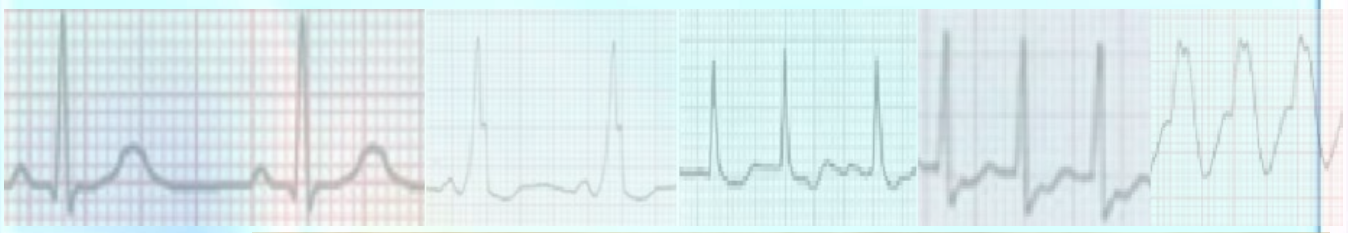
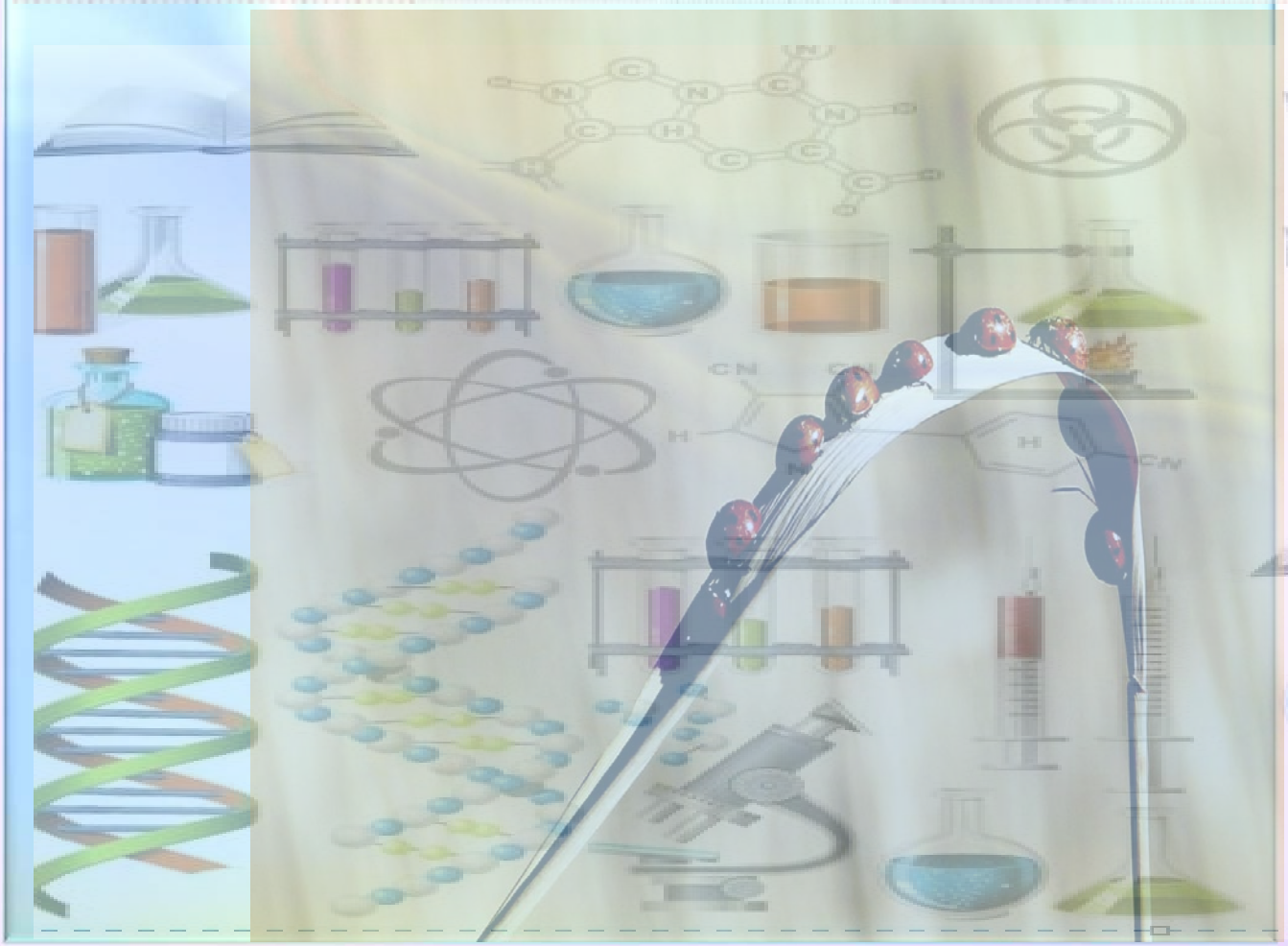
IJCMBS

International Journal of Current Medical and Biological Sciences

Volume 2 Issue 2 August-2022

e-ISSN:2791-8815





Journal Editorial Board

Overview

The International Journal of Current Medical and Biological Sciences is an international, peer-reviewed, open-access electronic and conventional published journal in the English language. The journal accepts clinical and experimental studies, editorial articles, technical and educational reviews, clinical case reports, original images, letters to the editor, and congress abstracts that contribute to general medicine and academic studies.

Information About the Journal

IJCMBS was established in 2021as open access and peer-reviewed journal that accepts articles in English. IJCMBS is published 3 times a year. Articles submitted should not have been previously published or be currently under consideration for publication any place else and should report original unpublished research results. The journal does not expect any fees for publication. All articles are available on the website of the journal with membership.

Journal Management Location and Address

Harran University Faculty of Medicine Osmanbey Campus Haliliye/ SANLIURFA/TURKIYE

Web: https://ijcmbs.com

E-mail: info@ijcmbs.com

Manuscript Preparation Guidelines

Author Guidelines

WRITING RULES

Manuscripts submitted for publication should be written in Microsoft Word. All text, figures and graphics should be sent electronically. Except for the cover page, the name of the institution and the authors should not be mentioned anywhere in the article. The cover page must be loaded separately.

ALL ARTICLES

Title page,

Turkish Abstract,

English Abstract,

Article Section,

Descriptions,

Referances,

Tables,

Figures and Pictures,

It should be arranged as subtitles.

Authors' Open Researcher and Contributor ID **(ORCID ID)** information must be uploaded to the system during article submission.

The article part (except for the abstract, references, table, figure and subtitles) of the research reviews should not exceed **3500 words**, the abstract part should not exceed **250 words,** the references should **not exceed 40**,

the number **of tables 5** and the number of **figures 5 should** not exceed. Case reports should consist of Turkish Title, English title, Turkish and English abstract, introduction, presentation of the case(s), discussion and references. Case reports should not exceed **5 pages** in total. Articles on technical and medical developments and image presentations of original topics should not **exceed 3 pages.**

PREPARATION OF ARTICLES

A plain and understandable writing language should be used in the text, scientific writing style should be adopted and unnecessary repetitions should be avoided. Writing; It should be written with 1.5 line spacing, Times New Roman 12 points. Pages should be numbered in the lower right corner. Manuscripts should be uploaded to the system in 2 files.

1 – Title PAGE

The title of the article should not exceed 100 characters (letters) in research articles and 80 characters in case reports. The title should be written in both English and Turkish. The names and surnames of the authors who contributed to the study should be written clearly.

If the study has been presented in any congress before, the congress name, time (day-month-year and congress place) should be specified.

The name, surname, full address, postal code, telephone e-mail address of the author to be contacted should be written at the bottom of the title page. **Reviewer recommendation:** Assists the editor when selecting a referee for a specific study. Include the names and contact details of at least **2 potential reviewer** on the title page.

2- FULL TEXT

The full texts to be reviewed by the referees during the evaluation process should be uploaded to the system as a single file. The full text file should be composed of the following parts and should be arranged in this order.

A) Abstract

Title of the Article; It should be short, easy to understand and describe the content of the article. Turkish (Abstract) and English (Abstract) abstracts should have Turkish and English titles at the beginning. It should not exceed **250** words in research reviews and **150** words in case reports.

Abstracts, Bacground, Materials and Methods, Results, Conclusion, Keywords in Turkish research articles; In English research papers, it should consist of Background, Materials and Methods, Results, Conclusions, Key words sections.

Case reports do not require these sections.

Keywords; At the end of the Turkish Abstract and English Abstract section, under the heading Keywords and Keywords, there should be a maximum of five keywords that capture the main titles of the scientific article and are written in accordance with the Index Medicus Medical Subject Headings (MeSH). Care should be taken to select the keywords from the Science Terms of Turkey (www.bilimterimleri.com).

Abbreviations in the text should be explained in parentheses where they are first used. Abbreviations should not be used in abstracts.

B) Article

It consists of Introduction, Material and method, Results, Discussion and Conclusion sections.

Introduction: Information that will explain the subject and the purpose of the study is given.

Material and method: The place, time, and planning of the study, as well as the elements and methods used,

should be reported. Data collection, characteristics of patients and individuals, characteristics of the experimental study, and statistical methods should be explained in detail.

Results: The obtained data should be given together with the statistical results.

Discussion: The results of the study should be evaluated by comparing with the literature data.

All spellings must comply with Turkish spelling rules and must be in accordance with punctuation marks. Abbreviations should be avoided as much as possible. References, figures, tables and pictures should be numbered in the order they appear in the text.

Conclusion: Findings from the study should be reported briefly without adding comments.

C) Referances

References should be written with 1.5 line spacing. Reference numbers should be given in ( ) without a period at the end of the sentence, and the period should be placed later. If more than one reference number is given, "," should be placed between them, and if more than two consecutive reference numbers are given, "-" should be placed between the numbers [eg. such as (1,2), (1-3)].

If the journal is used as a source: year, volume, issue, start and end pages are given.

If a book is used as a source: only year, start and end pages are given. The surnames and initials of the authors should be written in the references. If the number of authors is **3** or less in the references, all author names are written. If the name of the author is more than **3,** instead of the names of the following authors, "et al." is written in English sources and "et al." in Turkish sources. Journal names should be abbreviated according to Index Medicus. The way of writing the reference should be like the examples below. References should be arranged in the language of the article and as seen in the examples below.

Sources from journals: Tascanov MB, Tanriverdi Z, Gungoren F, et al. Comparisons of microbiota-generated metabolites in patients with young and elderly acute coronary syndrome. Anatol J Cardiol. 2020;24(3):175-82.

Books; 1. Wagner G. S. Marriott's Practical electrocardiography, Tenth ed. Lippincott Williams Publisher, 2000: 124-129

Web article: Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: www.nursingworld.org/AJN/2002/june/wawatch.htm

Website; Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 July 2002]. Available from: www.cancer-pain.org

Thesis; Gezer R: Morphological Characteristics and Individual Differences of Rugae Palatines. Master Thesis, Şanlıurfa: Harran University Institute of Health Sciences, 2016.

D) Explanations

Financial resources, contributing institutions, organizations and individuals, if any, should be specified in this section.

E) Tables

Tables should be written on a separate page with 1.5 line spacing, each table should have a number and an explanatory name. If there are abbreviations in the table, their expansions should be written in alphabetical order under the table. Table numbering (Table 1. , Table 2., ...) should be given consecutive numbers and Roman numerals should not be used. Tables should be uploaded in the article on a separate page.

F) Figures and Pictures

Figures and pictures must be named and numbered. Numbering (Figure 1., Figure 2., …) should be given consecutive numbers and Roman numerals should not be used. Images should be clear and have a minimum resolution of 300 dots per inch (dpi). Images should be uploaded within the article on a separate page. If abbreviations are used under the figure and picture, the explanation of the abbreviations should be stated under the subtitle in alphabetical order. The magnification and technique of microscopic images should be explained. The editorial board can make any changes it deems necessary without changing the essence of the article.

COMPLIANCE WITH PUBLICATION ETHICS

It is a must to prepare the manuscripts in accordance with research and publication ethics. The authors should state that they accept ethical principles in all human clinical research and that they conduct the research in accordance with these principles. Regarding these, in the Materials and Methods section: they obtained approval for each prospective and retrospective study from the ethics committee of the institution where the clinical research was conducted, and they obtained informed consent from the people who participated in the study or their guardians; In experimental studies on animals, they have to state that they protect animal rights and that they have received approval from the relevant experimental animal ethics committee. Regarding the results of experimental studies on humans or experimental animals, it is obligatory to submit an ethics committee approval document during the application to the journal. In the presence of the author(s), commercial link or institution providing financial support for the study; used commercial product, drug, company, etc. should inform the Editor on the presentation page. The absence of such a situation should also be stated on a separate page.

In studies that require ethics committee approval, the Ethics Committee Approval Document should be uploaded to the system during the article submission process, and the information about the permission (board name, date and issue number) should be stated in the material and method section, as well as in the explanations section after the discussion section of the article. Studies that require the approval of the Ethics Committee are as follows.

All kinds of research conducted with qualitative or quantitative approaches that require data collection from the participants by using survey, interview, focus group work, observation, experiment and interview techniques.

Use of humans and animals (including material/data) for experimental or other scientific purposes, Clinical studies on humans, Animal studies,

Retrospective studies in accordance with the law on the protection of personal data, (If requested, the permission document obtained from the institution where the study was carried out should also be uploaded to the system when the archive is scanned.)

The patient's clear identity should not be shared in the Case Report - Series.

EVALUATION AFTER THE REFEREE'S REPORT

Authors should write the topics that are requested to be corrected in the referee report as an answer in the answer section reserved for them. In addition, they should make the necessary changes in the article and resubmit them online by specifying (painting) them in the article

Contents

Original Article

Review

Case Report

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

Determinants of Premenstrual Syndrome Among Medical Students- Samsun sample

Tıp Öğrencilerinde Premenstrüel Sendromun Belirleyicileri- Samsun örneği

Gülşah ÖZTÜRK[1*]

[1] Gökberk Family Healthcare Center, Samsun, TURKİYE

Corresponding author:

Dr. Gülşah ÖZTÜRK
Adress:
Gökberk Family Healthcare Center, Samsun/TURKİYE
email:
dr.gulsahozturk@yahoo.com
Received: 03.04.2022
Accepted: 23.04.2022
Cite as: OZTURK. G Determinants of Premenstrual Syndrome Among Medical Students- Samsun sample IJCMBS 2022;2(2):82-89
doi.org/10.5281/zenodo.6523510

Abstract

Background: Premenstrual syndrome (PMS) is defined as a term involving physical, psychical and behavioral alterations that repeat in several cycles. The present study aimed to analyze the prevalence and affecting factors of premenstrual syndrome in students in a medical faculty. **Material and Methods:** We designed a sectional study conducted in between July and October 2016 among the single students aged between 17 and 49 years. "Survey of PMS" and "Menstrual Distress Questionnaire" were performed on the participants. **Results:** The mean age of the participants (n=286) was 20.67±1.91 years and the mean age of first menstruation was 13.07±1.19 years. PMS was present in 18.9% (n=54). The mean age of initial menstruation of students having PMS was observed to be lower ($p<0.05$). The ratio of students diagnosed with PMS who considered that this period was a physiological period was lower than those not diagnosed with PMS ($p<0.05$). For having diagnosis of PMS, the most common complaints before the menstruation were anxiety (78.8%), during the menstruation period was stomachache (72.2%), and skin spot and ache (29.6 %) in other days. Negative sensation subgroup was the overall most common complaints in the pre-menstruation period, in the course of menstruation and post-menstruation period. **Conclusions:** The recorded complaints about PMS are negatively affecting the familial conditions and social integration of the patients. It is important to define the disease and provide treatment to avoid biopsychosocial factors in women and their families.

