epigenetic regulation of placental formation and function by changing the embryonic environment, placental gene expression, and placental adaptive response to embryonic development (9). Adequate utero-placental circulation is required for proper development of the placenta and fetus. Pathological changes in the fetal-placental circulation as a result of placental dysfunction are the main cause of FGR (10). Few authors have suggested that transferring more than one embryo may increase the risk of placental pathology and adverse obstetric outcomes, such as preterm birth and low birth weight (BW) (11,12). The purpose of this study was to compare the morphological and histological features of placentae obtained from normotensive IVF singleton pregnancies with those of normotensive natural pregnancies and evaluate their effects on fetal growth.

Methods

Study design

This cross-sectional study was conducted by collecting placentae from Indian pregnant women aged between 20-45 years who delivered in Milan Fertility Center and Birthing Hospital, Bangalore from June 2022 to December 2023. This study was approved by an IIRRH-BACC Healthcare Institutional Ethics Committee (project no: 90/P/22/03, date: 03.06.2022) and informed consent was obtained from the participants.

Inclusion and exclusion criteria

The study included placentae from both IVF and natural conception pregnant mothers with age groups between 20 and 45 years, who had normal and singleton pregnancy. Pregnant women with hypertension, diabetes mellitus, anemia, multiple pregnancies, or preterm pregnancies were excluded from the study.

Method of data collection

Clinical data of the mothers were collected from case records, which included their demographic parameters, obstetric and medical history, and laboratory investigations report, which included their blood sugar, urea, creatinine, hemoglobin, platelet, and liver enzyme values. The modes of delivery and weight of newborns are also recorded.

Placentae with umbilical cords were collected soon after delivery and washed thoroughly in running tap water to remove all blood clots. Abnormalities in the cord and membrane were noted. The placental weight was measured using a weighing machine graduated in grams. Placental diameter, thickness, number of cotyledons, site of umbilical cord insertion, presence of infarction, calcification, accessory lobes, and hematomas were noted.

Fresh placentae were preserved in 10% formalin for microscopic examination. Tissue sections were obtained from the center of the placenta and from different areas of the margin for histological studies. Placental Tissue sections were processed, fixed, and stained using hematoxylin, eosin, Masson's trichrome, and Van Giessen's Stain. Slides were examined under a compound microscope to assess placental villi, syncytial knot formation, fibrinoid necrosis, stromal fibrosis, calcification, infarction, and intervillous hemorrhage.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences 20 statistical software. Continuous

variables are presented as means with standard deviations. Variables in both groups were compared using the non-parametric Mann-Whitney U test. The non-normality of the distribution of our data was determined using the Shapiro-Wilk test. Categorical data are presented as frequencies with percentages and were compared using the Chi-square test. Fisher's exact test was used to compare individual variables with a cell size was <5. A p value 0.05 was considered statistically significant.

The sample size for this study was calculated using G*Power software version 3.1.5.1 (REF) with α=0.05, power (1-β)=0.8, and effect size (0.58). The estimated sample size was 48 participants for each group (13).

Results

The study included a total of 100 placentae, 50 of which were from IVF pregnancy and 50 from natural pregnancy (age, mean±standard deviation: 31.5±3.0 years, IVF pregnant mothers; 30.9±4.0 years, natural pregnant mothers; p=0.254). In the IVF group, the highest number of subjects were in the 31-35 years age group (56%) and in the natural pregnancy group, the highest number of subjects were in the 30 years. In the IVF group, 62% were primigravidae, 38% were multigravidas, and all women underwent cesarean section. In the spontaneous group, 62% of the samples were multigravidas, and 38% were primigravidae. The mean gestational age was shorter in the IVF group than in the natural pregnancy group. This difference was statistically significant (p=0.001) (Table 1).

Morphometric findings of placenta

The placental morphological features are presented in Tables 1 and 2. The mean placental thickness was significantly increased in the IVF group (p=0.043). No significant differences were observed in placental weight, placental diameter, and mean number of cotyledons between the two groups (p>0.05). The mean BW of babies in the IVF group was lower than that of natural pregnancy. This difference was statistically significant (p=0.001) (Table 1).

In the IVF group, a higher incidence of hematoma, accessory lobes, and bilobed placentae was observed compared with the natural pregnancy group. The placentae of the IVF group showed a higher incidence of velamentous and marginal insertion of the umbilical cord, whereas the incidence of eccentric attachment of the umbilical cord was higher (Figure 1, Table 2).

Histology of placental tissues

Histological features of placentae are presented in Table 3. In the IVF group, the rates of calcification, syncytial knots, and fibrinoid necrosis were significantly higher than those in the natural pregnancy group. Distal villous hypoplasia was observed in eight placentae (16%) of the IVF group. Other histological features like infarction and stromal fibrosis were also comparatively increased in the IVF group, but the difference was statistically insignificant (p>0.05) (Figure 2, Table 3).

Discussion

The placenta plays a crucial role in supporting fetal development, and any abnormalities in its formation and function can lead to various pregnancy complications, including preterm birth. Alterations in placentation in IVF pregnancies have been a subject of research, and some studies have provided evidence of differences compared with naturally conceived pregnancies. There are very few studies that have compared the morphological and histological differences between IVF conception and spontaneous conception of pregnancy placentae.

The first controlled study on placental morphology and histology in ART pregnancies was reported by Joy et al. (14) who analyzed a total of 89 placentae from the Royal Fertility Center, Belfast and the Royal Jubilee Maternity Service, Belfast. Out of the 89 placentae, 39 were from the spontaneous conception group, 17 were from the untreated infertility group, and 33 were from the ART (IVF and microinjection) group. This study reported significantly increased placental thickness and greater frequency of retroplacental or marginal hematoma in the ART group compared with the control and infertility groups. In our study, we also observed significantly increased placental thickness and a higher incidence of hematoma in the IVF group

Table 1. Comparison of morphological parameters between IVF and natural pregnancy

Parameters	IVF pregnancy	Natural pregnancy	*p value
	Mean±SD	Mean±SD	
Gestational age in weeks	37.0±1.3	38.1±0.9	**0.001**
Weight of placenta (g)	503.2±95.7	526.5±76.8	0.163
Diameter of placenta (cm)	16.5±1.6	17.1±1.9	0.151
Thickness of placenta (cm)	2.43±0.36	2.17±0.39	**0.043**
Number of cotyledons	13.5±3.7	14.6±3.1	0.197
Birth weight (kg)	2.7±0.5	3.0±0.5	**0.001**

*p value-Mann-Whitney U test, p<0.05 indicates significance.
IVF: In vitro fertilization, SD: Standard deviation

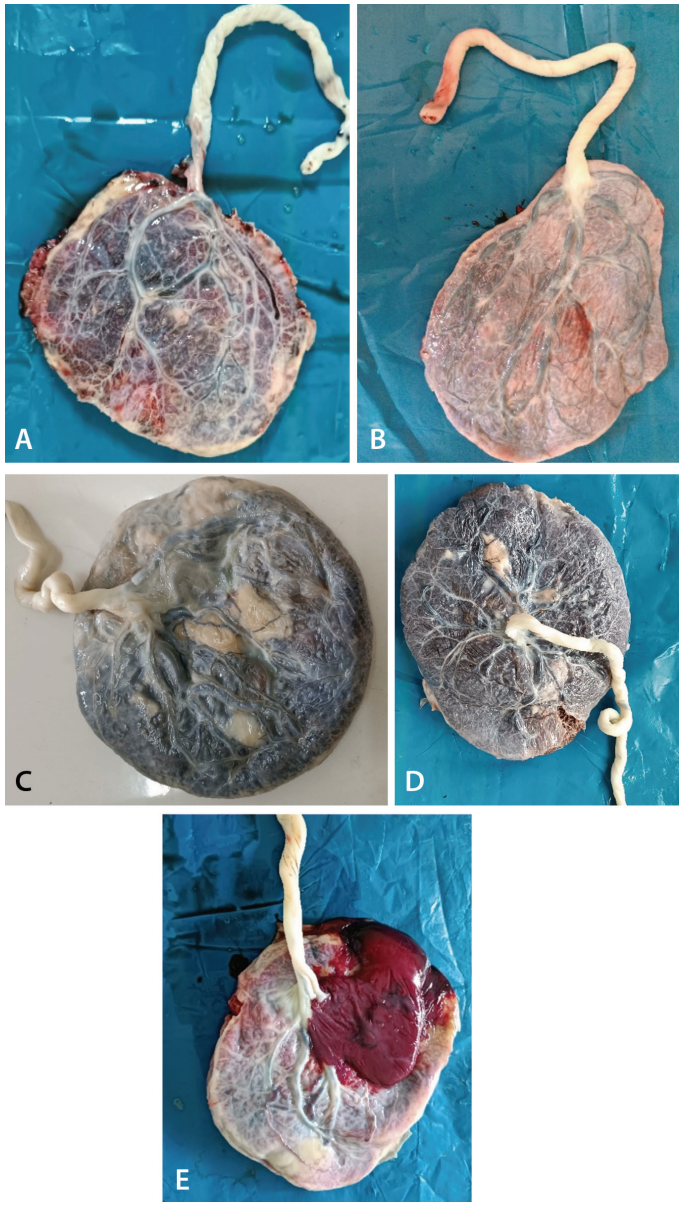

Figure 1. Placentae of IVF pregnancy showing attachment of the umbilical cord. A) Vestal, B) marginal, C) eccentric, D) central, and E) placenta with hematoma