Keywords: Premenstrual syndrome, faculty, students

ÖZ

Amaç: Premenstrüel sendrom (PMS), birkaç döngüde tekrarlayan fiziksel, ruhsal ve davranışsal değişiklikleri içeren bir terim olarak tanımlanmaktadır. Bu çalışmada bir tıp fakültesinin öğrencilerinde premenstrüel sendrom prevalansı ve etkileyen faktörlerin incelenmesi amaçlanmıştır. **Gereç ve Yöntem:** Yaşları 17 ile 49 arasında değişen bekar öğrenciler arasında Temmuz ve Ekim 2016 arasında yürütülen bir kesitsel çalışma tasarladık. Katılımcılara "PMS Anketi" ve "Menstrüel Distres Anketi" uygulandı. **Bulgular:** Katılımcıların (n=286) yaş ortalaması 20,67±1,91 yıl ve ortalama ilk adet görme yaşı 13,07±1,19 yıl idi. %18,9'unda (n=54) PMS mevcuttu. PMS olan öğrencilerin ortalama ilk adet görme yaşının daha düşük olduğu gözlendi ($p<0,05$). Bu dönemi fizyolojik bir dönem olarak gören PMS tanısı alan öğrencilerin oranı PMS tanısı almayanlara göre daha düşüktü ($p<0,05$). PMS tanısı için adet öncesi en sık şikayetler anksiyete (%78,8), adet döneminde karın ağrısı (%72,2), diğer günlerde cilt lekesi ve ağrısı (%29,6) idi. Negatif duyum alt grubu menstrüasyon öncesi, menstrüasyon seyri ve menstrüasyon sonrası dönemde genel olarak en sık görülen şikayetlerdi. **Sonuç:** Kaydedilen PMS şikayetleri hastaların ailevi durumlarını ve sosyal bütünleşmelerini olumsuz etkilemektedir. Kadınlarda ve ailelerinde biyopsikososyal faktörlerden kaçınmak için hastalığın tanımlanması ve tedavisinin sağlanması önemlidir. **Anahtar Kelimeler:** Premenstrüel sendrom, fakülte, öğrenciler

Highlights

- The present study aimed to analyze the prevalence and affecting factors of premenstrual syndrome in students in a medical faculty.
- The recorded complaints about PMS are negatively affecting the familial conditions and social integration of the patients.

Introduction

Premenstrual syndrome (PMS) is a common cyclical disorder characterised by biopsychosocial problems, often observed in young and middle-aged women, developing during the luteal phase of a woman's menstrual cycle and ending with the onset of menstruation. The indisputable prevalence, frequent failure in diagnosis, and difficulty of treatment make this disorder significant. Being a pathology brought about by a cycle lasting approximately 30–35 years, PMS has the potential to turn the lives of women into a nightmare. PMS has been added to DSM-V under the heading of depressive disorders as 'Premenstrual Dysphoric Disorder'. Based on this definition, there must have been certain complaints (e.g. decreased interest in activities, significant depressive mood, significant anxiety) during the last week of the luteal phase in most menstrual cycles on a regular basis during the last year; these complaints must have disappeared within a few days after the onset of menstrual bleeding; these complaints must have been at such a severe level so as to cause disturbances in work, school life or routine activities and must not have been present for at least 1 week after menstruation (1).

Although many authors consider that PMS is one of the most common diseases of the world, some authors suggest that it is a manifestation of physiological changes and not a disease. It may be difficult to make this distinction. According to the literature, 70%–90% of women in their reproductive period have some complaints associated with menstruation. However, 20%–40% of women have more advanced mental and physical dysfunctions (2). Mood and behavioural disorders play a major role in PMS complaints (3).

The present study aimed to analyse the presence of PMS and affecting factors in students of a faculty of Medicine. The analysis of these factors may contribute to the prevention or treatment of PMS.

Materials and Methods

Study Design

This study was conducted as a single-centre survey and included single women aged between 17 and 49 years who had been studying at OMU Faculty of Medicine. There were approximately 1,200 students in the preparatory, 1st, 2nd, 3rd, 4th, 5th and 6th year classes. Approximately 50% (600) of these students were females. When this population size was analysed with a 95% confidence interval (alpha = 0.05) and a possible sampling error of d = 0.05, it was noted that at least 235 participants were needed. Among the current female students, 316 were reached, 19 were excluded from the study because they refused to participate and 11 were excluded because of missing data in their questionnaires. A total of 286 participants were included in the study.

Participants were interviewed in their classrooms, in laboratories, on campus and in the hospital. Two questionnaires were conducted. In addition to the questions about demographic characteristics, the first questionnaire prepared by the researcher comprised questions about the prevalence of PMS among the participants. Names, national ID numbers or photographs of the participants were not disclosed. In addition, a second questionnaire that comprised the 'Menstrual Distress Complaint List' was given to the participants. During the collection of data, the participants were instructed on how to fill out the data collection form and the Menstrual Distress Complaint List, and they were asked to fill out the forms on their own so that there would be no interference by the researcher. The filling of the forms took approximately 25–30 minutes.

PMS Prevalence Questionnaire

This questionnaire comprised 15 + 3 questions. In the first part comprising 15 questions, the participants' smoking, tea, coffee, nutritional habits and menstrual characteristics; views about menstruation and complaints in the premenstrual period were examined. The 3 questions in the second part were asked to those who had one or more of the indications and characteristics, and the effect of bleeding on their daily life was questioned.

Menstrual Distress Complaint List

It is a measure developed by Rudolf H. Moos by application to 839 women in 1968 (4). It was adapted to the Turkish society by Kızılkaya in 1992 (5). In terms of reliability, internal consistency was obtained by applying it twice to 30 students who were educated at Istanbul University Florence Nightingale School of Nursing, and reliability coefficients were found to be 0.71 and 0.97, respectively. All r values were found to be extremely statistically significant ($p < 0.001$).

In the Menstrual Distress Complaint List, 47 complaints were individually interrogated for menstrual, premenstrual and intermenstrual periods. Complaints in the Menstrual Distress Complaint List were listed under 8 subgroups, including pain (muscle stiffness, headache, stomach cramps, backache, fatigue, general aches), water retention (weight gain, skin disorders, pimples, breast tenderness, swelling in breasts and stomach), autonomic reaction (dizziness, faintness, cold sweats, nausea, vomiting, hot flashes), negative affectivity (loneliness, anxiety, mood swings, crying, restlessness, irritability, depression and sadness, hyperactivity), impaired concentration (insomnia, forgetfulness, confusion, lowered judgement, difficulty in concentrating, tension, little accidents due to carelessness, lowered motor coordination), behavioural changes (lowered school or work performance, taking naps; staying in bed, staying at home, avoiding social activities, decreased efficiency), arousal (affectionate, orderliness, excitement, feelings of well-being, bursts of energy, activity) and control (feeling of suffocation, chest pains, ringing in the ears, palpitation, numbness and tingling in extremities, blind spots, fuzzy vision, sensory disturbances). Increased appetite was not included in any group (6).

Statistical analysis

The sociodemographic data of the participants and the data of the questionnaire forms were evaluated using SPSS for Windows 20.0 statistics software. Descriptive statistics are given as mean ± standard deviation for continuous variables and as number and percentage for categorical variables. Normal distribution of data was examined by the Kolmogrov–Smirnov test. Comparison of the groups (PMS Yes-No) was performed using Mann–Whitney U

test. The statistical significance of the changes in the Menstrual Distress Complaint List subscales within 3 periods was analysed using the Freidman test. Differences between the groups were compared using two-way Wilcoxon test and assessed using Bonferroni correction. The level of statistical significance was set at a p value of <0.05.

Results

The mean age of the participants (n = 286) in this study was 20.67 ± 1.91 years, the mean height was 164.63 ± 5.93 cm and the mean weight was 57.28 ± 8.33 kg. The mean age of the participants at the first menstrual period was 13.07 ± 1.19 years. It was observed that the mean age at the first menstrual period of PMS participants was significantly lower than that of non-PMS participants (Table 1).

Table 1. Comparison of some measures of the participants with and without PMS

	PMS		
	Yes (n = 54) Mean±SD	**No (n = 232) Mean ±SD**	**P**
BMI (kg/m2)	20.73 ± 3.19	21.16 ± 2.93	0.959
Age (year)	20.98 ± 2.00	20.60 ± 1.89	0.173
Height (cm)	162.56 ± 16.19	164.69 ± 6.05	0.934
Weight (kg)	55.93 ± 10.97	57.38 ± 8.41	0.707
Age at first menstrual period (year)	12.56 ± 1.98	13.14 ± 1.14	0.019
Length of menstrual flow (day)	6.02 ± 1.62	5.93 ± 1.34	0.846

Abbeartions: BMI : (Body mass index) SD: standard deviation, : $p < 0.05$,

The mean duration of the menstrual period was 5.94 ± 1.39 days. The premenstrual complaints started approximately 4.16 ± 2.57 days before and disappeared 2.52 ± 1.74 days after the onset of bleeding.

When it was investigated whether the participants had knowledge about this subject before their first menstrual period, it was observed that 89.9% of them had knowledge, and when they were asked from whom they obtained that knowledge, 64% of them answered 'family'. When they were asked about their thoughts of the menstrual period, it was found that 78% of them thought it was a natural and physiological process. Among those who thought that it was a natural and physiological process, the rate of those participants who did not have PMS was found to be significantly higher than that of those with PMS. Among those who thought that menstruation was one of the most important factors negatively affecting life, the rate of those participants with PMS was found to be significantly higher than that of those who did not have PMS ($p < 0.05$).

When eating habits were examined, it was observed that 43% of the participants regularly consumed 3 main meals and plenty of vegetables every day and 33.2% consumed 3 or more cups of tea/coffee per day. It was observed that among those who consumed 3 or more cups of tea/coffee per day, the rate of women with PMS was significantly higher than that of those who did not have PMS ($p < 0.05$). The rate of those who smoked was 3.8%, and the rate of those who exercised 2–3 days a week was 11.9%.

With respect to the factors that could cause social discomfort before menstruation, 53.8% indicated sleep disorders (Figure 1). When the symptoms that the participants had within a week before menstruation were investigated, it was found that 66.8% of them had physical symptoms and 55.2% of them had emotional fluctuations (Figure 2).

When the menstrual cycles in the last one year were considered, the rate of participants who said that complaints generally occurred was 69.9% (n = 200). The rate of participants who stated that these symptoms caused a serious problem in daily, school, work or social activities was 30.8% (n = 88). According to the analysis based on the diagnostic criteria, PMS was present in 18.9% of the participants (n = 54).

It was found that the rate of PMS was significantly higher in women with marital conflict, legal problems and attempted suicide ($p < 0.05$).

The frequency of complaints in the Menstrual Distress Complaint List was compared between 3 groups: 1 week period before menstruation, menstruation period, and other days. The complaints with a statistical difference between the participants with PMS and without PMS are shown in Table 2 with the sign '✓'.

Table 2. Periodical changes between the participants with and without PMS in terms of complaints

Complaints	**1 week before the last menstrual period**	**During the last menstrual period**	**Other days**
1. Weight gain	x	x	X
2. Insomnia	✓	x	X
3. Crying	✓	✓	X
4. Lowered school or work performance	✓	✓	x
5. Muscle stiffness	x	x	x
6. Forgetfulness	✓	x	x
7. Confusion	✓	✓	x
8. Taking naps, staying in bed	✓	x	✓
9. Headache	✓	x	x
10. Skin disorders	✓	x	x
11. Feeling of loneliness	✓	x	x

12. Feeling of suffocation	✓	✓	x
13. Affectionate	✓	✓	✓
14. Orderliness	x	x	x
15. Staying at home	✓	✓	x
16. Stomach cramps	✓	x	x
17. Dizziness, faintness	✓	✓	x
18. Excitement	✓	x	x
19. Chest pains	✓	x	✓
20. Avoiding social activities	✓	✓	x
21. Anxiety	✓	✓	x
22. Backache	✓	x	x
23. Cold sweats	✓	x	x
24. Lowered judgement	✓	✓	x
25. Fatigue	✓	✓	x
26. Nausea, vomiting	✓	✓	✓
27. Restlessness	✓	✓	✓
28. Hot flashes	✓	x	x
29. Difficulty in concentrating	✓	✓	x
30. Pain and tenderness in breasts	✓	x	x
31. Feelings of well-being	✓	✓	✓
32. Ringing in the ear	✓	✓	x
33. Tension	✓	x	✓
34. Swelling	✓	x	✓
35. Accidents	✓	✓	x
36. Irritability	✓	x	x
37. General aches and pains	✓	x	x
38. Mood swings	✓	x	✓
39. Palpitation	✓	x	x
40. Depression	✓	x	x
41. Decreased efficiency	✓	✓	x
42. Lowered motor coordination	✓	x	✓
43. Numbness and tingling in extremities	✓	x	x
44. Increased appetite	✓	x	x
45. Tension	✓	✓	✓
46. Blind spots, fuzzy vision	✓	✓	x
47. Burst of energy, activity	x	x	x

It was examined whether there was a difference between the Menstrual Distress Complaint List subscale averages for the three periods. According to this, it was observed that there was a significant difference between control, arousal, behavioural changes, concentration, negative affectivity, autonomic reaction, water retention and pain subscale averages during 1 week before the last menstrual period ($p < 0.05$). It was found that during the last menstrual period, there was a statistically significant difference between control, behavioural changes, concentration, negative affectivity and autonomic reaction subscale averages ($p < 0.05$). On the other days, a statistically significant difference was found between negative affectivity and water retention subscale averages ($p < 0.05$). Negative affectivity was the most common complaint in all periods (Table 3). It was investigated whether there was any difference in the Menstrual Distress Complaint List subscale averages between the 3 periods. According to this, it was found that there was a significant difference between the measurement averages taken in the 3 periods in terms of control, behavioural changes, concentration, negative affectivity, autonomic reaction, water retention and pain subscales ($p < 0.05$). There was no statistically significant difference in the arousal subscale when the averages were compared between each period (Figure 3)

Table-3. Comparison of the Menstrual Distress Complaint List subscales in participants with and without PMS

		PMS		
		Yes (n = 54) Mean ±SD	**No (n = 232) Mean±SD**	**P**
Other Days	**Control**	0.91±2.03	0.54±1.72	0.124
	Arousal	2.02±3.59	1.14±2.42	0.135
	Behavioural Changes	0.78±1.72	0.57±1.59	0.342
	Impaired concentration	2.30±3.61	1.49±2.88	0.173
	Negative Affectivity	2.24±3.49	1.62±3.59	0.046*
	Autonomic Reaction	0.35±0.97	0.29±0.92	0.495
	Water Retention	1.04±1.64	0.63±1.49	0.046*
	Pain	1.33±2.46	1.02±2.20	0.358
During the Last Menstrual Period	**Control**	2.52±3.52	1.31±2.53	0.012*
	Arousal	1.37±2.22	1.10±2.18	0.184
	Behavioural Changes	5.31±5.67	2.85±3.89	0.003*
	Impaired concentration	4.89±5.86	2.57±4.22	0.001*

	Negative Affectivity	7.81±7.66	4.46±5.69	0.003*
	Autonomic Reaction	2.83±3.37	1.34±2.59	0.001*
	Water Retention	3.41±3.36	2.64±2.86	0.180
	Pain	6.30±5.86	5.03±4.80	0.221
During 1 week Before the Last Menstrual Period	**Control**	3.78±3.27	1.31±2.40	0.001*
	Arousal	2.17±3.06	1.02±1.99	0.004*
	Behavioural Changes	4.76±4.40	1.60±2.76	0.001*
	Impaired concentration	6.91±7.45	2.44±3.96	0.001*
	Negative Affectivity	11.76±7.94	4.68±5.81	0.001*
	Autonomic Reaction	3.15±3.59	0.77±1.79	0.001*
	Water Retention	6.11±3.62	3.49±3.08	0.001*
	Pain	6.69±5.23	3.44±3.65	0.001*

Abberations: SD: standard deviation. *: $p < 0.05$, Mann–Whitney U Test was used.

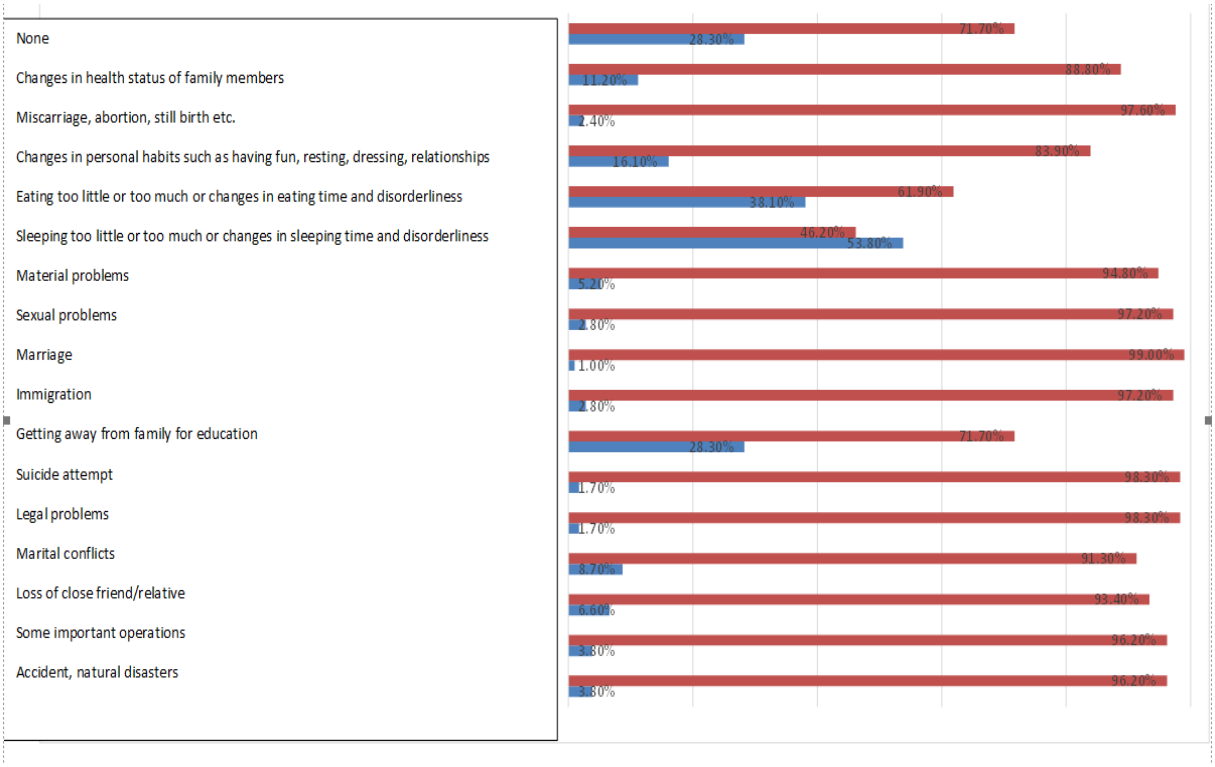


Figure 1. Various factors that might lead to premenstrual disturbances

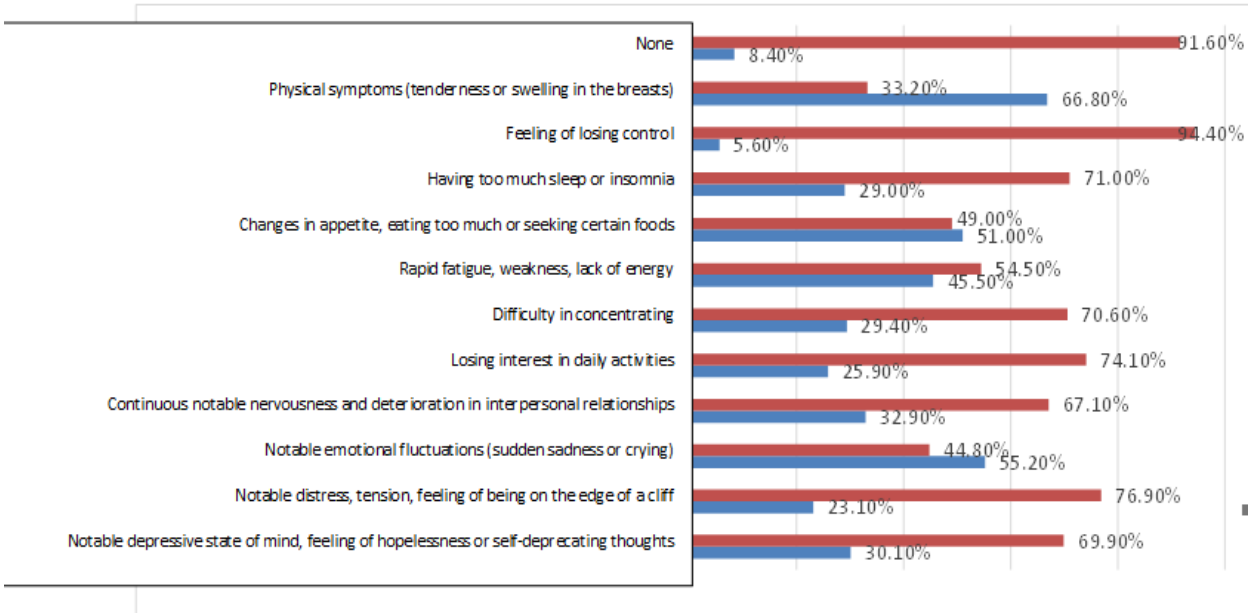


Figure 2. Symptoms that occurred during 1 week before the menstrual period

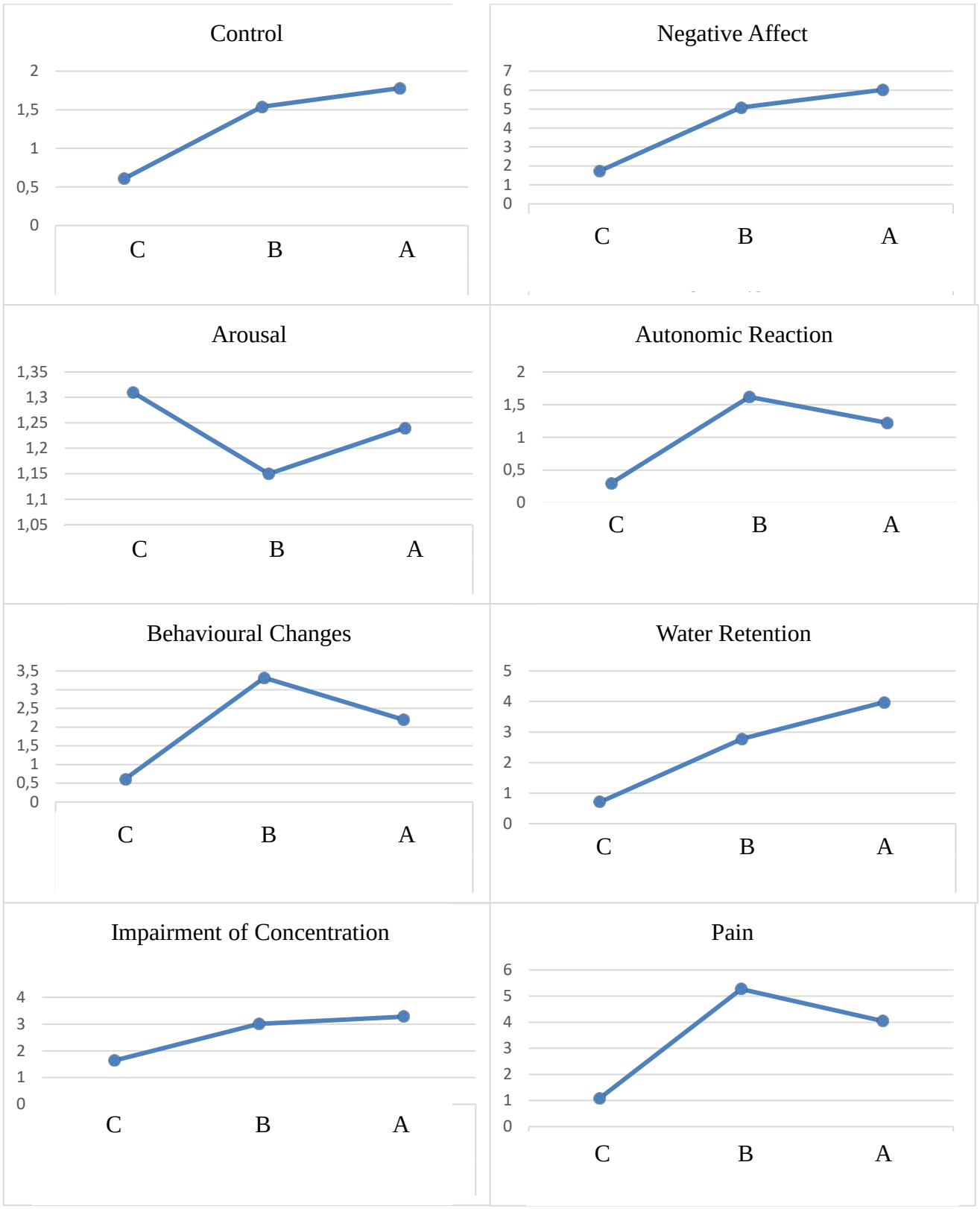


Figure 3. Comparison of the Menstrual Distress Complaint List Subscale Averages between Periods

A: One week before the last period, B: During the last period, C: Other days

Discussion

In the present study, a significant health problem was analysed via a specific group. PMS is common in women of reproductive age, particularly in young women. For a woman who menstruates approximately 12 times a year every year, there is a difficult period of 7–10 days every month, which corresponds to a total period of 3–4 months per year (approximately 25%–30% of the year).

Recent studies on PMS have been conducted mostly in Asia, and the prevalence of PMS has been found in a very wide range in these studies. Prevalence studies conducted in Turkey, India, Pakistan, the Arabian Peninsula and Taiwan have presented results in the range of 30%–80% (7-11). Comparing with these rates, in the present study, we obtained a much lower prevalence. Cheng et al. found no difference between PMS and non-PMS participants in terms of mean age and BMI (11). In the study by Alpaslan, there was no significant difference in age, BMI, first menstrual period and duration of menstruation between PMS and non-PMS participants (12). In our study, there was no significant difference in the means of BMI, age, height, weight and menstrual duration based on the presence or absence of PMS in women. However, it was observed that the mean age at the first menstrual period of PMS participants was significantly lower than that of non-PMS participants.

In a study by Masho et al., It was found that obese women have 2.8 times more risk for PMS than women of normal weight (13). Ellen also stated that obesity is a risk factor for PMS (14). In our study, the number of obese participants was not an ideal number to be compared with the duration of the menstrual cycle.

Studies conducted with women in different cultures have shown that social and cultural factors, activity status and stress play important roles in the development of premenstrual symptoms (15). Women who work or have a responsibility in the society feel themselves under more societal oppression, and for this reason, they experience more mental troubles. It is known that academic stress and intensive training in medical facilities exacerbate PMS (16). All the participants in our study were college students in accordance with the methodology. Despite this, the rates we obtained were below those reported in the literature based on the level of education comparisons.

Restriction of chocolate, cola, coffee, alcohol, nicotine, red meat and food containing sugar and salt is recommended in patients with PMS (17). Cohen et al. reported that the prevalence of premenstrual symptoms is higher in people who smoke more than 4 times a day (18). In another study, it was reported that those who consumed 3 or more cups of tea/coffee a day had 1.9 times more pain symptoms than those who consumed 1–2 cups (19). In our study, it was observed that there was a statistically significant difference between the levels of tea/coffee consumption and PMS, and among people who consumed 3 or more cups of tea/coffee, this difference was significantly higher in those with PMS than those without PMS. Caffeine by changing the levels of estrogen, testosterone and progesterone is known to have a ground preparatory effect on PMS (20,21). In our target group, we found that the smoking rate in the participants with PMS was significantly lower than the average smoking rate of the country. Nevertheless, it should be noted that smoking should be restricted in order to reduce PMS symptoms (2).

Although most women with PMS have only a few of the identified symptoms, almost every woman with PMS has experienced all of these complaints. For example, it is possible that a patient only experiences anxiety and this symptom typically ends within a few days from the onset of menstruation (22,23). Adıgüzel et al. reported that the most common PMS symptoms are nervousness and restlessness (24). In the study by Demir et al., the most common PMS symptoms were back pain and restlessness (2).