IVF: In vitro fertilization

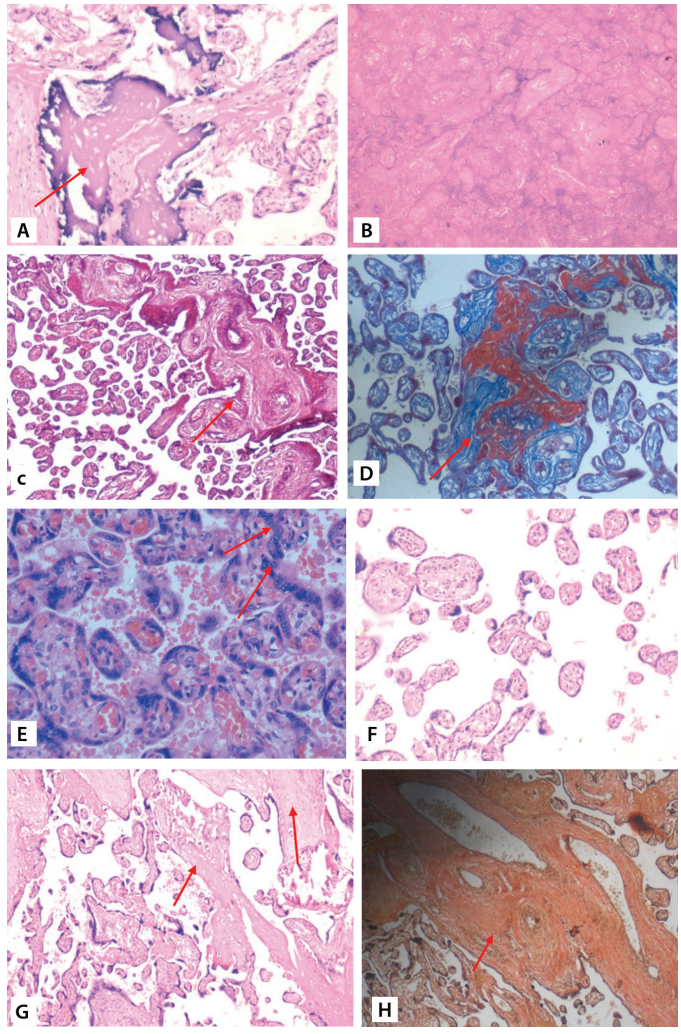

Figure 2. Histological findings of IVF pregnancy placentae. A) Calcification (H&E stain, 20x), B) infarction (H&E stain, 10x), C) stromal fibrosis (H&E stain, 10x), D) Masson's trichrome (20x), E) syncytial knotting (H&E stain, 20x), F) distal villous hypoplasia (H&E stain, 10x), G) fibrinoid necrosis of villi (H&E stain, 10x), H) Van Giessen's stain (20x)

IVF: In vitro fertilization

Table 2. Attachment of umbilical cord and gross features of placenta

	IVF pregnancy	Natural pregnancy	p value
Attachment of the cord, n (%)			
Central	3 (6)	8 (16)	0.001
Eccentric	18 (36)	38 (76)	
Marginal	19 (38)	4 (8)	
Velamentous	10 (20)	1 (2)	
Haematoma, n (%)	11 (22)	5 (10)	0.102
Accessory lobes, n (%)	4 (8)	3 (6)	1.00
Bilobed placenta, n (%)	3 (6)	3 (6)	1.00

IVF: In vitro fertilization

Table 3. Placental histology

Histological findings	IVF pregnancy	Natural pregnancy	Chi-square	Df	p value
Calcification, n (%)	37 (74)	23 (46)	8.167	1	**0.004**
Infarction, n (%)	21 (42)	12 (24)	3.664	1	0.056
Stromal fibrosis, n (%)	26 (52)	14 (28)	0.372	1	0.542
Increased syncytial knot, n (%)	22 (44)	19 (38)	6.000	1	**0.014**
Fibrinoid necrosis, n (%)	15 (30)	6 (12)	4.882	1	**0.027**
DVH, n (%)	8 (16)	-			

IVF: In vitro fertilization, DVH: Distal villous hypoplasia

compared with the natural pregnancy group. Increased placental thickness is strongly associated with potentially serious maternal and neonatal complications (15).

In the present study, there were no significant differences in mean placental weight, number of cotyledons, and placental diameter between the IVF and natural pregnancy group (p>0.05). Studies by Jauniaux et al. (16), Gavriil et al. (17), and Yanaihara et al. (18) reported no significant difference in placental weight between singleton ART and natural pregnancies. Daniel et al. (19) and Haavaldsen et al. (20) reported larger placentas and a higher placental weight/birthweight ratio among pregnancies conceived by ART compared with spontaneous pregnancies. Many studies have reported a higher incidence of velamentous insertion of the umbilical cord in IVF pregnancy (18-23). In the present study, we observed a higher incidence of marginal and velamentous insertion in the IVF group than in the natural pregnancy group. Velamentous cord insertion is an abnormal insertion of the umbilical cord that occurs when the umbilical vessels migrate between the placental membranes before reaching the placental mass. This abnormal insertion is associated with adverse pregnancy outcomes, including preterm delivery, and FGR (24). The exact cause of increased velamentous insertion in IVF pregnancy is unknown, but malrotation of the blastocyst during the implantation process in IVF may be the cause of placenta previa and velamentous insertion of the cord (25). In the present study, the mean BW of the babies was lower in the IVF group than in the natural pregnancy group. Similar findings were reported by Pandey et al. (26) and Szymusik et al. (27). A major risk factor for adverse perinatal outcomes in ART singleton pregnancy is subfertility, and hormones related to stimulation and IVF procedures may also be the cause for it (28). Insufficient transfer of nutrients from the placenta to the fetus may result in decreased BW and a larger placenta (29).

Herman et al. (30) reported that the placental weight and rates of maternal vascular malperfusion (MVM) and fetal vascular malperfusion (FVM) lesions were similar between the groups. They also found villitis of unknown etiology in the IVF group. In our study, histological examination of placentae showed a higher incidence of infarction, calcification, syncytial knotting, fibrosis, and distal villous hypoplasia in the IVF group than in the natural pregnancy group (Figure 2). Early calcification can be a pathological change resulting from the effects of environmental factors on the placenta. Calcifications may indicate placental tissue exposure to hypoxia. Preterm placental calcification and infarction are associated with a higher incidence of poor uteroplacental blood flow, fetal growth, and perinatal death (31). Increased fibrosis and syncytial knot formation in placental villi indicate hormonal factors that may alter placental morphometry (32). Sacha et al. (33) observed more frequent vascular pathology in patients with ART pregnancies, and the frequencies of MVM and FVM were similar between the groups. In ART, the endometrium is exposed to high levels of estrogen and progesterone, and external manipulation of gametes may affect implanting and placentation. Thus, the alteration of utero placentation circulation or disruption of the metabolism of the placenta could be the cause of the anatomical and histological changes observed in the IVF group (34).

The limitations of the study include the small sample size and variables like maternal age, parity, gestational weeks at the time of delivery, and mode of delivery were not matched between the two groups. Factors such as the causes of infertility, specific IVF procedures and medications used, and whether embryos were transferred fresh or frozen are all important variables that might influence outcomes but were not accounted for in the sampling process.

Conclusion

In the present study, placentae from IVF pregnancy showed a higher incidence of marginal and velamentous insertion of the umbilical cord, increased placental thickness, greater incidence of hematomas, and low BW. Histological examination of IVF placentae showed a higher rate of early calcification, syncytial knots and fibrinoid necrosis of villi. Infarction and stromal fibrosis were also increased in the placenta of IVF recipients, but the difference was not statistically significant. Therefore, further exploration is required with a greater number of samples. This study lays the pioneering cornerstone for future pathological studies on eclampsia in patients undergoing IVF given the increasing number and complications of IVF pregnancies.

Ethics

Ethics Committee Approval: This study was approved by an IIRRH-BACC Healthcare Institutional Ethics Committee (project no: 90/P/22/03, date: 03.06.2022).

Informed Consent: Informed consent was obtained from the participants.