The most common PMS symptoms in the study by Pinar were back pain, stress, anger and tenderness and swelling in breasts (7). In our study, the women's monthly cycles were assessed in 3 periods: '1 week before the last menstrual period', 'during the last menstrual period' and 'other days'. Among the complaints included in the Menstrual Distress Complaint List, the most frequent complaint in participants with PMS was anxiety during 1 week before the last menstrual period, stomach cramps during the last menstrual period and skin disorders during the other days. In participants without PMS, the most common complaint was skin disorders during 1 week before the last menstrual period, stomach cramps during the last menstrual period and insomnia during the other days. In the comparison of complaints of the participants with and without PMS based on the subscales, all the symptoms in the Menstrual Distress Complaint List were statistically significantly higher in the participants with PMS during 1 week before the last menstrual period. During the last menstrual period, there were statistically significantly more complaints among those with PMS in the control, behavioural changes, concentration, negative affectivity and autonomic reaction subscales. On the other days, there were statistically significantly more complaints among those with PMS in the negative affectivity and water retention subscales. As can be seen, the negative affectivity subscale (loneliness, anxiety, mood swings, crying, restlessness, irritability, depression-sadness, hyperactivity) manifested itself every day more frequently in the participants with PMS than in those without PMS.

According to a population-based study conducted in 2010 with 4,085 women aged 14–50 years living in France, Germany, Hungary, Italy, Spain, the United Kingdom, Brazil and Mexico, physical and mental complaints observed in patients with PMS were reported to negatively affect women's daily lives significantly (25). In our study, 25.9% of the participants with PMS considered menstruation as one of the most important events that negatively affects life. The same answer was given by only 2.6% of those without PMS.

Because the participants diagnosed with PMS in the present study were students of a medical faculty of one university only, the results may not be generalised to people of all ages, education levels and geographical areas. Because the scales evaluating complaints of individuals were not supported by biochemical measurements, the inferences were based on verbal expressions.

Conclusions

The need to use health care services for PMS has been gradually increasing. The number of applications to clinics with PMS-associated complaints has been increasing. Physicians play an important role in the process from the onset of

associated complaints to the diagnosis of PMS. Preventing women, and therefore families, from living with biopsychosocial problems will be possible by the diagnosis of the disorder and the provision of treatment.

Acknowledgements: Thanks to Prof. Mustafa Fevzi Dikici for his consultancy.

Ethical Approval: *OMU Clinical Researches Ethics Committee, B.30.2.ODM.0.20.08/398*

Author Contributions: *Author Contributions: Concept: G.Ö. Literature Review: G.Ö. Design: G.Ö. Data acquisition: G.Ö. Analysis and interpretation: G.Ö. Writing manuscript: G.Ö. Critical revision of manuscript: M.F.D*

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

Financial Disclosure: *Authors declared no financial support.*

References

1.Eisenlohr-Moul TA, Girdler SS, Schmalenberger KM, et al. Toward the reliable diagnosis of dsm-5 premenstrual dysphoric disorder: the carolina premenstrual assessment scoring system (C-PASS). Am J Psychiatry. 2017;174(1):51-9.
2. Demir B, Algül LY, Güvendağ ESG. Sağlık çalışanlarında premenstrüel sendrom insidansı ve etkileyen faktörlerin araştırılması. Türk Jinekoloji ve Obstetrik Derneği Dergisi. 2006;3(4): 262–70.
3. Koci A, Strickland O. Relationship of adolescent physical and sexual abuse to premenstrual symptoms (PMS) in adulthood. Issues in Mental Health Nursing. 2006;28: 75–87.
4. Moos RH. The Development of a menstrual distress questionnaire. Psychosomatic Medicine 1968; 30:853-67.
5. Kızılkaya N. Perimenstrual distres ve hemşirelik yaklaşımı. Hemşirelik Bülteni. 1994;8(31): 84-90.
6. Ross C, Coleman G, Stojanovska C. Factor structure of modified moos menstrual distress questionnaire: assessment of prospectively reported follicula menstrual and premenstrual symptomatology. J Psychosom Obstet Gynaecol. 2003; 24(3):163-74.
7. Pinar G, Colak M, Oksuz E. Premenstrual syndrome in Turkish college students and its effects on life quality. Sexual & Reproductive Healthcare. 2011;2 (1): 21 - 8.
8. Sarkar AP, Mandal R, Ghorai S. Premenstrual syndrome among adolescent girl students in a rural school of West Bengal, India. International Journal of Medical Science and Public Health. 2016;5:03.
9. Singh A, Kiran D, Singh H, et al. Prevalence and severity of dysmenorrheal: a problem related to menstruation, among first and second year female medical students. Indian J Physiol Pharmacol. 2008;52(4):389-97.
10. Balaha MH, Amr MA, Saleh Al Moghannum M, et al. The phenomenology of premenstrual syndrome in female medical students: a cross sectional study. Pan Afr Med J. 2010;23: 5:4.
11. Cheng SH, Shih CC, Yang YK, et al. Factors associated with premenstrual syndrome - a survey of new female university students. Kaohsiung J Med Sci. 2013;29(2):100-5.
12. Alpaslan AH, Avcı K, Soylu N, et al. Association between premenstrual syndrome and alexithymia among Turkish university students. Gynecol Endocrinol. 2014;30(5):377-80.
13. Mosho SW, Adera T, South- Paul J. Obesity as a Risk Factor for Premenstrual Syndrome. J Psychosom Obstet Gynaecol. 2005; 26(1):33-9.
14. Ellen W Freeman. Epidemiology and Etiology of Premenstrual Syndromes. 2014.
15. Özkan S. Psikiatrik tıp: konsültasyon-liyezon psikiatrisi. pp:216-20. İstanbul,1993.
16. Nazzal Z, Hasan N, Al-Sukhon M. Moderate to Severe premenstrual syndrome and contributing factors among university students in Palestine. J J Commun Med. 2015;1(2):016.
17. London LM, Patricia, Ladewig W, et al. Women's health care Maternal Newborn & Child Nursing Family Centered Care, Prenticet hall. 2003; 47.
18. Cohen LS, Soares CN, Otto MW, et al. Prevelance and predictors of premenstrual dysphoric disorder in older premenopausal women the Harward study of moods and cycles. J Affective Dısorder. 2002; 70(2): 125-32.
19. Gökçe G. Bir grup çalışan kadında premenstrual semptom görülme sıklığı ve etkileyen faktörler, Yayınlanmamış Yüksek Lisans Tezi, Mersin Üniversitesi Sağlık Bilimleri Enstitüsü, Mersin, 2006.
20. Erbil N, Karaca A, Kırış T. Investigation of premenstrual syndrome and contributing factors among university students. Turk J Med Sci. 2010; 40:565-73.
21. Hong JP, Park S, Wang H-R, et al. Prevalence, correlates, comorbidities, and suicidal tendencies of premenstrual dysphoric disorder in a nationwide sample of Korean women. Soc Psychiatry Psychiatr Epidemiol. 2012;47:1937–45.
22. Kapur N. Premenstrual symptoms and social disability. International Journal of Innovative Knowledge Concepts. 2016;4(1):1-11.
23. Hoyer J, Burmann I, Kieseler ML, et al. Menstrual cycle phase modulates emotional conflict processing in women with and without premenstrual syndrome (PMS) - a pilot study. PLoS ONE. 2013; 8 (4):e59780.
24. Adıgüzel H, Taskın O, Danacı AE. Manisa ilinde premenstrüel sendrom belirti örüntüsü ve belirti yaygınlığının araştırılması. Türk Psikiyatri Dergisi. 2007;18(2): 1-8.
25. Dennerstein L, Lehert P, Bäckström T, et al. The effect of premenstrual symptoms on activities of daily life. Fertil Steril. 2010;94(3): 1059 - 64.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

An Investigation on the Oral Hygiene Tools Knowledge of the Personnel of Nyala Sudan Turkey Training and Research Hospital

Nyala Sudan Türkiye Eğitim ve Araştırma Hastanesinde Çalışan Personelin Oral Hijyen Araçları Konusundaki Bilgilerinin Araştırılması

Elif Esra OZMEN[1*], Metin Ocak[2]

[1]Department of Oral Surgery, Ahmet Keleşoglu Faculty of Dentistry, Karaman,TURKİYE
[2]Gazi State Hospital Emergency Clinic, Samsun, TURKİYE

Corresponding author:

Elif Esra OZMEN, Dt
Adress:
Department of Oral Surgery, Ahmet Keleşoglu Faculty of Dentistry, Karaman,TURKEY
email: elifesraozmen89@gmail.com
Received: 23.03.2022
Accepted: 15.04.2022
Cite as: OZMEN. E,E. OCAK. MAn Investigation on the Oral Hygiene Tools Knowledge of the Personnel of Nyala Sudan Turkey Training and Research HospitalIJCMBS 2022;2(2):90-95
doi.org/10.5281/zenodo.6523612

Abstract

Background: Dental studies have shown that caries or periodontal disorders in the mouth are the result of dental plaque. An adequate oral hygiene is possible with sufficient removal of the dental plaque. Different tools are used in order to achieve a healthy oral hygiene.

Materials and Methods: The study was conducted on adults working in Nyala Sudan Training and Research Hospital. The present study included 104 individuals including 43 (41.35%) males and 61 (58.65%) females with age ranging between 20 and 57 years. Individuals were asked to fill out a questionnaire aiming to measure their oral hygiene habits and dental plaque knowledge.

Result: In the present study where oral hygiene habits were examined, a significant difference was detected between the genders in terms of tooth brushing habits and women fulfill these habits more regularly ($p<0.05$). Furthermore, it was concluded that women use mouthwash more regularly ($p<0.05$). On the other hand, it was observed that their knowledge level about dental plaque was lower than men ($p<0.05$). The review of the age variable revealed that individuals over 40 years of age had brushing habits once a day when compared to other age groups ($p<0.05$). Different levels of knowledge about age could not be reached in individuals.

Conclusion: It was reached out that the knowledge and attitudes of the participants on oral hygiene and the frequency of visits to the dentist were insufficient. It was observed that knowledge and habits on oral hygiene vary between individuals

Key words: Dental plaque, Toothbrush, Oral hygiene

ÖZ

Amaç: Diş hekimliği ile ilgili olarak yapılan araştırmalar göstermiştir ki ağız içerinde meydana gelen çürük ya da periodontal rahatsızlıklar dental plağın sonucudur. Dental plağın yeterince uzaklaştırılabilmesi ise iyi bir ağız hijyeni ile mümkün olur. Sağlıklı bir ağız hijyeni sağlayabilmek için farklı araçlar kullanılmaktadır. Bu çalışmada Nyala Sudan Türkiye Eğitim ve Araştırma hastanesinde çalışan personelin oral hijyen alışkanlıklarını, oral hijyen araçlarını ve bilgilerini değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: Çalışma Nyala Sudan Türkiye Eğitim ve Araştırma Hastanesinde çalışan yetişkinler üzerinde gerçekleştirildi. Çalışmaya yaşları 20-57 arasında olan 43 (%41,35) erkek ve 61 (%58,65) kadın olmak üzere toplam 104 kişi katılmıştır. Bireylerin oral hijyen alışkanlıklarını ve dental plak bilgilerini ölçmeyi amaçlayan anket formunu doldurmaları istendi.

Bulgular: Oral hijyen alışkanlıklarının incelendiği bu çalışmada cinsiyetler arasında diş fırçalama alışkanlığı açısından kadınların bu alışkanlıklarını daha düzenli yerine getirdikleri yönünde anlamlı bir farklılık görüldü ($p<0.05$). Ayrıca gargara kullanım alışkanlıkları konusunda da kadınların daha düzenli kullandıkları sonucuna ulaşıldı ($p<0.05$). Öte yandan dental plak konusunda erkeklere göre bilgi düzeylerinin daha düşük olduğu görülmüştür ($p<0.05$). Yaş değişkenine bakıldığında ise 40 yaş üstü bireylerin günde 1 kez fırçalama alışkanlıklarının olması diğer yaş gruplarına göre anlamlı olarak görülmüştür($p<0.05$). Bireyler arası yaşla ilgili farklı bilgi düzeylerine ulaşılamamıştır.

Sonuç: Çalışma sonuçları ele alındığında bireylerin oral hijyen alışkanlıklarının neler olduğu ve hangi sıklıkla kullandıkları hususunda bazı sonuçlara ulaşılmıştır. Oral hijyen bilgi ve alışkanlıklarının bireyler arası değişiklik gösterdiği görülmüştür.

Anahtar Kelimeler: Dental plak, Diş fırçası, Oral hijyen

Highlits

In this study, it was aimed to measure the knowledge level of the participants about oral hygiene. Questionnaires were filled in by the participants. Evaluations were made with statistical analyzes and it was concluded that the level of knowledge is important in the light of the literature. While conducting the surveys, help was received from an interpreter who knew the local people's language and Turkish.

Introduction

Studies conducted for many years indicated that dental plaque is the main cause of dental caries and periodontal diseases (1-3). It is important for the patient to have a well oral hygiene knowledge in order to remove the plaque (3,4). The goal of all recommended tools for maintaining oral hygiene is to remove the plaque effectively. If the plaque cannot be removed, the discomfort in the gums and hard tissues may lead to tooth loss (5). According to the World Health Organization (WHO), “Oral health means not only having good teeth, but being free from diseases and ailments that affect the mouth and oral cavity (6).

Healthcare services in Sudan is very below the average. For instance, the infant death rate was detected 62% in Sudan. Despite infectious diseases such as tuberculosis, malaria and hepatitis A, the budget allocated by the government for health expenditures is less than 10% of the total expenditure. Furthermore, the ratio of dentists per population in rural areas is very low, and the studies showed that approximately three dentists serve for 100,000 people. There is limited number of dentists in Sudan. This restricts the access to dental therapies. The population of Sudan is around 45.5 million (7). Majority of the population is located in rural areas. Approximately 40% of the population lives below the poverty line (8). A previous study conducted in Sudan in 2013 showed that the share of expenditure on health services by individuals is 78.9% (9). Individuals spend 1% of these health expenditures for dental treatments only (10).

There have been many studies showing that cultural beliefs and practices have a higher impact on access to health services (8,11,12). However, there is a widely accepted view that majority of the researches on healthcare at global scale are performed in developed countries. Relatively limited number of researches is available in developing countries (8). Africa including rural part of Nyala is a region where health research is limited. The aim of this study was to evaluate the oral hygiene knowledge and habits of individuals in this region.

Materials and Methods

Following approval from the ethics committee of Sudan Nyala Training and Research Hospital, 104 personnel were informed about the study and their written consents were obtained from the Dental Clinic of Nyala Training and Research Hospital between June 2019 and January 2020. Adult volunteer patients without any psychological disorder were enrolled into the study. Patients with anxiety disorder, minors and patients over 65 years of age were excluded. Assistance was obtained from a translator who was fluent in the language of the region as well as Turkish language while preparing the questionnaire, and care was taken to ensure that the questions were in their own language. Demographic data were included in the questions related to gender, age and reason for referral in the first part of the questionnaire. In the second part, oral hygiene habits and knowledge about dental plaque were questioned. The literature was benefited in order to decide the scale measuring the Periodontal Awareness information (15). Alwaeli et al. used a scale of which the reliability was confirmed. In this section, 5 questions were asked to the individuals and they were asked to give the correct answer or answers by putting 4 options for each question (13,15). An explanatory scale derived from the short version of the SOC scale proposed by Antonovsky was used (16,17) Four questions were asked and appropriate answers were requested in order to measure Oral Hygiene Motivation. Dental procedures planned were performed to the patients after filling in the questionnaires.

Statistical Analysis

After filling out the questionnaire forms, statistical analyzes were carried out with the help of SPSS version 17.0 program. Consistency with normal distribution was evaluated with histogram graphics and Kolmogorov-Smirnov test. Mean, standard deviation, and median values were used in descriptive analyses. Pearson's Chi-Square test was used when evaluating categorical variables. In the statistical analysis part, since the groups showed normal weight, Student's t test was applied. Any p value below 0.05 was accepted as statistically significant.

Results

The present study included 104 individuals including 43 (41.35%) males and 61 (58.65%) females with age ranging between 20 and 57 years. The mean age of the individuals participating in our study was 32.22±9.02. One hundred and one (101) individuals brush their teeth; however, 3 individuals stated that they do not brush their teeth. Ten (10) individuals stated that they brushed their teeth once a day, 86 individuals brush their teeth twice or more, and 5 individuals reported that they have an irregular tooth-brushing habit. There are 22 people who use dental floss and/or interdental brushes. Five (5) individuals use dental floss and/or interdental brushes once a day whereas 7 individuals use these twice or more in a day. Twenty fine (25) individuals use mouthwash and/or mouth spray. When the last dentist appointment of the participants was investigated, 23 people were examined in the last 6 months; however, 21 individuals had dental examination 1 year ago, the number of people who have referred to the dentist 3 years ago is 4, and the number of people who have never been to the dentist is 40. Among all participants, 35 individuals stated that dental plaque is a soft attachment, 25 individuals said that it is a hard attachment, 3 individuals said that it was a stone, and 41 individuals stated that they had no idea about this. There are 49 individuals who say that if the dental plaque persists in the mouth for

a long time would cause discoloration, 5 people who say it would cause malformation, 18 people think that it would cause gingival discomfort, and 32 individuals do not have an opinion on this issue.
Eighty-eight individuals interpret a possible bleeding as gingivitis; 5 individuals consider that bleeding is an indicator for healthy gingiva; 4 individuals think that this might cause gingival recession, and 4 individuals have no idea on this issue. There are 7 individuals who believe dieting is the method to prevent gum diseases; 88 individuals who see it as oral care, and 9 people think that vitamin C supplementation is necessary. In addition to these, there were 89 individuals who said "we should brush our teeth in the morning"; 3 people found it important to brush at noon. The number of people who say "we should brush before going to bed" is twelve (Table 1).

Table 1. Demographic Data, Attitudes and Information about Oral Hygiene of the Participants

Age, year		32.22±9.2
Gender, n, (%)	Male	43(41.35)
	Female	61(58.65)
Do you brush your teeth?n, (%)	No	3(2.88)
	Yes	101(97.12)
How frequent do you brush your teeth?n, (%)	Once in a day	10(9.90)
	Twice or more times	86(85.15)
	Irregularly	5(4.95)
Do you use dental floss and/orinterdental brushes?n, (%)	No	82(78.85)
	Yes	22(21.15)
How frequent do you use these? n, (%)	Once in a day	5(22.73)
	Twice or more times	7(31.82)
	Irregularly	10(45.45)
Do you use mouthwash and/ormouth spray?n, (%)	No	79(75.96)
	Yes	25(24.04)
When did you haveyour last dentist examination?n, (%)	6 months ago	23(22.12)
	1 year ago	21(20.19)
	2 years ago	16(15.38)
	3 years or more than 3 years ago	4(3.85)
	Never	40(38.46)
What is a dental plaque?n, (%)	Soft attachment on tooth surface	35(33.65)
	Hard attachment on tooth surface	25(24.04)
	Tartar	3(2.88)
	I don’t know	41(39.42)
What can occur as a result ofnot removing dental plaque from the mouth for a long time?n, (%)	Colorings	49(47.12)
	Malformation on tooth surface	5(4.81)
	Gingival Disease	18(17.31)
	I don’t know	32(30.77)
What does gingival bleeding indicate?n, (%)	Gingivitis	88(84.62)
	Healthy gingival	5(4.81)
	Gingival Recession	4(3.85)
	I don’t know	7(6.73)
How can you beprevented from gingival diseases?n, (%)	Through having Diet	7(6.73)
	Through brushing teeth and using dental floss	88(84.62)
	Through taking Vitamin C supplement	9(8.65)
At what time of the dayis brushing the gumsmore important?n, (%)	In the mornings	89(85.58)
	At noon	3(2.88)
	Before sleeping	12(11.54)

When evaluated in terms of gender variable, the rate of brushing teeth is higher in women than in men ($p<0.05$). The rate of using mouthwash and/or mouth spray is higher in males than females ($p<0.05$). The rate of those who say that dental plaque is a soft attachment on the tooth surface is higher in women than in men ($p<0.05$). There was not any significant difference between genders about frequency of brushing and referring to a dentist ($p>0.05$) (Table 2).

Table 2. Comparison of Participants' Attitudesand Knowledge Levels on Oral Hygiene by Gender

		Male	**Female**	**P**
Do you brush your teeth?n, %	No	3(6.98)	0(.00)	0.036
	Yes	40(93.02)	61(100.00)	
How frequent do you brush your teeth? n, %	Once in a day	4(10.00)	6(9.84)	0.999
	Twice or more times	34(85.00)	52(85.25)	
	Irregularly	2(5.00)	3(4.92)	

Do you use dental floss and/orinterdental brushes? n, %	No	34(79.07)	48(78.69)	0.963
	Yes	9(20.93)	13(21.31)	
How frequent do you use these? n, %	Once in a day	1(11.11)	4(30.77)	0.434
	Twice or more times	4(44.44)	3(23.08)	
	Irregularly	4(44.44)	6(46.15)	
Do you use mouthwash and/ormouth spray? n, %	No	24(55.81)	55(90.16)	<0.001
	Yes	19(44.19)	6(9.84)	
When did you haveyour last dentist examination? n, %	6 monthsago	8(18.60)	15(24.59)	0.162
	1 yearago	9(20.93)	12(19.67)	
	2 yearsago	10(23.26)	6(9.84)	
	3 yearsormorethan 3 yearsago	3(6.98)	1(1.64)	
	Never	13(30.23)	27(44.26)	
What is a dental plaque? n, %	Soft attachment on tooth surface	11(25.58)	24(39.34)	0.021
	Hard attachment on tooth surface	17(39.53)	8(13.11)	
	Tartar	1(2.33)	2(3.28)	
	I don’tknow	14(32.56)	27(44.26)	
What can occur as a result ofnot removing dental plaquefrom the mouth for a long time? n, %	Colorings	24(55.81)	25(40.98)	0.313
	Malformation on tooth surface	2(4.65)	3(4.92)	
	Gingival Disease	8(18.60)	10(16.39)	
	Idon’tknow	9(20.93)	23(37.70)	
What does gingival bleeding indicaten,%	Gingivitis	39(90.70)	49(80.33)	0.411
	Healthy gingival	2(4.65)	3(4.92)	
	Gingival Recession	1(2.33)	3(4.92)	
	Idon’tknow	1(2.33)	6(9.84)	
How can you beprevented from gingival diseases? n, %	Through having Diet	2(4.65)	5(8.20)	0.660
	Through brushing teeth and using dental floss	38(88.37)	50(81.97)	
	Through taking Vitamin C supplement	3(6.98)	6(9.84)	
At what time of the dayis brushing the gumsmore important? n, %	In the mornings	36(83.72)	53(86.89)	0.100
	At noon	3(6.98)	0(.00)	
	Before sleeping	4(9.30)	8(13.11)	

When a comparison is done for the age variable, the age of those who use dental floss and/or interdental brush once a day is higher than those who use it twice or more and those who use it irregularly ($p<0.05$). No significant difference was observed in other habits and knowledge in consideration of the age variable (Table 3).

Table 3. Comparison of Participants' Attitudesand Knowledge Levels on Oral Hygiene by Age

		Mean± S.D.	**P**
Gender	Male	32.47±8.90	0.574
	Female	32.05±9.18	
Do you brush your teeth?	No	44.33±13.58	0.071
	Yes	31.86±8.70	
How frequent do you brush your teeth?	Once in a day	34.50±9.24	0.633
	Twice or more times	31.67±8.83	
	Irregularly	29.80±4.32	
Do you use dental floss and/or interdental brushes?	No	31.60±8.97	0.145
	Yes	34.55±9.06	
How frequent do you use these?	Once in a day	44.40±8.88	0.045
	Twice or more times	30.86±6.59	
	Irregularly	32.20±7.54	
Do you use mouthwash and/or mouth spray?	No	32.18±8.87	0.939
	Yes	32.36±9.67	
When did you have your last dentist examination?	6 monthsago	36.17±10.36	0.327
	1 yearago	31.19±7.63	
	2 yearsago	30.94±8.46	
	3 yearsormorethan 3 yearsago	30.50±13.48	
	Never	31.17±8.44	

What is a dental plaque?	Soft attachment on tooth surface	32.49±9.40	0.895
	Hard attachment on tooth surface	32.12±9.42	
	Tartar	35.00±7.00	
	I don't know	31.85±8.84	
What can occur as a result of not removing dental plaque from the mouth for a long time?	Colorings	32.08±8.84	0.970
	Malformation on tooth surface	30.00±6.82	
	Gingival Disease	33.22±9.48	
	I don't know	32.22±9.64	
What does gingival bleeding indicate?	Gingivitis	32.11±9.33	0.737
	Healthy gingival	35.60±8.29	
	Gingival Recession	29.50±4.43	
	I don't know	32.71±8.08	
How can you be prevented from gingival diseases?	Through having Diet	34.14±8.78	0.652
	Through brushing teeth and using dental floss	31.95±9.16	
	Through taking Vitamin C supplement	33.33±8.54	
At what time of the day is brushing the gums more important?	In the mornings	32.84±9.27	0.115
	At noon	24.00±4.36	
	Before sleeping	29.67±6.60	

Discussion

Having good oral health is very important for the continuation of general health (17). It is extremely important to train the individuals for oral and dental health. There are many studies advocating the correctness of giving these trainings in the very early years so that the person may provide adequate oral hygiene (13).

A study was conducted in Saudi Arabia in which oral hygiene habits and dental plaque knowledge were questioned among primary school teachers. The aforesaid study concluded that female teachers have more knowledge. Furthermore, it was observed that the frequency of tooth brushing of female teachers was more intense than male teachers. There was not any significant difference between teachers about referring to a dentist. It was concluded in the aforesaid study that both men and women do not have sufficient knowledge about dental plaque, and this aspect is not similar to the study conducted in Saudi Arabia. However, the outcome that women brush their teeth more frequently in the present study is consistent with the more frequent tooth brushing habit of female teachers. Moreover, it is similar to this study and some studies in the literature in that there is no significant difference between the frequency of women and men going to the dentist (18,19).

Some studies demonstrated that the increase in the income level of individuals may lead to an increase in oral hygiene habits (18). Since the income of the personnel working in the hospital is higher than the local people, the outcome may not reflect the oral hygiene habits of the local people. It is possible to conclude that the oral hygiene habits of these people who have a better income compared to the local people are more ideal than the general population.

It was concluded in the review of the literature that individuals do not regularly visit the dentist in many studies (3,18). The rate of referring to the dentist in the last year was 42.3% in the study stated above. The rate of the individuals who have not visited a dentist was 57.7%. This may suggest that the importance of oral-dental health is not known and ignored. However, we may consider that they visit a dentist when they had very important complaints. It is consistent from this point of view. On the other hand, Limited number of dentists restricts access to dentists according to the density of the population in Sudan. The reason for the scarcity of visits is understandable when such restriction is considered

It was concluded in a study conducted with patients receiving orthodontic treatment in Sudan that the habit of brushing twice a day was 46% (3). In a study conducted in Turkey, the rate of regular tooth-brushing twice a day was 39% (18). Such rate was detected 82.6% in this study. We believe that this positive result is due to the convenience of reaching the dentist in the institution where they work.

It was observed in another study conducted in Turkey that individuals do not attach the necessary importance to interface cleanliness (18). When the interface cleaning habits of the individuals were examined in this study, it was seen that 21% of them did not use any tool. It was stated that very limited number of people use dental floss and interface brushes. It was detected that the importance of interface care in this regard was not sufficiently understood, and it was seen that it was compatible with similar studies conducted in other countries.

It was found in a study conducted in Turkey about the knowledge of individuals on dental plaque that they did not have enough information (18). The knowledge of the individuals on dental plaque was examined in this study and it was concluded that 66.3% of the participants did not know what dental plaque was. This result seems normal to us in a society where the rate of going to the dentist is lower. It is a fact that psychological factors can affect oral hygiene habits. In a study, it was seen that depression will increase in pregnant women. This affects oral hygiene habits. Since the pregnancy status of the women participating in the study was not questioned, no conclusion could be reached regarding this (20).

Conclusion

As a result of the research, it was seen that approximately 40 of the patients who have referred to the dental clinic had never been to the dentist and the majority of them did not have sufficient knowledge about dental plaque. It is observed in

the light of these results that the lack of knowledge about oral hygiene continues to exist in the whole society; however, this rate increases in geographies where it is difficult to reach the treatment. These studies should be conducted on large patient series in wider geographies.

Limitations

One of our limitations is that the study is conducted in a single center. The main limitations of this study are not questioning the use of auxiliary dental hygiene tools such as miswak as an alternative to toothbrush, using it on a limited number of people, and getting help from an interpreter for communication.