Footnotes

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Saddler TW: Langmans Medical Embryology. 9th ed. Philadelphia: Lippincott Williams and Wilkins Company; 2004: 51-134.
2. Benirschike K, Kaufmann P, Baergen R. Pathology of the human placenta. 5th ed. New York: Springer;2006.
3. Benirshke K. The Placenta: How to examine it and what you can learn. Contemp Obstet Gynaecol. 1981;17:117-119.
4. Sunderam S, Kissin DM, Crawford SB, et al. Assisted Reproductive Technology Surveillance - United States, 2015. MMWR Surveill Summ. 2018;67:1-28.
5. Volodarsky-Perel A, Ton Nu TN, Buckett W, et al. Effect of embryo stage at transfer on placental histopathology features in singleton live births resulting from fresh embryo transfers. Fertil Steril. 2021;115:673-682.
6. Larcher L, Jauniaux E, Lenzi J et al. Abnormalities of the placenta in in vitro fertilisation pregnancies: a prospective controlled study. Ultrasound Obstet Gynecol. 2021;58:5.
7. Kong F, Fu Y, Shi H, et al. Placental Abnormalities and Placenta-Related Complications Following In-Vitro Fertilization: Based on National Hospitalized Data in China. Front Endocrinol (Lausanne). 2022;13:924070.
8. Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. Hum Reprod Update. 2003;9:515-522.
9. Choux C, Carmignac V, Bruno C, Sagot P, Vaiman D, Fauque P. The placenta: phenotypic and epigenetic modifications induced by Assisted Reproductive Technologies throughout pregnancy. Clin Epigenetics. 2015;7:87.
10. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. Am J Obstet Gynecol. 2018;218:S745-S761.
11. Harris AL, Sacha CR, Basnet KM, et al. Vanishing Twins Conceived Through Fresh In Vitro Fertilization: Obstetric Outcomes and Placental Pathology. Obstet Gynecol. 2020;135:1426-1433.
12. Luke B, Stern JE, Kotelchuck M, et al. Adverse pregnancy outcomes after in vitro fertilization: effect of number of embryos transferred and plurality at conception. Fertil Steril. 2015;104:79-86.
13. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-191.
14. Joy J, Gannon C, McClure N, Cooke I. Is assisted reproduction associated with abnormal placentation? Pediatr Dev Pathol. 2012;15:306-314.
15. Elchalal U, Ezra Y, Levi Y, Bar-Oz B, Yanai N, Intrator O, Nadjari M. Sonographically thick placenta: a marker for increased perinatal risk--a prospective cross-sectional study. Placenta. 2000;21:268-272.
16. Jauniaux E, Englert Y, Vanesse M, Hiden M, Wilkin P. Pathologic features of placentas from singleton pregnancies obtained by in vitro fertilization and embryo transfer. Obstet Gynecol. 1990;76:61-64.
17. Gavriil P, Jauniaux E, Leroy F. Pathologic examination of placentas from singleton and twin pregnancies obtained after in vitro fertilization and embryo transfer. Pediatr Pathol. 1993;13:453-462.
18. Yanaihara A, Hatakeyama S, Ohgi S, et al. Difference in the size of the placenta and umbilical cord between women with natural pregnancy and those with IVF pregnancy. J Assist Reprod Genet. 2018;35:431-434.
19. Daniel Y, Schreiber L, Geva E, Lessing JB, Bar-Am A, Amit A. Morphologic and histopathologic characteristics of placentas from twin pregnancies spontaneously conceived and from reduced and nonreduced assisted reproductive technologies. J Reprod Med. 2001;46:735-742.
20. Haavaldsen C, Tanbo T, Eskild A. Placental weight in singleton pregnancies with and without assisted reproductive technology: a population study of 536,567 pregnancies. Hum Reprod. 2012;27:576-582.
21. Cochrane E, Pando C, Kirschen GW, Soucier D, Fuchs A, Garry DJ. Assisted reproductive technologies (ART) and placental abnormalities. J Perinat Med. 2020;48:825-828.
22. Schachter M, Tovbin Y, Arieli S, Friedler S, Ron-El R, Sherman D. In vitro fertilization is a risk factor for vasa previa. Fertil Steril. 2002;78:642-643.
23. Matsuzaki S, Ueda Y, Matsuzaki S, et al. Assisted Reproductive Technique and Abnormal Cord Insertion: A Systematic Review and Meta-Analysis. Biomedicines. 2022;10:1722.
24. de Los Reyes S, Henderson J, Eke AC. A systematic review and meta-analysis of velamentous cord insertion among singleton pregnancies and the risk of preterm delivery. Int J Gynaecol Obstet. 2018;142:9-14.
25. Zhu L, Zhang Y, Liu Y, et al. Maternal and Live-birth Outcomes of Pregnancies following Assisted Reproductive Technology: A Retrospective Cohort Study. Sci Rep. 2016;6:35141.
26. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012;18:485-503.
27. Szymusik I, Kosinska-Kaczynska K, Krowicka M, Sep M, Marianowski P, Wielgos M. Perinatal outcome of in vitro

fertilization singletons - 10 years' experience of one center. Arch Med Sci. 2019;15:666-672.

28. Pinborg A, Wennerholm UB, Romundstad LB, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19:87-104.
29. Hayward CE, Lean S, Sibley CP, et al. Placental Adaptation: What Can We Learn from Birthweight:Placental Weight Ratio? Front Physiol. 2016;7:28.
30. Herman HG, Tamayev L, Feldstein O, et al. Placental-related disorders of pregnancy and IVF: does placental histological examination explain the excess risk? Reprod Biomed Online. 2020;41:81-87.
31. Puri M, Soni S, Gaikwad V, Mann M. Study of the calcified placenta and its correlation with feto-maternal outcome. Int J Appl Res. 2017;3:616-619.
32. Ranga M.K.SS. Thangam T.F.A, M.C., Mallika V, Indira M.V. Morphological and Histological variations of Human placenta in hypertensive disorders of pregnancy. Int J Anat Res. 2017;5:3591-3598.
33. Sacha CR, Mortimer RM, James K, et al. Placental pathology of term singleton live births conceived with fresh embryo transfer compared with those conceived without assisted reproductive technology. Fertil Steril. 2022;117:758-768.
34. Belak U, Pinter B, Ban Frangež H, Velikonja M, Korošec S. Pathology of the Placenta in Singletons after Assisted Reproductive Technology Compared to Singletons after Spontaneous Conception: A Systematic Review. Fetal Pediatr Pathol. 2023;42:438-449.

DOI: 10.4274/gulhane.galenos.2024.16046
Gulhane Med J 2024;66(4):203-210

Understanding the role of perfectionism in adult expectations of dietary restriction and thinness

Emine Merve Ekici[1], Zeyneb Yıldırım[2], Sibel Erdem[3]

[1]University of Health Sciences Türkiye, Gülhane Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Türkiye
[2]Ankara Medipol University, Institute of Health Sciences, Department of Nutrition and Dietetics, Ankara, Türkiye
[3]Muğla Sıtkı Koçman University, Fethiye Faculty of Health Sciences, Department of Nutrition and Dietetics, Muğla, Türkiye

Cite this article as: Ekici EM, Yıldırım Z, Erdem S. Understanding the role of perfectionism in adult expectations of dietary restriction and thinness. Gulhane Med J. 2024;66(4):203-210

Date submitted:
24.04.2024
Date accepted:
24.07.2024
Online publication date:
22.11.2024

Corresponding Author:
Emine Merve Ekici, M.D., University of Health Sciences Türkiye, Gülhane Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Türkiye
eminemerve.ekici@sbu.edu.tr
ORCID:
orcid.org/0000-0001-5409-6309

Keywords: Perfectionism, eating disorders, thinness, dietary restriction

ABSTRACT

Aims: This study aimed to evaluate the relationship between perfectionism and expectations of thinness and dietary restriction in adults.

Methods: This descriptive and cross-sectional study was conducted with adults 18-65 years old. Demographic characteristics and anthropometric measurements were questioned using a questionnaire form. Perfectionism characteristics of individuals were evaluated with "Frost Multidimensional Perfectionism Scale (FMPS)" developed to make a multidimensional assessment of perfectionism, and the findings regarding their expectations from thinness and food restriction were evaluated with the "Thinness and Restricting Expectancy Inventory (TREI) Scale". Data collection was facilitated using a web-based questionnaire. Those who were not in the appropriate age range and did not tick the "I consent to participate in this study voluntarily" tab were excluded from the study.

Results: The study population was 1,376 adults (mean age: 27.42±10.99 years; 74.4% female). The TREI total score showed a weak positive correlation with the FMPS total score ($r=0.351$, $p<0.001$), and body mass index (BMI) ($r=0.288$, $p<0.001$). In multiple regression analysis, BMI, marital status, and TREI score showed an independent association with the FMPS total score. Male sex, TREI score, fear of making mistakes, and dieting showed independent associations with BMI.

Conclusions: Perfectionism is associated with body dissatisfaction, BMI, expectations regarding thinness, and dietary restriction. It is considered an influential personality trait for identifying the risk of developing eating disorders and assessing the treatment process in the future.

Introduction

Perfectionism is characterized by striving for flawlessness, setting unattainably high standards, engaging in harsh self-criticism, and fearing negative evaluations from others (1). It is assessed both unidimensionally and multidimensionally (2). In the unidimensional perspective of perfectionism, individuals tend to focus more on themselves. They set unreachable criteria, engage in excessive self-criticism, and evaluate their worth solely in terms of productivity and success (3). In multidimensional perfectionism, Frost et al. (2) and Hewitt and Flett (4) analyses revealed evaluations of both self and social relationships. Frost et al. (2) highlights not only individuals' high standards regarding performance but also their excessive concern about mistakes when evaluating perfectionism and its subdimensions. Hewitt and Flett (4), on the other hand, specified that perfectionism comprises three components: self-oriented perfectionism (setting high standards for oneself), other-oriented perfectionism (setting high standards for others), and socially prescribed perfectionism (others setting high standards for oneself). These dimensions can be adaptive or maladaptive (2). Adaptive perfectionism is associated with success and leads to positive health indicators, such as psychological well-being, self-efficacy, perceived social support, coping, lower vulnerability, and fewer self-defeating behaviors. On the other hand, maladaptive perfectionism is linked to excessive stress and hypersensitivity to health problems, resulting in negative outcomes such as eating disorders, depression, and anxiety (5,6). Eating disorders, which are associated with increased depression and anxiety, significantly impact quality of life, well-being, and health (7).

Dietary restriction is defined as the deliberate limitation of the total amount of food consumed, the types of food consumed, or both, which are often motivated by a desire to reduce body weight or maintain a specific body shape (8). It is known that some individuals engage in behaviors such as restrictive eating, emotional eating, binge eating, adhering to very low-calorie diets, and using laxatives for purging (9,10). Individuals excessively restrict their diets to control their body weight and body shape due to the individual's evaluation of their worth based on eating, body weight, and body shape. Individuals engage in very low-energy dieting to transform their bodies into the "valued" shape, which can lead to subsequent psychopathological binge eating or starvation syndrome (11). In this context, perfectionism is considered a strong risk factor for the development and continuation of eating disorders due to eating restriction (12).