Acknowledgements: None

Ethical Approval: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article

Author Contributions: *Author Contributions: Concept:.E.E.Ö., M.O. Literature Review:E.E.Ö. Design:E.E.Ö., M.O . Data acquisition: E.E.Ö., M.O. Analysis and interpretation: E.E.Ö., M.O . Writing manuscript: E.E.Ö. Critical revision of manuscript: M.O.*

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

Financial Disclosure: *Authors declared no financial support*

REFERENCES

1. Carranza FA. NMG. Clinical Periodontology, Sixth ed. W.B. Saunders Co. 1996.
2. Lindhe, J., Karring T, Niklaus P.L. Klinische Parodontologie und Implantologie. Ouintessenz Verlags-GmbH, 1999.
3. Develioglu H, Gedik R. Investigation of Oral Hygiene tools and knowledge of individuals. Cumhur Üniversitesi DişHekim. 2001;4(1).
4. Kandemir Ş, Atilla G. A Comparative Study of the Effectiveness of Motivation Applied by Different Persons inChildren in Youth Detention Centers. EÜ Faculty of Dentistry Bulletin. 1991;12:56–60.
5. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. Journal of Industrial Microbiology. 1995;15(3):169-175.
6. Petersen PE. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century - The approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003;31:3–24.
7. Pengpid S, Peltzer K. Prevalence and correlates of dental service utilisation among a national general adult population sample in Sudan. BMC Oral Health. 2021;21(1):1-8.
8. Owens J, Saeed SM. Exploring the oral health experiences of a rural population in Sudan. Int Dent J. 2008;58(5):258–64.
9. World Health Organization (WHO) Regional Ofce for the Eastern Mediterranean. Sudan health profle 2015.URL: https://rho.emro.who.int/sites/defau lt/fles/Profles-briefs-fles/EMROPUB_EN_19610-SUD.pdf. 2021.
10.Sudan Federal Ministry of Health. Health System Financing Review Report May 2014. URL:fle:///C:/Users/user/Downloads/HealthSystemFinancingRe_viewReport.2014.pdf 20 Jan 21. 2014.
11. Nations MK, Nuto S de AS. “Tooth worms”, poverty tattoos and dental care conflicts in Northeast Brazil. Soc Sci Med. 2002;54(2):229–44.
12. Ensor T, Cooper S. Overcoming barriers to health service access: Influencing the demand side.Health Policy and Planning. 2004;19, 69–79.
13. Asa FA, Rahman G, Al Mahmoud N et al. Periodontal disease awareness among pregnant women in the central and eastern regions of Saudi Arabia. Wiley Online Libr. 2015;6(1):8–15.
14. Üstün K, Özdemir E, Sezer U. Hamile Bireylerde Periodontal Farkındalık ve Dental Anksiyetenin Sosyo-Demografik Veriler Işığında Değerlendirilmesi. İnönü University Helath sicences Bulletin. 2013;2(2)1-10.
15. H A Alwaeli, SHA-J. Periodontal disease awareness among pregnant women and its relationship with socio-demographic variables. Int J Dent Hyg. 2005;3(2):74-82.
16. Antonovsky A. Unraveling the mystery of health: How people manage stress and stay well. 1987.
17. Allen PF. Assessment of oral health related quality of life. Health Qual Life Outcomes. 2003;1(1):1-8.
18. Almas K,Al-Malik T, Al-Shehri M, et al. The knowledge and practices of oral hygiene methods and attendance pattern among school teachers in Riyadh, Saudi Arabia. Saudi Med J. 2003;24(10):1087–91.
19 . Loupe MJ. Knowledge and attitude of schoolteachers toward oral health programs and preventive dentistry. J Am Dent Assoc. 1983;107:229–34.
20 .Yaksi N, Tastekin N. Effects of novel coronavirus (covıd-19) pandemic on pregnant psychology. International Journal of Current Medical and Biological Sciences 2022;2(1):1-14

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

New regular candidates to the emergency department; lasting symptoms following acute COVID -19 infection: the example of northwestern Syria

Acil Servislerin Yeni Müdavim Adayları; Geçmeyen COVID-19 Semptomları: Suriye'nin Kuzeybatısı Örneği

Burak ÇELİK [1*], Bahadır Karaca[2]

[1] Kirsehir Training and Research Hospital Kırşehir, TURKİYE

[2] Sancaktepe Şehit Prof.dr. Ilhan Varank Training and Research Hospital Istanbul, TURKIYE

Corresponding author:

Dr. Burak ÇELİK

Adress:

Kirsehir Training And Research Hospital Kırşehir, TURKİYE

email: burakcelik57@hotmail.com

Received: 29.03.2022

Accepted: 22.04.2022

Cite as: CELİK B, KARACA B New Regular Candidates to the Emergency Department; Lasting Symptoms After COVID-19: The Example of Northwestern Syria IJCMBS 2022;2(2):96-102 doi.org/ 10.5281/zenodo.6524489

Abstract

Background: Symptoms decrease during the first 4-weeks after the onset of COVID -19. The situation of patients whose symptoms persist longer or do not seems to be one of the current research topics. In regions with internal turmoil, such as Northwest Syria, emergency departments that carry the burden of emergency and trauma patients were occupied during the COVID-19 pandemic. This study aimed to investigate the post-discharge symptoms of COVID -19 patients hospitalized by the emergency department in northwestern Syria.

Materials and Methods: Between 07/1/2020 and 01/05/2021, 163 patients who were hospitalized and discharged from the emergency department due to COVID-19 in northwest Syria were included in the study. A questionnaire based on the COVID-19 Yorkshire Rehabilitation Scale was administered to all patients. The obtained data were processed into the data collection form and analyzed with explanatory and comparative statistics.

Results: After they developed COVID-19, we noted decreased dry cough, loss of taste and smell, headache symptoms, and increased fatigue and joint pain symptoms. We found that patients who were re-hospitalized following acute COVID-19 infection received longer treatment and those re-hospitalized patients had increased memory impairment, hoarseness, fatigue, and weakness in the post-COVID-19 period.

Conclusion: We think that symptoms and complaints that do not resolve after COVID -19 may be among the common reasons for admission to the emergency department in the future. Emergency departments need to be better prepared to manage persistent COVID -19 symptoms.

Key words: COVID -19, Emergency department, long COVID, post-COVID, Syria

ÖZ

Amaç: Bu çalışmada Suriye'nin kuzeybatısında acil servisten yatırılan COVID-19 hastalarının taburculuk sonrası devam eden semptomlarının araştırılması amaçlanmıştır.

Gereç ve Yöntem: 1.07.2020-01.05.2021 tarihleri arasında Suriye'nin kuzeybatısında acil servisten COVID-19 nedeniyle yatışı yapılan ve taburcu olan 163 kişiye COVID-19 Yorkshire Rehabilitation Scale anketi baz alınarak hazırlanmış bir anket uygulandı. Elde edilen veriler veri kayıt formuna işlendi ve açıklayıcı ve karşılaştırmalı istatistiksel analizler yapıldı.

Bulgular: Katılımcıların %54.6'sı erkek, yaş ortalaması 52.97∓19.45 yıldı ve %17.8'i COVID-19 nedeniyle yoğun bakımda tedavi görmüştü. COVID-19 enfeksiyonu akut dönemi sonrasında kuru öksürük, tat ve koku duyusu kaybı, baş ağrısı semptomlarında azalma, yorgunluk ve eklem ağrısı semptomlarında ise artış saptandı. COVID-19 enfeksiyonu sonrası yeniden hastaneye başvuran hastaların daha uzun süre tedavi aldıkları ve yeniden yatırılan hastaların COVID-19 sonrası dönemde hafızada zorlanma, ses kısıklığı, yorgunluk ve halsizlik şikayetlerinde artış olduğu tespit edildi.

Sonuç: Suriye'nin kuzeybatısında COVID-19 süreci ile pandemi aciller kurulmuş ve bu aciller üzerinden pandemi süreci tüm yönleriyle yönetilmiştir. Gelecekte COVID-19 sonrası geçmeyen semptom ve şikayetlerin acil servise sık başvuru nedenleri arasına girebileceği ve geçmeyen COVID-19 semptomlarına yaklaşımda acillerin daha hazırlıklı olması gerekebileceğini düşünmekteyiz.

Anahtar Kelimeler: COVID -19, Acil Servis, uzamış COVID, post-COVID, Suriye

Highlights

- Due to the persistent of symptoms after COVID-19, emergency room applications continue to increase.
  - It affects the number of hospitalizations.
  - Post-COVID-19 patients also joined the caravan of those who applied to the emergency department due to exacerbation or complications of common chronic diseases.
  - Emergency services should be prepared for patients whose symptoms continue after COVID-19. Even though we are in conflict zones such as Northwest Syria.
- Patients rehospitalized for persistent symptoms after COVID-19 have 3 hallmarks:
  - They were discharged from the hospital earlier.
  - They have stayed in the hospital longer.
  - One of every two patients was taken to the intensive care unit.

Introduction

COVID -19 is a heterogeneous disease affecting the respiratory system and other organs. It has sickened more than 350 million people worldwide, but the pandemic bill does not yet appear to have been met (1). Some survivors of COVID-19 continue to have symptoms, and a new picture, also known as the post-COVID syndrome, is emerging (2).

Acute COVID-19 infection, like other viral infections, may present with symptoms such as fever, fatigue, dry cough, myalgia, and dyspnea (3). Additionally, symptoms such as headache, sore throat, rhinorrhea, gastrointestinal symptoms, conjunctivitis, decreased sense of taste and smell are also common (4). In addition to its routine symptoms, the risk of mortality may increase with the cardiovascular effect of COVID-19 (5).

Generally, symptoms resolve within 4-weeks of starting COVID -19. The situation of patients whose symptoms persist longer or do not resolve, seems to be one of the current research topics. In the literature, COVID -19 symptoms lasting longer than 4-weeks were defined as "long COVID" (6). However, the definition of "long COVID" needed to be expanded as this clinical picture occurred in an increasing number of patients over a long period. Therefore, “ongoing COVID -19” was defined for symptoms lasting between 4 and 12 weeks, and the post-COVID syndrome was defined for symptoms lasting longer than 12 weeks. According to the literature, the most common symptom in more than 40% of patients evaluated as long COVID was found to be fatigue. However, sleep disturbance, dyspnea and cough are other common symptoms, respectively. The prevalence of these symptoms was also found to be more than 25% (7-9).

In northwestern Syria (10), which ranks in the top 5 on the global terror list, the healthcare system has been on the verge of collapse since 2010 due to internal turmoil (11). Since 2016, Turkey has opened hospitals in the region in cooperation with the Turkish Red Crescent. These hospitals started to provide holistic healthcare services under the leadership of emergency services. These hospitals have been converted into pandemic hospitals with the emergence of COVID -19 cases in the region. Applies due to COVID -19 were also handled by pandemic emergency services at these hospitals.

This study aimed to investigate the lasting symptoms after the discharge of COVID -19 patients admitted by the emergency services of the pandemic hospitals in northwestern Syria, where the health care system is in place as part of the humanitarian response.

Materials and Methods

Study Design

This study was conducted with 163 people who applied to the emergency department and were discharged from the pandemic hospital due to COVID-19 in northwest Syria between 01/07/2020-01/05/2021. Participants were given a questionnaire based on the COVID -19 Yorkshire Rehabilitation Scale (C19- YRS). Before the start of the study, ethical approval from the Ethics Committee of Hatay Mustafa Kemal University Non-Interventional Research (date of meetings: 06/05/2021 number of decision: 24), and institutional approval from the relevant hospital administration were obtained. Addition, the study was conducted in accordance with the ethical principles of the Declaration of the World Medical Association of Helsinki.

Selection of participants

In northwest Syria, applications due to COVID-19 have been accepted in the pandemic emergency services. Between 01.07.2020 and 01.05.2021, 3617 patients applied to the pandemic emergency. Patients who were not hospitalized to the pandemic service from these patients were excluded from the study. Among 610 patients who were hospitalized and discharged from the emergency room, 163 patients who came to the hospital for control or applied to the emergency service and volunteered to participate in the study were included in the study. The interviews were conducted face to face with these patients. Patients younger than 18 years, those who presented outside the emergency department, and those whose hospitalization was not due to COVID -19 were excluded from the study.

Obtaining the data

We administered a questionnaire based on C19-YRS to those who agreed to participate in the study [the questionnaire is available free of charge at https://acnr.co.uk/. C19- YRS was created by a multidisciplinary team at the College Hospitals of Leeds, Airedale, and Hull in Yorkshire, England (12). The aim was to assess the long-term symptoms and clinical problems that COVID -19 patients may encounter after discharge. Our survey asked

for general information: the location of the interview, demographic data (age, sex), how many days ago they were admitted to the hospital and whether they were admitted to the service or intensive care unit, the duration of hospitalization, whether they came to the hospital with the recurrent complaints or with the persistent complaints, and whether they were subsequently re-admitted to the hospital. Participants were then asked to rate the severity of shortness of breath, voice, difficulty eating or swallowing, loss of appetite, weight loss, fatigue, distraction, memory difficulties, sadness, and loss of interest complaints before and following COVID -19 using a 10-point Likert scale. They were also asked whether complaints of dry cough, shortness of breath, loss of sense of taste and smell, fatigue, cough with phlegm, sore throat, arthralgias, headache, and nosebleeds occurred during and following the start of COVID -19. We processed the obtained information in a prepared data collection sheet.

Statistical Analysis

Statistical analyses of the study were performed using Statistical Package software for Social Sciences version 25.0 for Windows (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp., USA). The normality assumption was tested with the tests Kolmogorov-Smirnov and Shapiro-Wilk. In addition to these tests, the Kurtosis and Skewness coefficients of the variables were also examined. Explanatory statistics of variables are reported as mean ± standard deviation, median (Min-Max) and n (%). For univariate analyses, the Chi-Square, Fisher's Exact, Fisher-Freeman-Halton exact test, Mann Whitney U and Wilcoxon Sign test were used, depending on the nature of the variables and the availability of assumptions.

Results

The study involved 163 individuals who had applied to the pandemic emergency department and agreed to participate. The mean (±sd) age of the participants was 52.97∓19.45 years, and 89 (54.6%) of the participants were male and 74 (45.4%) were female. The median application time between the diagnosis of COVID-19 and the interview was 21 (min-max: 15-250) days. The median time of application to the emergency department for pandemic post-discharge was 8 (1-150) days. The median length of hospital stay was 7 (1-25) days in the presence of acute COVID-19 infection. Twenty-nine (17.8%) of patients were treated in the intensive care unit due to acute COVID-19 infection. The number of those who applied to the emergency room for treatment due to the persistence of COVID-19 symptoms was 16 (9.8%).

When patients were asked to rate symptoms before and following COVID -19 on a scale of 0-10 (e.g.: none: 0, can't breathe: 10), the mean values obtained and group comparisons before and following COVID -19 are summarized in Table 1.

Table 1. Differences in symptoms before and following COVID -19 (n=163)

	Before COVID-19 Median (Min-Max)	**Following acute COVID-19** Median (Min-Max)	***p****
Shortness of breath	0(0-5)	3(1-10)	<0.001
Change of voice	0 (0-5)	2(1-10)	<0.001
Fatigue during eating and drinking	0(0-5)	2(1-10)	<0.001
Loss of appetite/weight	0(0-4)	3(1-10)	<0.001
Fatigue/weakness	0(0-4)	2(1-10)	<0.001
Distractibility	0(0-8)	2(0-8)	<0.001
Memory strain	0(0-5)	2(0-10)	<0.001
Hoarseness	0(0-7)	2(1-10)	<0.001
Sadness/Loss of interest	0(0-10)	1(0-10)	<0.001

*: Wilcoxon Signed Ranks Test

The frequency and percentages of patients' symptoms during and following acute COVID -19 is shown in Table 2. Following acute COVID -19 infection, patients had a significant decrease in dry cough, loss of taste and smell, headache symptoms, and a significant increase in fatigue and joint pain symptoms ($p < 0.05$).