Perfectionistic individuals often focus on body flaws, leading to dissatisfaction with body image and the development of unhealthy eating behaviors. Fear of failure in perfectionistic concerns can lead to overeating or weight gain. This situation contributes to negative self-criticism/evaluation and a more determined effort to strive for new goals, thus impacting eating disorders (13). Perfectionism is a risk factor for eating disorders (3). Individuals with eating disorders have rigid and restrictive individual eating rules. Individuals who rigidly adhere to dietary rules regarding what, when, and how foods should be eaten may develop eating disorders (14). Due to excessive concern about overeating, they avoid unhealthy foods, deprive themselves of certain foods, and restrict food intake by consuming only what they consider safe. This can lead to physiological deprivation and cravings for binge eating (4).

The importance of further investigating the relationship between perfectionism and dietary restriction to understand how individuals' tendencies toward perfectionism can be associated with body image and food restrictions, as well as in promoting nutrition awareness. Additionally, from a psychological assessment perspective, this approach will contribute to increasing awareness among individuals who have or are at risk of eating disorders. There are only a limited number of studies demonstrating the relationships between perfectionism, thinness perception, and dietary restrictions among adult individuals from various cultures. There is a need for more research on how these relationships vary across cultural contexts.

This study aims to understand the relationships among perfectionism, thinness, and eating habits among individuals and to investigate how these relationships impact them. Thus, this study examines how expectations regarding perfectionism and food restrictions influence each other, the effects of this interaction on adult individuals, and their emotional states.

Methods

Participants and procedure

This cross-sectional, descriptive study was planned to be conducted between October 2023 and February 2024 and aimed to include individuals aged 18-65 years, which is the adult age range. The "Snowball Sampling Method" was used to determine the sample created by the researchers through Google Forms, and individuals were reached via social media. Data collection was facilitated using a web-based questionnaire. The study sample comprised individuals who actively consented to participate by selecting the designated option at the onset of the questionnaire and subsequently completed it in its entirety. Those who were not in the appropriate age range and did not tick the "I consent to participate in this study voluntarily" tab were excluded from the study.

Assessments

Sociodemographic data form: Demographic variables (gender, age, educational attainment, income level) and anthropometric measures (body weight and height) were assessed using a questionnaire. Among the information about the general characteristics of individuals, such as their educational status, how they perceived themselves in terms of

appearance, and their food preferences were also questioned. Additionally, individuals' perfectionism traits were measured using the "Frost Multidimensional Perfectionism Scale (FMPS)", designed for a comprehensive evaluation of perfectionism, while their attitudes toward thinness and food restriction were gauged using the "Thinness and Restricting Expectancy Inventory (TREI) Scale".

Anthropometric measurements: Anthropometric measurements (body weight, height, and waist circumference) were self-reported by participants. Detailed instructions on how to perform these measurements were provided in the questionnaire. Body mass index (BMI) was calculated by dividing body weight (in kilograms) by the square of height (in meters). BMI values below 18.50 kg/m^2 were categorized as underweight, between 18.50-24.99 kg/m^2 as normal, between 25.0-29.99 kg/m^2 as overweight, and above 30.0 kg/m^2 as obese (15).

Frost Multidimensional Perfectionism Scale: The FMPS was developed by Frost et al. (2) to assess perfectionism in a multidimensional manner. This scale consists of 35 items distributed among 6 subscales, rated from 1 (strongly agree) to 5 (strongly disagree). The subscales of the scale are personal standards (7 items) and organization (6 items), concern over mistakes (9 items), parental expectations (5 items), parental criticism (4 items), and doubts about actions (4 items). Internal consistency in the current study was acceptable for both factors (α=0.86 and 0.91, respectively). The reliability and validity of the scale in Turkey was conducted by Kağan (16). Each item in the scale is a five-point Likert-type measurement scored between 1 and 5. There are no reverse-scored items in the scale. Evaluation is performed by adding the scores of the seven items. The scores obtained from the scale range from 1 to 175. Evaluation was performed according to the total scores of the measurement tool, with an increase in total scores indicating an increase in perfectionism.

Thinness and Restricting Expectancy Inventory Scale: The TREI scale was developed by Hohlstein et al. (17) on the basis of expectancy learning theory. The reliability and validity study of the scale in Turkey was conducted by Sapmaz Yurtsever and Tekinsav Sutcu (18). This unidimensional scale provides information about an overgeneralized expectation of goodness for thinness and dieting. The 44-item scale, which includes items such as "I feel like I could get some things more easily if I were thin" and "Others will care more about me when I limit what I eat", is answered on a 7-point scale. As the scale score increased, the desire to be thin and eating restriction behaviors also increased (18).

Ethics

Ethical approval for the study was obtained prior to commencement and was granted by the University of Health Sciences Türkiye, Gülhane Scientific Research Ethics Committee under decision number: 2023-333, date: 26.09.2023. All procedures in this study adhered to the principles outlined in the Declaration of Helsinki.

Statistical Analysis

The data analysis in this study was conducted using Statistical Package for Social Sciences 27.0 software. The normality distribution was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics are presented as frequences (n), percentages, and median lower and upper values. For comparisons between two independent groups, non-parametric tests such as the Mann-Whitney U test for numerical/quantitative data and the Pearson Chi-square test for qualitative data were employed. Spearman's rank correlation analysis, a non-parametric method, was used to examine the relationships between numerical variables. Regression analysis was used to predict total FMPS. Logarithmic transformation was applied to non-normally distributed variables prior to linear regression analysis to approximate normality. Statistical significance was determined at $p<0.05$.

Results

Descriptive findings

This descriptive and cross-sectional study included 1,376 adults (1,018 female, 358 male) aged 18-65 years. The average age of the participants was 27.42±10.99 years and 70.1% were single. A significant proportion of participants (73.2%) held university degrees. Among the participants, men preferred protein foods more than women, whereas women preferred carbohydrate foods more ($p<0.05$). The average BMI of the participants was 23.1 (15.4-40.2) kg/m^2. Regarding BMI classification, 60.2% of participants were classified as having normal weight, 23.0% were overweight, and 8.2% were obese. The mean total FMPS score was 102.0 (39-170), whereas the mean total TREI score was 2.77 (1-7). Although the mean FMPS total score did not differ according to sex, the mean TREI total score was higher among females. The general characteristics of the participants are presented in Table 1.

Relationship between FMPS, TREI Scale, and BMI

The simple correlations between the FMPS, TREI, and BMI are presented in Table 2.

Predictors of multidimensional frost perfectionism

The linear regression analysis results of the prediction of multidimensional perfectionism are presented in Table 3. The linear regression model created to evaluate the factors associated with FMPS total score was significant ($R2=0.212$; $p<0.001$). BMI, marital status, and TREI score were significantly associated with the FMPS total score ($p<0.001$), whereas sex was not.

Table 1. General characteristics of individuals

	Gender						
	Female		Male		Total		Statistical analysis*
Variables	**n**	**%**	**n**	**%**	**n**	**%**	
Gender	1018	74.0	358	26.0	1376	100.0	-
Marital status							
Single	714	70.1	251	70.1	965	70.1	p=0.993
Married	304	29.9	107	29.9	411	29.9	
Education level							
Primary school	35	3.4	4	1.1	39	2.8	
Middle school	18	1.8	13	3.6	31	2.3	**p<0.001***
High school	166	16.3	82	22.9	248	18.0	
University	745	73.2	234	65.4	979	71.1	
Master's degree/doctorate	54	5.3	25	7.0	79	5.7	
Food preference							
Fatty foods (e.g. fried foods, fatty meats, fatty dishes, margarine and butter on bread, etc.)	125	12.3	69	19.3	194	14.1	
Carbohydrate foods (e.g. bread, rice, pasta, sweets, etc.)	450	44.4	100	27.9	550	40.0	**p<0.001***
Protein foods (e.g. meat/chicken/fish/eggs)	256	25.1	173	48.3	429	31.2	
Vegetable-based foods (dark green leafy vegetables, root vegetables)	187	18.4	16	4.5	203	14.8	
Assessing body weight							
Underweight	115	11.3	44	12.3	159	11.6	
Normal	492	48.3	159	44.4	651	47.3	**p=0.164**
Overweight	322	31.6	131	36.6	453	32.9	
Obese	89	8.8	24	6.7	113	8.2	
BMI classification							
Underweight (<18.50 kg/m^2)	112	11.0	6	1.7	118	8.6	
Normal (18.50-24.99 kg/m^2)	625	61.4	203	56.7	828	60.2	**p<0.001***
Overweight (25.00-29.99 kg/m^2)	199	19.5	118	33.0	317	23.0	
Obese (≥30.0 kg/m^2)	82	8.1	31	8.7	113	8.2	
Past weight loss diet history							
Yes	489	48.5	99	27.7	588	43.0	**p<0.001**
	Median (min-max)		**Median (min-max)**		**Median (min-max)**		
Age (years)	22.00 (18-65)		28.41 (18-65)		27.45 (18-65)		**Z=-1.314 p=0.189**
BMI (kg/m^2)	22.37 (15.4-38.2)		24.41 (16.1-40.2)		23.1 (15.4-40.2)		**Z=8.795 p≤0.001***
Total FMPS score	101.0 (39-170)		103.0 (48-162)		102.0 (39-170)		**Z=-0.632 p=0.528**
Total TREI score	2.85 (1-7)		2.54 (1-6.8)		2.77 (1-7)		**Z=-3.408 p<0.001***