Table 2. Frequency values of the patients' symptoms during and following COVID -19 (n=163)

	During COVID-19 n, (%)	**Following acute COVID-19 n, (%)**	***p****
Dry cough	127(77.9%)	51(31.3%)	<0.001
Shortness of breath	87(53.4%)	83(50.9%)	0.657
Loss of taste and smell	100(61.7%)	68(41.0%)	0.000
Fatigue	68(41.7%)	85(52.1%)	0.059
Cough with phlegm	67(41.1%)	75(46.0%)	0.372
Throat ache	74(45.4%)	84(51.5%)	0.268
Joint pain	72(44.2%)	91(55.8%)	0.035
Headache	90(55.2%)	70(42.9%)	0.027
Nosebleeds	26(16.0%)	15(9.2%)	0.066

*: Chi-Square test

When examining the radar chart of symptoms (Figure 1), an increase in symptoms of fatigue, cough with phlegm, sore throat, and joint pain was noted following acute COVID-19 infection.
Additionally, 56.3% (n=9) of the patients who were applied to the emergency department with persistent complaints following acute COVID-19 infection (n=16) were patients who had previously received acute COVID-19 treatment in the intensive care unit. We found that those who came to the hospital with complaints that did not improve following the COVID-19 infection stayed longer in the hospital (p=0.034). We found that they received longer treatment (p<0.05). The mean scores of the because of persistent symptoms following acute COVID-19 infection hospitalized patients (n=16) for symptoms before and following COVID -19 are shown in Table 3.

Table 3. Mean scores of patients hospitalized with persistent symptoms following acute COVID-19 infection, based on their symptoms (n=16)

	Before COVID-19 Median (Min-Max)	**Following acute COVID-19** Median (Min-Max)	**p***
Shortness of breath	1(0-3)	8(1-10)	<0.001
Change of voice	1(0-10)	3(1-10)	<0.001
Fatigue during eating and drinking	1(0-3)	9.5(1-10)	<0.001
Loss of appetite/weight	1(0-3)	7.5(1-10)	<0.001
Fatigue/weakness	1(0-4)	10(1-10)	<0.001
Distractibility	1(0-3)	2(1-7)	<0.001
Memory strain	1(0-3)	3(1-10)	<0.001
Hoarseness	1(0-4)	7(1-10)	<0.001
Sadness/Loss of interest	1(0-4)	4(1-8)	<0.001

*: Wilcoxon Signed Ranks Test

It was determined that patients (n=19) who were due to persistent or recurrent symptoms following acute COVID-19 infection hospitalized after recurrent admissions left the hospital a short time ago and received treatment for a longer period of time ($p < 0.05$). The mean scores of these patients for their symptoms and group comparisons are summarized in Table 4. According to these results, complaints of memory difficulties, hoarseness, fatigue, and weakness increased in hospitalized patients during the post-COVID-19 period ($p < 0.05$).

Table 4. The mean values for the persistent or recurrent symptoms of the patients who were treated at the time of readmission to the hospital (n=19)

	During COVID-19 Median (Min-Max)	**Following acute COVID-19** Median (Min-Max)	**p***
Shortness of breath	3(1-5)	3(1-10)	0.096
Change of voice	2(1-4)	2(1-5)	0.352
Fatigue during eating and drinking	2(1-5)	2(1-4)	0.206
Loss of appetite/weight	3(1-4)	2(1-10)	0.494
Fatigue/weakness	3(1-4)	10(1-4)	<0.001
Distractibility	3(1-8)	3(1-7)	0.914
Memory strain	1(1-4)	4(1-6)	0.016
Hoarseness	1(1-5)	6(2-10)	0.020
Sadness/Loss of interest	1(1-9)	5(1-9)	0.096

*: Wilcoxon Signed Ranks Test

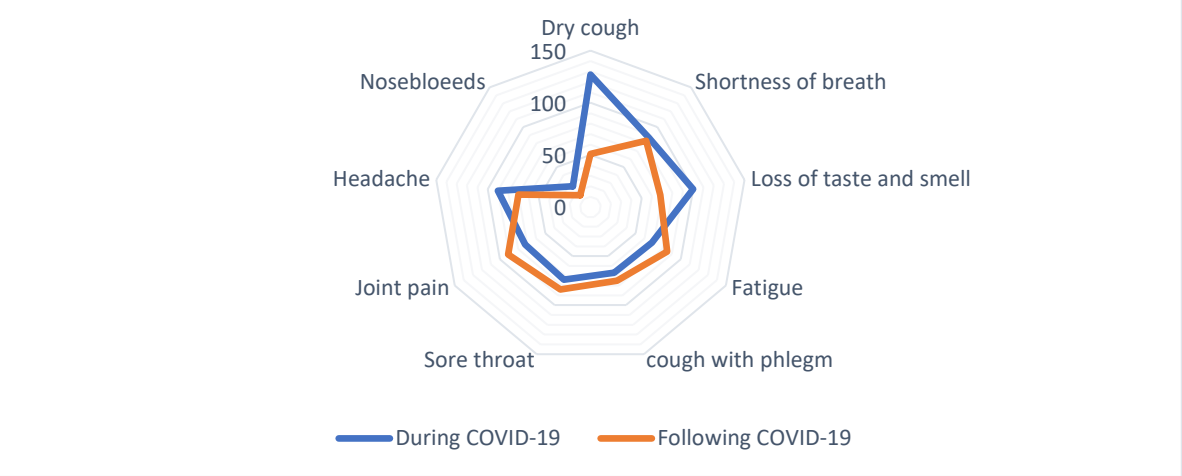


Figure 1. Distribution of patients' complaints during and following COVID -19 (n=163)

Discussion

In regions where there is conflict and turmoil, health care focuses on emergency and trauma patients. However, socioeconomic difficulties and epidemics and their consequences are unknown in these regions.

In a survey conducted through social media, it was found that 26% of people who had COVID -19 had painful symptoms (13). When meta-analyses were examined, muscle and joint pain were present in 35% to 50% of all COVID -19 cases (14), and these symptoms were observed to persist in 1 in 4 patients after treatment for COVID -19 (15). In our study, in accordance with the literature, it was found that approximately half of the patients had joint pain both during and following acute COVID-19 infection. In general, joint pain seems to be one of the most common complaints following acute COVID-19 infection.

A review of the literature shows that 5% to 20% of COVID -19 cases complain of sore throat and that sore throat usually persists after other symptoms of infection have resolved. (14,16) Painful necrotic and aphthous ulcers reported in patients may contribute to changes in taste and smell in addition to sore throat (14). In our study, we observed that almost half of the patients suffered from sore throats. Conditions such as poor oral hygiene and tobacco use may cause this symptom more frequently in Syria, where socioeconomic status is poor.

Zayet et al. (15), found that 74% of patients with persistent symptoms after COVID -19 had a loss of smell, and 31.5% had a loss of taste. While in our study, 61.7% of patients suffered from a loss of taste and smell at the beginning of COVID -19, this rate decreased to 41.0% after COVID -19, and it is one of the most common symptoms that persist in parallel with the literature. The loss of taste and smell likely affects the quality of life of patients with COVID -19.

The rate of depression after COVID -19 varies from 11% to 28% in the literature (16). In studies conducted in Turkey, it has been shown that COVID-19 and related effects cause psychiatric effects in healthcare workers and also in pregnant women (17,18,19). Some studies also reported cases of psychosis (20). Women and individuals with previous psychiatric disorders are more psychopathologically affected by COVID-19. However, depression and anxiety symptoms are reported more frequently in individuals with a shorter length of hospital stay (21). In our study, we observed an increase in sadness and loss of interest in patients readmitted to the hospital with unresolved symptoms. COVID-19 can affect the emotional states of patients who experience social isolation and have difficulties in the treatment process. It may have caused further distress and loss of interest in patients who felt inadequately treated and did not receive adequate medical care after re-hospitalization. It has been shown that social and economic factors increase the tendency to depression in patients following acute COVID-19 infection (22-24). It is reasonable to assume that the social and economic difficulties associated with poverty and the inability to cope with it, especially in northern Syria, may lead to depression in the current patient population after COVID -19.

A literature review shows that during COVID -19, headaches occur in 11% to 40% of cases. In our study, we noted headaches in about half of the patients. While headache is one of the ongoing symptoms like other pain, patients with chronic headaches may also be triggered by COVID -19. While cerebrovascular events due to thromboembolic conditions can be seen with COVID -19, subcortical changes have also been noted in some brain MRIs. Associations with various diseases, such as Parkinson's disease and post-flu psychosis, were found in the 1918 Spanish flu (14). COVID -19 has not yet completed its impact, can leave many lasting traces, and what we learn from this infection can influence the rest of our lives (25). Because of the conditions in the northern Syria's region, few advanced neurologic examination tests are available. However, it is suspected that patients with headaches may have other associated symptoms or may have neurologic problems in the future.

In our study, we observed an increase in memory difficulties, distractibility, sadness, and loss of interest between the onset of COVID -19 and later hospitalizations. Cognitive impairment was observed in 67% of patients presenting to the neurology outpatient clinic in Germany after COVID -19, whereas depressive symptoms were observed in 65% (25). In addition to the neuropsychiatric findings, the meta-analysis by Jennings et al. (8), found that the rate of fatigue in ongoing COVID -19 was 43%. In our study, consistent with the general literature, fatigue was observed in more than half of the participants. According to neuroinflammatory models, the hypothalamic paraventricular nucleus is affected in severe infections, and the persistence of fatigue is a effect of severe infections, along with neuropsychiatric effects (26). In areas of civil turmoil, as in the regions where our study was conducted, emergency services may provide the closest contact with patients. Once such complex neuropsychiatric syndromes are identified, it would likely be more beneficial for patients to be referred by any emergency department that provides initial evaluation and medical attention. Of course, it is known that it is more convenient in terms of cost effectiveness to establish post covid polyclinics where routine control and rehabilitation of these patients can continue. however, the regional conjuncture does not allow this.

In our study, more than half of the patients who came to the hospital with complaints that did not improve following the COVID-19 infection was treated in the intensive care unit. Those treated in the intensive care unit were hospitalized more frequently when they were applied to the hospital again. Also, the average length of treatment in hospital in acute COVID-19 time was longer for patients who were re-hospitalized because of persistent or recurrent COVID-19 symptoms than for those who were not hospitalized because of persistent or recurrent COVID-19 symptoms. This may have resulted in the need to seek medical attention again, depending on the presence of persistent symptoms or progress of comorbidities, even if the treatment of the critically ill had been completed in the hospital. For this reason, the people and institutions that provide health care must be prepared for this situation, especially in the follow-up of patients treated in the intensive care unit and the emergency services.

Hospitalization may be required for patients with symptoms that do not resolve after COVID -19. Therefore, a thorough examination should be done in the emergency department.

The most common GIS symptom in COVID-19 patients is anorexia, and symptoms such as nausea, vomiting, diarrhea, gastrointestinal bleeding, and abdominal pain have also been noted. It has even been noted that at the onset of COVID-19, gastrointestinal symptoms may occur even before fever or other symptoms of COVID-19 (27, 28). In another review, it was observed that weight and appetite loss have been complained in ongoing COVID -19 (8). In our study, we showed that weight and appetite loss complaints increased after the disease. The aforementioned GIS symptoms and decreased sense of taste and smell suggested that appetite loss may increase. The nutritional deficiency of patients living in difficult conditions due to socioeconomic problems is already a problem. As mentioned in our study, it can be assumed that weight loss may increase with COVID-19 and accompanying medical problems. Even if the COVID-19 treatment is completed, there may be an increase in recurrent emergency applications with such comorbidities that may arise with COVID-19.

Complaints of cough, shortness of breath, sore throat, and hoarseness were persistent symptoms in our study as well as in the literature (15). In our study, the complaints of dyspnea were prevalent. The effects of polluted air in conflict areas and increased tobacco use in underdeveloped societies may exacerbate respiratory symptoms with COVID -19. Although some studies have shown that tobacco users do not suffer more COVID -19 than nonsmokers (29), there are insufficient data on persistent symptoms. The overlap of these respiratory illness with other respiratory illness or symptoms may increase the emergency department and ICU admissions. In Bangladesh, one of the socioeconomically similar countries, shortness of breath is shown as one of the main problems of hospitalizations both during and following acute COVID-19 infection. Moreover, one-third of the patients still had shortness of breath 2 months later (30). Looking at developed countries, it was found that in countries such as France and Italy, 40% of patients suffer and continue to suffer from shortness of breath. The incidence of respiratory tract diseases may vary depending on climate and air pollution. However, the uncertainty of how long the symptoms of dyspnea last and the difficulty of emergency care and medical management of these patients indicate that the costs of COVID-19 are and will be high. In addition to conditions such as chronic obstructive pulmonary disease and asthma, complaints of shortness of breath that do not resolve after COVID -19 appear to be among the reasons for emergency department routine applications.

Conclusion

In northwestern Syria, pandemic emergency departments were established for the COVID -19 pandemic, and the pandemic process was managed by these departments in all aspects. In this study, the persistent symptoms of COVID-19 after discharge of COVID-19 patients hospitalized from the emergency room were investigated. It was observed that the patients applied to the emergency service due to the persistence of the symptoms and their hospitalizations were repeated. Possibly, symptoms and complaints that do not resolve following COVID-19 and exacerbation of comorbidities of COVID-19 will become reasons for routine emergency department applications; and patients with such complaints will become new regulars of emergency departments. We believe this research will help improve the preparedness of emergency departments in dealing with persistent COVID-19 symptoms.

Limitation

Because of in regions where civil turmoil and terrorism are prevalent, the inability to reach elderly patients who have completed acute COVID -19 treatments in the hospital is a limitation of our study. The lack of telephone or other means of communication with Internet options for socioeconomic reasons makes it difficult to reach patients or their relatives.