BMI: Body mass index, FMPS: Frost Multidimensional Perfectionism Scale, TREI: Thinness and Restricting Expectancy Inventory Scale, Z: Mann-Whitney U test, min-max: Minimum-maximum, Chi-square test, *p<0.05

Table 2. Correlation coefficients between scale scores and BMI

		1	2	3	4	5	6	7	8	9
1. TREI total score	**r**	**-**								
	p	-								
2. FMPS total score	**r**	0.351	-							
	p	**<0.001**								
3. CM	**r**	0.389	0.840	-						
	p	**<0.001**	**0.000**							
4. PS	**r**	0.195	0.746	0.533	-					
	p	**<0.001**	**<0.001**	**<0.001**						
5. PE	**r**	0.230**	0.699	0.489	0.447**	-				
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**					
6. PC	**r**	0.326**	0.639**	0.548**	0.267**	0.587**	-			
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				
7. D	**r**	0.302**	0.643**	0.615**	0.344**	0.324**	0.449**	-		
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**			
8. O	**r**	-0.018	0.263**	-0.027	0.313**	0.032	0.155**	-0.044	-	
	p	0.505	**<0.001**	0.322	**<0.001**	0.233	**<0.001**	0.105		
9. BMI	**r**	0.288**	-0.033	-0.068*	-0.019	-0.008	0.048	-0.051	0.007	-
	p	**<0.001**	0.217	**0.012**	0.490	0.774	0.076	0.058	0.790	

Spearman's correlation analysis was used to calculate the p value.
BMI: Body mass index, CM: Concern over mistakes, D: Doubts about actions, FMPS: Frost Multidimensional Perfectionism Scale, O: Organization, PC: Parental criticism, PE: Parental expectations, PS: Personal standards, TREI: Thinness and Restricting Expectancy Inventory Scale

Table 3. Multiple linear regression analysis of the predictors of Frost's Multidimensional Perfectionism Level

	Frost Multidimensional Perfectionism Total Score				
Model	***Beta***	**t**	**p**	**95% confidence interval**	
Gender	0.056	2.255	**0.024**	0.320	4.599
BMI	-0.102	-3.640	**<0.001**	-0.707	-0.212
Marital status	0.172	6.604	**<0.001**	5.098	9.407
TREI score	0.448	17.648	**<0.001**	0.158	0.197
		R^2=0.212; **p<0.001***			

*Significant at p value <0.05.
BMI: Body mass index, TREI: Thinness and Restricting Expectancy Inventory Scale

Predictors of BMI

The linear regression analyses for the prediction of BMI are presented in Table 4. The linear regression model created to evaluate the factors associated with BMI was significant (R2=0.243; p<0.001). Sex, TREI score, concerns over mistakes score, and dieting status were significantly associated with the BMI (p<0.001), whereas FMPS total score was not.

Discussion

Perfectionism was observed to be associated with body dissatisfaction, BMI, expectations regarding thinness, and dietary restriction. The main findings of the study were as follows: The level of expectations related to being thin and restricting eating was positively related to the FMPS total score and all subcategories. Increasing BMI is associated with increased thinness and an increased level of expectations from restricting eating. The main factors affecting FMPS levels were BMI, marital status, gender, and TREI.

Perfectionism is a multidimensional concept that encompasses both maladaptive and adaptive characteristics (19). Perfectionism in terms of physical appearance is viewed as a feature associated with body dissatisfaction and eating behavior disorder (20). Expectations that shape eating and non-eating behaviors can lead to eating behavior disorders (21). Body dissatisfaction is a major risk factor for eating behavior disorder (22) and is considered a normative concern (23). Some studies

Table 4. Multiple linear regression analysis of the predictors of BMI

	BMI level				
Model	***Beta***	**t**	**p**	**95% confidence interval**	
Gender	0.272	11.295	**<0.001**	2.172	3.086
TREI score	0.301	10.874	**<0.001**	0.022	0.031
FMPS score	0.017	0.347	0.729	-0.017	0024
Concerns regarding misusing	-0.213	-4.398	**<0.001**	-0.177	-0.068
Dieting status	-0.286	-11.275	**<0.001**	-2.885	-2.030
		R^2=0.243; **p<0.001***			

*Significant at p value <0.05.
BMI: Body mass index, FMPS: Frost Multidimensional Perfectionism Scale, TREI: Thinness and Restricting Expectancy Inventory Scale

have suggested the importance of perfectionism and affect in the psychopathology of eating behavior disorder (24,25).

Studies conducted to date have demonstrated the influential role of personality traits such as perfectionism, obsessiveness, and impulsivity, along with early life experiences such as abuse, traumatic events, and adverse family environments, in the development and perpetuation of eating disorders. In addition to these effects, expectations about "eating" have been suggested as a factor leading to disordered eating behaviors (17,25,26). This view was proposed within the framework of expectancy learning theory, which explains the mechanisms of human behavior (17,27). Accordingly, people increase or restrict their eating behavior to meet many expectations, such as relaxation, alleviation of negative emotions, feeling more secure, and being more attractive. Research has shown that expectations about eating, thinness, and restriction evolve into eating behavior disorders. It has also been shown that these expectations can determine the treatment of eating behavior disorders, and it is possible to prevent eating behavior disorders and evaluate the treatment process during the treatment phase with a good understanding of expectations from eating and thinness (17,28,29).

According to the study gender is closely related to body satisfaction and TREI. Sex, maladaptive perfectionism, and psychological well-being are risk factors for body dissatisfaction (30). One study found that than non-dieters, dieting women had higher reward expectations for thinness (31). In this study, although the average FMPS total score did not differ by gender, the average TREI total score and tendency to diet were higher in women. At the same time, although 61.4% of the women were in the normal range according to the BMI classification, 48.3% stated that they perceived themselves as having normal weight, and most of the difference was due to the fact that they perceived themselves as overweight. This suggests that body dissatisfaction is higher in women. In a study conducted with women aged between 28 and 40 years, the relationship between different dimensions of perfectionism and body dissatisfaction was confirmed (22). In this study, although the TREI score affected the state of perfectionism, the average TREI score showed a positive relationship with the FMPS and FMPS subdimensions of worry about making mistakes, personal standards, familial expectations, criticism from the family, and not being sure of what one is doing.

Perfectionist evaluation concerns and distrust of body sensations (i.e., not feeling safe in one's own body; are key components in the relationship between perfectionism, intrapersonal sensitivity, and eating symptomatology (32). Personality traits are associated with body dissatisfaction in both genders. Personality traits influence how a person perceives external pressure to meet social ideals, which can lead to body image concerns (22). Perfectionism behavior increases the individual's dissatisfaction with body image and tendency to develop unhealthy behaviors by intensifying perceptions of certain body-related flaws (33). The fact that people with high perfectionism have high standards of body image increases their body image dissatisfaction scores by focusing on the difference between the ideal and the real body (34).

There are studies in the literature that examined the relationship between anorexia nervosa and perfectionism (35,36). The results obtained in a study indicate that the prevalence of anorexia nervosa among female students is 0.2%, and the importance of insecure attachment styles, perfectionism, and body shape anxiety as risk factors are pointed out (37). In the study by Świerczyńska (35), the anorectic group had the highest FMPS score. As the tendency toward body weight loss increases, individuals' concerns about their own mistakes and doubts about their actions also escalate (38). The likelihood of engaging in compensatory behaviors related to restriction (such as fasting, intense exercise) is associated with perfectionism, including high standards. However, binge eating, laxative misuse, and purging behaviors are not associated with these standards; instead, they are associated with impulsivity (39). This study showed that there was a significant positive correlation between the FMPS and TREI scores.

Unsuccessful weight management often leads to a weight cycle characterized by repeated weight loss and

unintentional weight gain. Individuals with a higher weight cycle, independent of age, gender, BMI, and weight loss attempts, have higher expectations of thinness (40). Although it has been shown that emotional or behavioral disorder (EBD) symptoms, especially restrictive eating and body control, increase with perfectionism, individuals with low perfectionism have been shown to be the group that exhibits the most appropriate functioning in terms of EBD pathology and affective symptoms (depression, anxiety, negative affect) (36). The results of this study are consistent with the existing literature. The level of expectations regarding thinness and dietary restriction showed a positive relationship with the total FMPS score and all its subcategories. As individuals' expectations of thinness increase, their perfectionism levels also increase. In addition, we concluded that increasing BMI was associated with increasing expectations regarding thinness and dietary restriction. According to the results of the regression analysis conducted in this study; BMI, marital status, and TREI score significantly influenced the FMPS total score. Additionally, factors such as gender, TREI score, concern over mistakes score, and dieting status were found to significantly impact BMI.This study evaluated the relationship between perfectionism and thinness expectations and dietary restriction in a large sample. Perfectionism; It is related to thinness expectations and body satisfaction. Personality characteristics may be effective in determining the risk of developing an eating disorder and evaluating the treatment process in the future. Accordingly, this study discussed the relationship between perfectionism, thinness expectations, and dietary restriction in detail. The findings of this study may serve as a guide for future studies examining the relationships between personality traits and eating disorders.

The findings of this study should be evaluated in light of some limitations. Since the data in this study were collected based on personal statements via the survey method, there may have been errors. The cross-sectional design of the study precludes the establishment of a causal relationship between perfectionism and thinness expectations and dietary restriction. The study was conducted with a limited sample; more studies should be conducted with larger samples to generalize the findings to the general population.

Conclusion

In conclusion, perfectionism has been observed to be associated with body dissatisfaction, BMI, expectations regarding thinness, and dietary restriction. It is considered an influential personality trait for identifying the risk of developing eating disorders and assessing the treatment process in the future. In this context, it is thought that it would be useful to consider and evaluate the concept of perfectionism in relation to the formation, risk, and treatment of eating behavior disorder.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained prior to commencement and was granted by the University of Health Sciences Türkiye, Gülhane Scientific Research Ethics Committee under decision number: 2023-333, date: 26.09.2023.

Informed Consent: Survey study.

Footnotes

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Flett GL, Hewitt PL. Perfectionism and maladjustment: An overview of theoretical, definitional, and treatment issues. American Psychological Association. 2002;5-31.
2. Frost RO, Marten P, Lahart C, Rosenblate R. The dimensions of perfectionism. Cognitive therapy and research. 1990;14:449-468.
3. Shafran R, Mansell W. Perfectionism and psychopathology: a review of research and treatment. Clin Psychol Rev. 2001;21:879-906.
4. Hewitt PL, Flett GL. Perfectionism in the self and social contexts: conceptualization, assessment, and association with psychopathology. J Pers Soc Psychol. 1991;60:456-470.
5. Bieling PJ, Israeli AL, Antony MM. Is perfectionism good, bad, or both? Examining models of the perfectionism construct. Personality and Individual Differences. 2004;36:1373-1385.
6. Stoeber J, Otto K. Positive conceptions of perfectionism: approaches, evidence, challenges. Pers Soc Psychol Rev. 2006;10:295-319.
7. Vo M, Accurso EC, Goldschmidt AB, Le Grange D. The Impact of DSM-5 on Eating Disorder Diagnoses. Int J Eat Disord. 2017;50:578-581.
8. Williamson DA, Martin CK, York-Crowe E, et al. Measurement of dietary restraint: validity tests of four questionnaires. Appetite. 2007;48:183-192.
9. Croll J, Neumark-Sztainer D, Story M, Ireland M. Prevalence and risk and protective factors related to disordered eating behaviors among adolescents: relationship to gender and ethnicity. J Adolesc Health. 2002;31:166-175.
10. Quick VM, Byrd-Bredbenner C, Neumark-Sztainer D. Chronic illness and disordered eating: a discussion of the literature. Adv Nutr. 2013;4:277-286.

11. Solomon-Krakus S, Uliaszek AA, Sabiston CM. The differential associations between self-critical perfectionism, personal standards perfectionism, and facets of restrictive eating. Personality and Individual Differences. 2022;184:111178.
12. Egan SJ, Wade TD, Shafran R. Perfectionism as a transdiagnostic process: a clinical review. Clin Psychol Rev. 2011;31:203-212.
13. Fairburn CG, Cooper Z, Shafran R. Cognitive behaviour therapy for eating disorders: A "transdiagnostic" theory and treatment. Behaviour research and therapy. 2003;41:509-528.
14. Brown AJ, Parman KM, Rudat DA, Craighead LW. Disordered eating, perfectionism, and food rules. Eat Behav. 2012;13:347-353.
15. Madden AM, Smith S. Body composition and morphological assessment of nutritional status in adults: a review of anthropometric variables. J Hum Nutr Diet. 2016;29:7-25.
16. Kağan M. Frost Çok Boyutlu Mükemmeliyetçilik Ölçeği'nin Türkçe Formunun Psikometrik Özellikleri. Anadolu Psikiyatri Dergisi. 2011;12:192-197.
17. Hohlstein LA, Smith GT, Atlas JG. An application of expectancy theory to eating disorders: Development and validation of measures of eating and dieting expectancies. Psychological Assessment. 1998;10:49-58.
18. Sapmaz Yurtsever S, Tekinsav Sutcu S. Yemeden Beklentiler Ölçeği ile Zayıflıktan ve Yemeyi Kısıtlamaktan Beklentiler Ölçeğinin Türkçeye Uyarlanması ve Psikometrik Özelliklerinin İncelenmesi. Psikoloji Çalışmaları. 2022;42:133-164.
19. Costa S, Hausenblas HA, Oliva P, Cuzzocrea F, Larcan R. Maladaptive perfectionism as mediator among psychological control, eating disorders, and exercise dependence symptoms in habitual exerciser. J Behav Addict. 2016;5:77-89.
20. McComb SE, Mills JS. Young women's body image following upwards comparison to Instagram models: The role of physical appearance perfectionism and cognitive emotion regulation. Body Image. 2021;38:49-62.
21. Williams-Kerver GA, Steffen KJ, Mitchell JE. Eating Pathology After Bariatric Surgery: an Updated Review of the Recent Literature. Curr Psychiatry Rep. 2019;21:86.
22. Wade TD, Tiggemann M. The role of perfectionism in body dissatisfaction. J Eat Disord. 2013;1:2.
23. Lavender JM, Wonderlich SA, Engel SG, Gordon KH, Kaye WH, Mitchell JE. Dimensions of emotion dysregulation in anorexia nervosa and bulimia nervosa: A conceptual review of the empirical literature. Clin Psychol Rev. 2015;40:111-122.
24. Oldershaw A, Lavender T, Sallis H, Stahl D, Schmidt U. Emotion generation and regulation in anorexia nervosa: a systematic review and meta-analysis of self-report data. Clin Psychol Rev. 2015;39:83-95.
25. Vince EP, Walker IA. A set of meta-analytic studies on the factors associated with disordered eating. The Internet Journal of Mental Health. 2008;5:1-30.
26. Schmidt U. Aetiology of eating disorders in the 21(st) century: new answers to old questions. Eur Child Adolesc Psychiatry. 2003;12 Suppl 1:I30-37.
27. Tolman EG. Purposive behavior in animals and men. Univ of California Press. 1932.
28. Combs JL, Smith GT, Simmons JR. Distinctions Between Two Expectancies in the Prediction of Maladaptive Eating Behavior. Pers Individ Dif. 2011;50:25-30.
29. Fitzsimmons-Craft EE, Keatts DA, Bardone-Cone AM. Eating Expectancies in Relation to Eating Disorder Recovery. Cognit Ther Res. 2013;37:104.
30. Hicks RE, Kenny B, Stevenson S, Vanstone DM. Risk factors in body image dissatisfaction: gender, maladaptive perfectionism, and psychological wellbeing. Heliyon. 2022;8:e09745.
31. Boivin MK, Polivy J, Herman CP. An intervention to modify expectations of unrealistic rewards from thinness. Eat Disord. 2008;16:160-179.
32. Martini M, Marzola E, Brustolin A, Abbate-Daga G. Feeling imperfect and imperfectly feeling: A network analysis on perfectionism, interoceptive sensibility, and eating symptomatology in anorexia nervosa. Eur Eat Disord Rev. 2021;29:893-909.
33. Polivy J, Herman CP. Causes of eating disorders. Annu Rev Psychol. 2002;53:187-213.
34. Yokoyama H, Nozaki T, Nishihara T, Sawamoto R, Komaki G, Sudo N. Factors associated with the improvement of body image dissatisfaction of female patients with overweight and obesity during cognitive behavioral therapy. Front Psychiatry. 2022;13:1025946.
35. Świerczyńska J. The image of perfectionism in youth with diverse structure and intensity of anorexia readiness. Psychiatr Pol. 2022;56:523-534.
36. Haynos AF, Utzinger LM, Lavender JM, et al. Subtypes of Adaptive and Maladaptive Perfectionism in Anorexia Nervosa: Associations with Eating Disorder and Affective Symptoms. J Psychopathol Behav Assess. 2018;40:691-700.
37. Lazarević J, Batinić B, Vukosavljević-Gvozden T. Risk factors and the prevalence of anorexia nervosa among female students in Serbia. Vojnosanit Pregl. 2016;73:34-41.
38. Świerczyńska J. Coexistence of the features of perfectionism and anorexia readiness in school youth. Psychiatr Pol. 2020;54:239-252.
39. Schaumberg K, Wonderlich S, Crosby R, et al. Impulsivity and anxiety-related dimensions in adults with bulimic-spectrum disorders differentially relate to eating disordered behaviors. Eat Behav. 2020;37:101382.
40. Olson EA, Visek AJ, McDonnell KA, DiPietro L. Thinness expectations and weight cycling in a sample of middle-aged adults. Eat Behav. 2012;13:142-145.

DOI: 10.4274/gulhane.galenos.92063
Gulhane Med J 2024;66(4):211-214

Unilateral accommodative spasm arising upon stress

Bağım Ayçin Çakır İnce[1], Önder Ayyıldız[2], Gökhan Özge[2], Yusuf Uysal[2]

[1]Göksun State Hospital, Clinic of Ophthalmology, Kahramanmaraş, Türkiye
[2]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Ophthalmology, Ankara, Türkiye

Cite this article as: Çakır İnce BA, Ayyıldız Ö, Özge G, Uysal Y. Unilateral accommodative spasm arising upon stress. Gulhane Med J. 2024;66(4):211-214

Date submitted:
08.02.2024
Date accepted:
08.06.2024
Online publication date:
22.11.2024

Corresponding Author:
Bağım Ayçin Çakır İnce, M.D., Göksun State Hospital, Clinic of Ophthalmology, Kahramanmaraş, Türkiye
dr.aycin@hotmail.com

ORCID:
orcid.org/0000-0002-3108-6295

Keywords: Accommodation spasm, pseudomyopia, stress, unilateral

ABSTRACT

We present a young male patient with a 2-day history of vision loss and left eye pain. Refraction showed right eye was emmetropic, while the left eye had a refractive diopter measurement of -4.50. After the administration of mydriatic drops, an emmetropic measurement was performed in both eyes, and the patient's pain decreased. Being a military student and undergoing annual eye examinations suggested that the change in refraction was indicative of an acute problem. Repeat measurements and dynamic pupil analysis were conducted along with A-mode ultrasound. Mydriatic drops and eyeglasses were prescribed. Psychiatric consultation was recommended due to the patient's statements regarding anxiety. His symptoms regressed, and his vision improved excellently.

Introduction

Typically, accommodation spasm is caused by prolonged contraction of the ciliary muscle due to prolonged close viewing. Pseudomyopia may occur in accommodation spasm, which is different from myopia because there is no deterioration in the eye anatomy (1). It is common among students preparing for exams or working with a digital screen. There is an organic cause in 18-25% of cases (2,3). Its association with anxiety, depression, neurological diseases, and head trauma has been previously reported (4-7).

Case Presentation

A 20-year-old man with no significant medical history was admitted to the eye clinic with symptoms in the left eye, including pain, blurred vision, decreased visual acuity, and headache. We conducted a comprehensive ophthalmologic assessment, including visual acuity, pupillary response, ocular motility, anterior and posterior segment evaluations and intraocular pressure measurements. Retinal nerve fiber layer (RNFL) analysis (RNFL; OCT, Heidelberg, Germany), dynamic pupillometry (OPD-Scan 3, Nidek, Japan), and A-mode ultrasonography (USG) were performed.

We informed the patient about this case presentation and obtained his informed consent.

His eyes were orthotropic, and his visual acuity was complete without correction in the right eye and 0.2 (Snellen) in the left eye. Refraction was -0.25 diopters (dpt) in the right eye; -4.50 dpt myopia was detected in the left eye. Pupillary light reactions were natural, but the left eye was more miotics than the right. Slit-lamp and fundoscopic examinations were not affected in either eye. The eye pressure was 15-16 mmHg. In the examination performed with a dynamic pupillometry device, the right eye pupil diameter was 2.70 mm in the photopic environment, while the left eye pupil diameter was 2.48 mm; for the mesopic environment, the pupil diameter was 5.89 mm, and the pupil diameter was 3.47 mm (Figures 1, 2). Both eyes were -0.25 dpt on refraction examination performed after a 1.5% drop of cyclopentolate HCL. With A-scan USG, the axial lengths of the right and left eyes were 23.00 and 23.20 mm, respectively.

Since no other pathology was detected in the tests and repeated examinations, unilateral accommodation spasm was considered in the left eye, and neuroradiological imaging was performed for possible causes. Contrast-enhanced brain and orbital magnetic resonance (MR) examinations were normal. Tropicamide 0.5% drops were prescribed as the initial treatment. After 1 week, because of persistent symptoms in the left eye, the treatment was switched to cyclopentolate HCL 1.5% drops, administered twice daily.

The patient stated that he had recently been experiencing stress due to school/exam anxiety. Based on his statements, a psychiatric evaluation was recommended without subjecting him to any stress test. It has been reported that antidepressant treatment recommended by a psychiatrist for anxiety was rejected by the patient but was continued with psychotherapy sessions.

During the follow-up period of up to 6 months with intermittent topical drops treatment and psychotherapy sessions, the patient's complaints were resolved, vision was full in both eyes, and the pupils were isochoric and of normal size.

Discussion

Unilateral accommodation spasm is an extremely rare clinical manifestation in the available literature. The largest series in the literature included 17 cases (3), which reported symptoms of blurred vision (71-100%), diplopia (62%), headache (24-56%), ocular pain (37-47%), and photophobia (24%).

There are different recommendations for the treatment of accommodation spasms. The integration of plus lenses and orthoptic exercises proved successful in a 6-year-old girl with a history of esotropia, which had been previously managed with hyperopic correction (4). The patient was free of any diseases or trauma history and experienced a sudden onset of severe headache, blurred vision, diplopia, and esotropia following the death of her grandfather. Upon examination, her left pupil exhibited marked miosis, and alternating myopic refraction was noted upon retinoscopy. Despite previous examinations consistently showing a refraction of +5.50 dpt in both eyes, she was diagnosed with accommodation spasm. To counteract

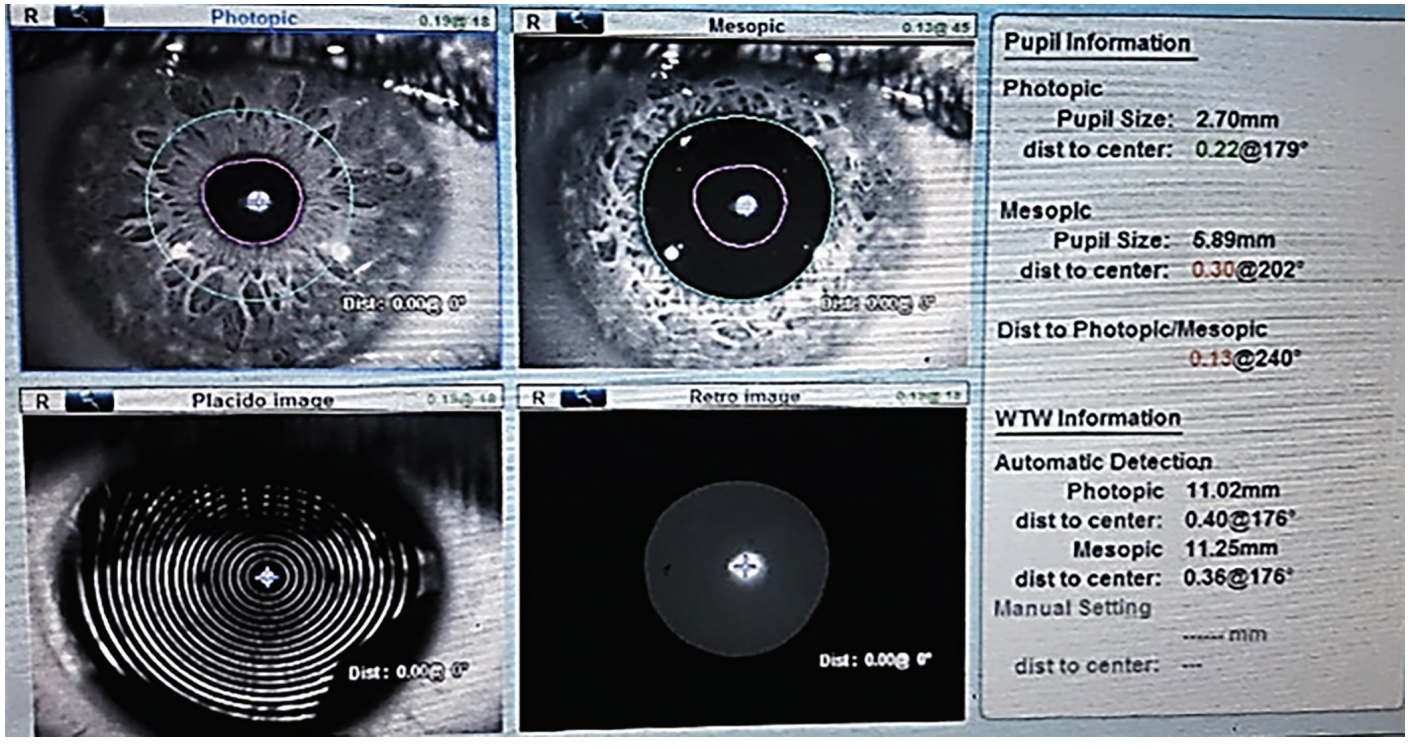

Figure 1. OPD scan 3 images of the right eye: photopic pupil, 2.70 mm; mesopic pupil, 5.89 mm in diameter

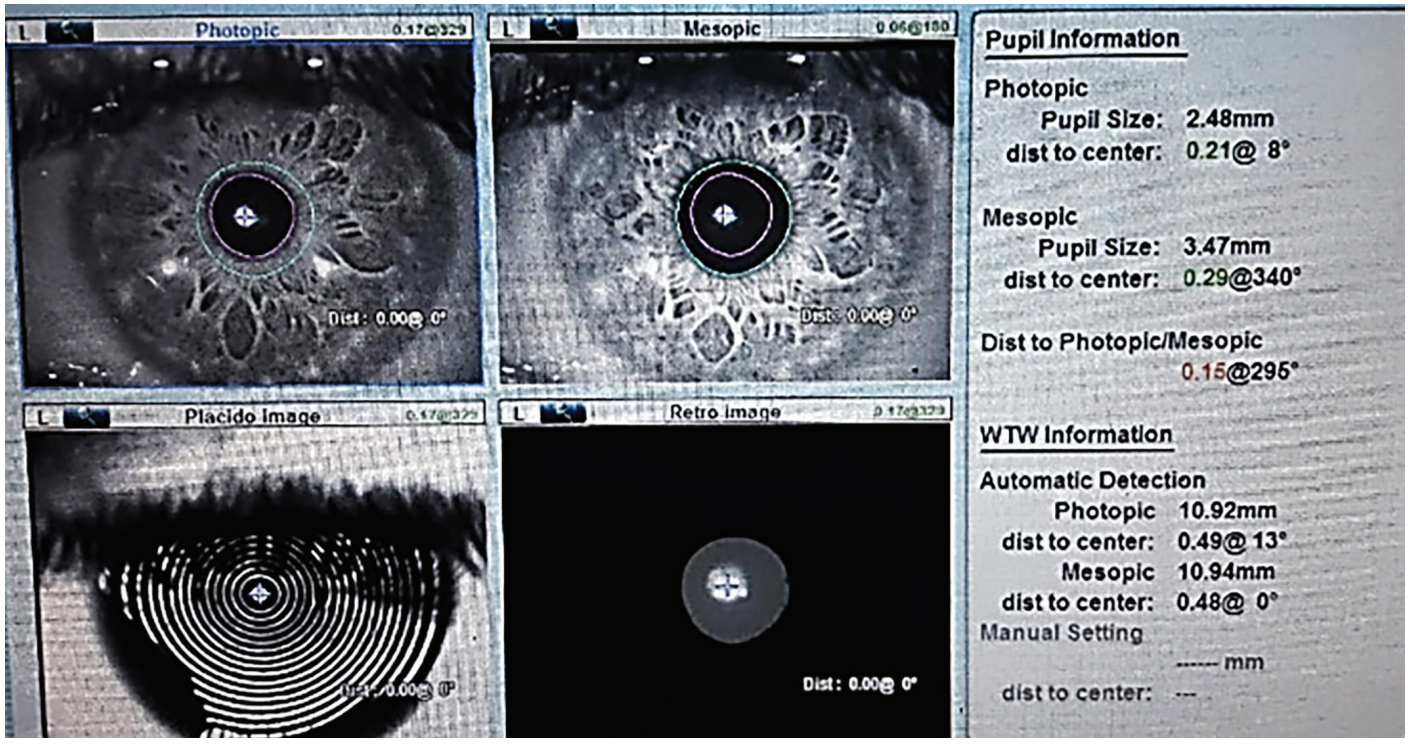

Figure 2. OPD scan 3 images of the left eye: photopic pupil 2.48 mm, mesopic pupil 3.47 mm in diameter

the accommodation, Fresnel press-on bifocal lenses of +2.50 dpt were applied to the patient's spectacles. Remarkably, the patient experienced spontaneous recovery within 2 months, underscoring the effectiveness of the intervention.

Murrah reported an 18-year-old female patient who developed unilateral accommodation spasm after a traffic accident in 1965 (5). In the current case, unilateral accommodation spasm is the expression of increased parasympathetic activity or decreased sympathetic activity due to possible ciliary ganglion trauma.

A 34-year-old female patient who developed sudden-onset blurred vision in her right eye after a car accident was also reported (6). There was no damage during examination or MR imaging. The patient was prescribed glasses -3.50 dpt for the right eye.

Various treatments were given to 16 patients with accommodation spasm (7). Atropine drops were administered to 7 patients, and improvements were observed in these patients. To treat accommodative spasms, we recommended myopic negative lenses to 10 patients, which led to significant improvements in vision quality and reduced complaints in all cases. Four patients who did not receive any treatment exhibited spontaneous recovery. These findings demonstrate the effectiveness of mydriatic drop application and myopic negative lenses in the treatment of accommodation spasms.

Botulinum toxin was applied to the medial rectus muscles, but no improvement was observed in symptoms or visual acuity (8). This report highlighted the rare occurrence of long-term functional accommodative spasms. The study also emphasized the importance of considering functional causes when organic reasons are ruled out. Correction of refractive errors and cycloplegic drops were recommended for symptom relief. Furthermore, the importance of psychological support was underscored.

In the current case, 1.5% cyclopentolate HCL drops were initiated to treat the accommodation spasm. To enhance visual acuity, plano lenses were prescribed for the right eye, while the left eye was corrected with 1.5-dpt myopic lenses.

Previous studies have underscored the importance of psychological support for these patients (8,9). Psychotherapy was beneficial for the current patient.

Conclusion

Unilateral accommodation spasm is a rare condition that often suggests an underlying potential organic pathology. It is important to exclude organic causes before evaluating functional etiologies. In addition to correcting refractive errors, comprehensive ophthalmological evaluation is required. The use of cycloplegic agents may relieve symptoms. In functional accommodation spasms, psychological support and patient assuring that their condition is temporary and treatable are important throughout the treatment process.

Ethics

Informed Consent: We informed the patient about this case presentation and obtained his informed consent.

Footnotes

Authorship Contributions

Concept: B.A.Ç.İ., Ö.A., Y.U., Design: G.Ö., Data Collection or Processing: B.A.Ç.İ., Analysis or Interpretation: B.A.Ç.İ., Ö.A., G.Ö., Y.U., Literature Search: B.A.Ç.İ., Writing: B.A.Ç.İ.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Lindberg L. Akkommodaatiospasmi [Spasm of accommodation]. Duodecim. 2014;130:168-173.
2. Goldstein JH, Schneekloth BB. Spasm of the near reflex: a spectrum of anomalies. Surv Ophthalmol. 1996;40:269-278.
3. Rutstein RP, Daum KM, Amos JF. Accommodative spasm: a study of 17 cases. J Am Optom Assoc. 1988;59:527-538.
4. Rutstein RP. Spasm of the near reflex mimicking deteriorating accommodative esotropia. Optom Vis Sci. 2000;77:344-346.
5. Murrah WF Jr. Recurrent Unilateral Accommodative Spasm And Miosis. South Med J. 1965;58:1135-1136.
6. Hughes FE, Treacy MP, Duignan ES, Mullaney PB. Persistent pseudomyopia following a whiplash injury in a previously emmetropic woman. Am J Ophthalmol Case Rep. 2017;8:28-30.
7. Kaczmarek BB, Dawson E, Lee JP. Convergence spasm treated with botulinum toxin. Strabismus. 2009;17:49-51.
8. Akar S, Gökyiğit B, Aygıt E, Kaynak P, Demirok A. Yakın Refleksin İstemli Kontrolü: Bir Olgu. Turk J Ophthalmol. 2014;44:161-163.
9. Peinado GA, Merino Sanz P, Del Cerro Pérez I, Gómez de Liaño Sánchez P. Unilateral accommodation spasm: Case report and literature review. Arch Soc Esp Oftalmol (Engl Ed). 2019;94:285-287.

2024 Referee Index

Abdullah Doğan
Abdurrahman Şimşek
Abdurrahman Tufan
Abdurrrahman Sönmezler
Ahmet Çağın
Alanur Çiftçi Şişman
Ali Kaplan
Ali Selçuk
Aniekan Monday Abasiattai
Arzu Şenol
Atilla Kazancı
Ayhan Dağ
Ayşe Gürol
Ayşe Özcan Edeer
Ayşe Saatçi Yaşar
Banu Bayar
Berivan Bitik
Bharath Kumar Abbavannagari
Burak Ahmet Bilekli
Burcu Yücel
Büşra Emir
Canan Ayabakan
Canan Uzun
Cem Atabey
Cem Haymana
Cengiz Zeybek
Chandni Gupta
Chie Bujo
Cihangül Bayhan
Coşkun Fırat Özkeçeci
Coşkun Meriç
Deniz Çankaya
Deniz Doğan
Dilek Orbatu
Dilek Tezcan
Dorina Esendağlı
Dorothy Kruger
Duray Şeker
Duygu Kaba
Duygu Mert
Eda Ünal
Edis Çolak
Emine Aydın
Emine Büşra Ata
Emre Ata
Erdem Aras Sezgin
Erkan Yıldırım
Ersin Sapmaz
Esma Asil
Esma Yüzügüldü
Evren Hızal
Fahri Burçin Fıratlıgil
Farzaneh Ahrari
Fatma Arslan
Fatma İlknur Çınar
Ferhat Cüce
Fevzi Demirel
Francesk Mulita
Gökhan Şahin
Gözde Şengül Ayçiçek
Gülali Aktaş
Hadim Akoğlu
Hakan Eren
Hakan Işık
Hakan Zeybek
Hasan Satış
Hatice Özışık
Hazan Karadeniz
Hüseyin İlter
Ildar Fakhradiyev
İlkay Erdoğan
İlker Taşçı
İlyas Öztürk
İsmail Oskay Kaya
İsmail Yaşar Avcı
İsmail Zafer Ecevit
İzzet Fidancı
İzzet Yavuz
Kadir Canoğlu
Kazım Emre Karaşahin
Kerim Bora Yılmaz
Kourosh Zarea
Lalla Asmaa Katir Masnaoui
Mahipal Singh Sachdev
Majid Ghyour-Mobarhan
Maryam Moradi
Mazlum Serdar Akaltun
Mehmet Ayhan Cöngöloğlu
Mehmet Burak Aşık
Mehmet Engin Tezcan
Mehmet Ferdi Kıncı
Mehmet Murat Beyzadeoğlu
Mehmet Talay Köylü
Melike Mercan Başpınar
Merita Emini Sadiku
Mesut Ortatatlı
Metin Keren
Miftah Fauzan
Mohamed Ali
Mohammad Hasan Chowdhury
Mouhammed Amir Habra
Murat Alan
Murat Büyükşekerci
Murat Çelik
Murat Koç
Murat Taşçı
Mustafa Candemir
Mustafa Kuzucuoğlu
Mustafa Salih Karatepe
Mustafa Volkan Yılmaz
Nazmi Liana Azmi
Necla İpar
Nezahat Müge Çatıkkaş
Nilgün Kuru Alıcı
Nuket Emine Ünsal
Nurdan Korkmaz
Okan Kuzhan
Onur Akın
Ökkeş Hakan Miniksar
Özlem Dikmetaş
Pavlina Peneva
Pulkit Khandelwal
Rabia Gönül Sezer
Ramazan Acar
Ramazan Gümral
Renato Franco
Sait Yeşillik
Sara Samur Ergüven
Seda Çevik
Seda Çolak
Sefa Aktı
Seniha Şenbayrak
Serkan Asil
Sevinç Mersin
Siti Nazihahasma Hassan
Sourav Karmakar
Süleyman Demir
Sumru Emine Savaş
Suzana Mustafa
Tolga Ege
Tolga Yıldırım

2024 Referee Index