Acknowledgments: *The authors would like to thank Prof. Dr. Özkan GORGULU (Kırşehir Ahi Evran University, Faculty of Medicine, Biostatistics and Medical Informatics Department) for his assistance in the statistical analysis of the study.*

Ethical Approval: *Non-Interventional Clinical Trials Ethics Committee of Hatay Mustafa Kemal University Tayfur Ata Sökmen Faculty of Medicine no:24/ 06.05.2021*

Author Contributions: *Concept:B.Ç., B.K. Literature Review: B.Ç., B.K. Design: B.Ç., B.K. Data acquisition: B.Ç., B.K. Analysis and interpretation: B.Ç., B.K. Writing manuscript: B.Ç., B.K. Critical revision of manuscript: B.Ç.*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019 [cited: 09.01.2022]
2. Yong SJ & Liu S. Proposed subtypes of the post-COVID-19 syndrome (or long-COVID) and their respective potential therapies. Reviews in medical virology. 2021; e2315.
3. Chen ATC, Coura-Filho GB, Rehder MHH. Clinical characteristics of COVID-19 in China. New Engl J Med 2020;382.
4. McIntosh K. Coronavirus disease 2019 (COVID-19): Epidemiology, virology, clinical features, diagnosis, and prevention. UpToDate, 2021. Available at: https://www.uptodate.com/contents/coronavirus-disease2019-COVID-19-epidemiology-virology-and-prevention

5. Ocak M, Tascanov MB, Yurt NŞ, et al. A new predictor for indicating clinical severity and prognosis in COVID-19 patients: Frontal QRS-T angle. The American Journal of Emergency Medicine, 2021;50, 631-35.
6. Greenhalgh T, Knight M, A'Court C, et al. Management of post-acute COVID-19 in primary care. BMJ. 2020;370: m3026.
7. Cevik M, Kuppalli K, Kindrachuk J, et al. Virology, transmission, and pathogenesis of SARS-CoV-2. BMJ. 2020;371:m3862.
8. Jennings G, Monaghan A, Xue F, et al. A systematic review of persistent symptoms and residual abnormal functioning following acute COVID-19: Ongoing symptomatic phase vs. post-COVID-19 syndrome. Journal of Clinical Medicine. 2021;10(24): 5913.
9. Oran DP & Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. Ann Intern Med. 2020;173(5): 362-67.
10. https://visionofhumanity.org/wp-content/uploads/2020/11/GTI-2020-web-1.pdf [cited:08.02.2022]
11. Akbarzada S & Mackey TK. The Syrian public health and humanitarian crisis: a 'displacement' in global governance? Global Public Health. 2018;13(7), 914-930.
12. O'Connor RJ, Preston N, Parkin A, et al. The COVID-19 Yorkshire Rehabilitation Scale (C19-YRS): application and psychometric analysis in a post-COVID-19 syndrome cohort. Journal of Medical Virology. 2022;94(3), 1027-34.
13. Lambert NJ & Corps S. COVID-19 "long hauler" symptoms survey report. 2020
14. Walitt B & Bartrum E. (2021). A clinical primer for the expected and potential post-COVID-19 syndromes. Pain reports, 6(1).
15. Zayet S, Zahra H, Royer PY, et al. Post-COVID-19 Syndrome: Nine Months after SARS-CoV-2 Infection in a Cohort of 354 Patients: Data from the First Wave of COVID-19 in Nord Franche-Comté Hospital, France. Microorganisms, 2021;9(8), 1719.
16. Renaud-Charest O, Lui LM, Eskander S, et al. Onset and frequency of depression in post-COVID-19 syndrome: A systematic review. Journal of Psychiatric Research. 2021;144, 129-37.
17. Sun P, Qie S, Liu Z, et al. Clinical characteristics of hospitalized patients with SARS-CoV-2 infection: a single-arm meta-analysis. Journal of medical virology, 2020;92(6), 612-17.
18. Ocak M, Yurt NŞ, Yurt YC, et al. The Burnout Levels of Emergency Employees in COVID-19 Pandemic and the Related Factors. Harran Üniversitesi Tıp Fakültesi Dergisi. 2021;18(2), 250-55.
19. Yaksi N & Tastekin N. Effects of COVID-19 pandemic on pregnant psychology. The International Journal of Current Medical and Biological Sciences. 2022;2(1):1-14.
20. Ignatova D, Krasteva K, Akabalieva K, et al. Post-COVID-19 psychosis: Cotard's syndrome and potentially high risk of harm and self-harm in a first-onset acute and transient psychotic disorder after resolution of COVID-19 pneumonia. Early intervention in psychiatry. 2021
21. Mazza MG, De Lorenzo R, Conte C, et al. COVID-19 BioB Outpatient Clinic Study Group. Anxiety and depression in COVID-19 survivors: Role of inflammatory and clinical predictors. Brain, behavior, and immunity. 2020;89, 594-600.
22. Chirumbolo A, Callea A, Urbini F. The effect of job insecurity and life uncertainty on everyday consumptions and broader life projects during COVID-19 pandemic. International Journal of Environmental Research and Public Health, 2021, 18.10: 5363.
23. Sepúlveda-Loyola W, Rodríguez-Sánchez I, Pérez-Rodríguez P, et al. Impact of social isolation due to COVID-19 on health in older people: mental and physical effects and recommendations. The journal of nutrition, health & aging, 2020;24(9), 938-47.
24. Szlamka Z, Kiss M, Bernáth S, et al. Mental health support in the time of crisis: Are we prepared? Experiences with the COVID-19 counselling programme in Hungary. Frontiers in psychiatry. 2021;12, 792.
25. Boesl F, Audebert H, Endres M, et al. A Neurological Outpatient Clinic for Patients With Post-COVID-19 Syndrome—A Report on the Clinical Presentations of the First 100 Patients. Frontiers in Neurology. 2021;12.
26. Mackay A. A paradigm for post-COVID-19 fatigue syndrome analogous to ME/CFS. Frontiers in Neurology. 2021;1334.
27. Schmulson M, Ghoshal UC, Barbara G. Managing the inevitable surge of post–COVID-19 functional gastrointestinal disorders. Official Journal of the American College of Gastroenterology ACG, 2021;116(1), 4-7.
28. Tian Y, Rong L, Nian W, et al. Review article: Gastrointestinal features in COVID-19 and the possibility of faecal transmission. Aliment Pharmacol Ther 2020; 51:843–51
29. Khorrami Z, Nili S, Sharifi H, et al. Association of cigarette smoking, obesity, and underlying medical conditions with COVID-19 hospitalization and mortality in Iran: A nationwide retrospective ecological study. Medical Journal of the Islamic Republic of Iran. 2020;34, 133.
30. Islam MK, Hossain MF, Molla MMA, et al. A 2-month post-COVID-19 follow-up study on patients with dyspnea. Health science reports, 2021;4(4), e435.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

Transition to Web-Based Asynchronous Education in Biostatistics Education During The Covid-19 Pandemic: A Case of Bursa Uludag University

Covid-19 Pandemisi Sırasında Biyoistatistik Eğitiminde Web Tabanlı Asenkron Eğitime Geçiş: Bursa Uludağ Üniversitesi Örneği

Güven ÖZKAYA[1*], Mevlüt Okan AYDİN [2]

[1] Bursa Uludag University, Faculty of Medicine, Department of Biostatistics Bursa,TURKİYE
[2] Bursa Uludag University, Faculty of Medicine, Department of Medical Education Bursa, TURKİYE

Corresponding author:

Dr. Güven Özkaya
Adress:
Department of Biostatistics, Faculty of Medicine,Bursa Uludag University Bursa,TURKİYE
email: ozkaya@uludag.edu.tr
Received: 07.04.2022
Accepted: 06.05.2022
Cite as: OZKAYA. G, AYDIN MO. Transition to Web - Based Asynchronous Education in Biostatistics Education During The Covid-19 Pandemic: A Case of Bursa Uludag University IJCMBS 2022;2(2):103-110 doi.org/ 10.5281/zenodo.6524527

Abstract

Background: Over time, the integration of advancing technology into education gained speed due to the pandemic. Alternative education strategies to traditional education emerged. The study aims to examine whether web-based asynchronous (WBA) education can be used as an alternative to traditional face-to-face (FTF) education if the views of post-graduate students are in line with it. **Materials and Methods:** The opinions of 19 students who enrolled in the post-graduate biostatistics course in the spring semester of 2019-2020 and participated in both FTF and WBA were consulted. Students were asked 17 questions about the distance education perception scale, educational strategies and 3 open-ended questions. Post-graduate students were grouped according to their answers to the question of which type of education would prefer if the students had to take the biostatistics course once again. **Results:** Thirteen of the students (68.4%) stated that if they would take this course once again, they would prefer WBA, 5 (26.3%) students FTF and only 1 (5.3%) student prefer online education. While FTF students were more satisfied with FTF training (p=0.014), WBA students stated that they were more satisfied with the WBA education (p=0.004). WBA students thought that WBA education provides more flexibility in terms of time (p=0.046) and resources (p=0.007). WBA students stated this education would be preferred more in the future (p=0.035). **Conclusion**: WBA education is a very good alternative for courses similar to biostatistics courses where computer programs are frequently used in practice. In case students and lecturers are provided with internet and necessary hardware and software support by the state and universities, the transition to WBA education may be permanent.

Keywords: Biostatistics education; Distance education perception scale; Face-to-face education; Web-based asynchronous education

ÖZ

Amaç: Zamanla, gelişen teknolojinin eğitime entegrasyonu pandemi nedeniyle hız kazandı. Geleneksel eğitime alternatif eğitim stratejileri ortaya çıktı. Bu çalışma, lisansüstü öğrencilerinin görüşleri doğrultusunda web tabanlı asenkron eğitimin geleneksel yüz yüze eğitime alternatif olarak kullanılıp kullanılamayacağını incelemeyi amaçlamaktadır. **Gereç ve Yöntem**: 2019-2020 bahar döneminde lisansüstü biyoistatistik dersine kayıt yaptıran ve hem yüz yüze hem de web tabanlı asenkron eğitime katılan 19 öğrencinin görüşlerine başvurulmuştur. Öğrencilere uzaktan eğitim algı ölçeği, eğitim stratejileri ile ilgili 17 soru ve açık uçlu 3 soru sorulmuştur. Lisansüstü öğrenciler tekrar biyoistatistik dersini almaları gerekse hangi eğitim türünü tercih ederler sorusuna verdikleri yanıtlara göre gruplandırılmıştır. **Bulgular**: Öğrencilerin 13'ü (%68,4) bu dersi bir kez daha alacak olsalar web tabanlı asenkron eğitimi, 5 öğrenci (%26,3) yüz yüze eğitimi ve sadece 1 öğrenci (%5,3) online eğitimi tercih edeceklerini belirtmişlerdir. Yüz yüze eğitimi tercih eden öğrenciler yüz yüze eğitiminden daha memnun iken (p=0,014) web tabanlı asenkron eğitimi tercih eden öğrenciler web tabanlı asenkron eğitiminden daha memnun olduklarını belirtmişlerdir (p=0,004). Web tabanlı asenkron eğitimi tercih eden öğrenciler, web tabanlı asenkron eğitiminin zaman (p=0,046) ve kaynaklar (p=0,007) açısından daha fazla esneklik sağladığını düşünmüşlerdir. Web tabanlı asenkron eğitimi tercih eden öğrenciler, bu eğitimin gelecekte daha çok tercih edileceğini belirtmişlerdir (p=0,035). **Sonuç**: Web tabanlı asenkron eğitim, uygulamada bilgisayar programlarının sıklıkla kullanıldığı biyoistatistik derslerine benzer dersler için çok iyi bir alternatiftir. Devlet ve üniversiteler tarafından öğrencilere ve öğretim görevlilerine internet ile gerekli donanım ve yazılım desteğinin sağlanması durumunda web tabanlı asenkron eğitimine geçiş kalıcı olabilir.

Anahtar Kelimeler: Biyoistatistik eğitimi; Uzaktan eğitim algı ölçeği; Yüz yüze eğitim; Web tabanlı asenkron eğitim

Highlights

- Web-based asynchronous education is one of the first educational methods to be applied in emergencies such as a pandemic.
- Interaction with students, which is the difference of face-to-face education, can be reduced by online interaction.
- Unlike other studies, the students participating in this study first received face-to-face and Web-based asynchronous education afterward.

Introduction

Due to the pandemic, which started at the end of 2019 and affected our country since the first period of 2020, there have been changes in many areas. During the quarantine period, there were serious problems in education around the world (1). The education was continued by providing the optimal way for students while the studies related to the vaccine and drugs were accelerated due to the pandemic. While the face-to-face education (FTF) of students was suspended due to current conditions, the uncertainty in the pandemic process prevented the time to return to face-to-face education from being clarified (2).

Distance education provided a short-term and emergent solution for education that could not be done FTF. This development has led to an increase in the number of studies on distance education This development has led to an increase in studies on distance education (3–5). Video communication became popular among students and researchers and often preferred video communication (6,7). During the pandemic period, many countries used Zoom, Skype, FaceTime, etc. for video communication and instant message applications started to offer distance education to students (8). Also, education platforms, where lesson programs are announced, instant meetings, online lessons, exams, homework, and applications are made, have become one of the important elements used in distance education. These training platforms have started to take place in information and communication technology studies.

Distance education has been widely used as a compulsory alternative to FTF education during the pandemic period. Online (synchronous) education and web-based asynchronous (WBA) education come to the fore in distance education, which is offered as an alternative to FTF education. Some researchers have suggested that the flexible nature of WBA education can be accomplished through video, thus combining the effects of FTF education with the flexibility of online environments (7,9,10). Distance education differs on levels of the education system and the characteristics of the courses (Theoretical and/or practical etc.). While transitioning to distance education instead of FTF education during the pandemic period, online or WBA education preferences differed according to countries/cities/regions. The biostatistics course, which is widely given as an online course and also taught as a course in colleges and universities, depends on the developments in information communication technologies due to the use of both software and hardware tools. The biostatistics course is not only precious in theory but also in practice. It involves multi-disciplines such as medicine, public health, and nursing. While the number of medical research increases, the demand of biostatistics course increased due to the increasing requirement of medical students as well as those working in pharmaceutical companies, research hospitals, states, and universities (11).

It is an important issue whether the compulsory strategy change due to the pandemic in biostatistics education, which is at the intersection of technology use, FTF and distance education, is sustainable in the following periods. In our university, the biostatistics course is carried out as a compulsory course in the Institute of Health Sciences in post-graduate education. Considering the post-graduate education process in our country, it is common for students to work in a job or study for a master's degree from a different city/region during their post-graduate education. Considering this situation, WBA education was preferred while providing distance education to graduate students in our study instead of online synchronous.

This study aims to examine the perspectives of postgraduate students on both education methods after the emergency transition from FTF education to WBA education due to the pandemic and to compare their preferences and reasons in the following periods. After the pandemic, it will guide us in the direction of continuing the biostatistics course in post-graduate education in the traditional way or with WBA education. While comparing the views of students who were educated with different methods in similar studies conducted before, our study also examines the views of students due to their participation in both FTF and WBA education. This feature reveals the difference between our study from other studies.

Methods

Post-graduate courses at Bursa Uludag University started for the spring semester on 10th February 2020. Biostatistics courses were held face-to-face between February 10 to March 16, and distance education from 16th March 2020 (Figure-1). Ethical approval was taken from the Bursa Uludag University, Faculty of Medicine, Clinical Research Ethics Committee (Date 10 June 2020; Number: 2020-10/24). We conducted this study according to the principles of the Declaration of Helsinki. Informed consent was obtained from all the participants.

The FTF education, was held in the computer laboratory of our department. Theoretical issues and practices were carried out in the same place. There is one computer for each student in the laboratory. Thus, the students carried out the practice alone. The course was conducted by only one lecturer during the spring semester. The course was held as WBA education over Google Classroom. Google Classroom has been used frequently in distance education and has been the subject of research (12–15). It was preferred owing to the opportunities for students and faculty members such as the fee, and the capacity of the programs. In this process, the lectures were recorded on video by the lecturer and shared with the students via Google classroom on the day of the lecture every week. Students were asked to watch the video, review the topics covered, and complete their homework until the next week. During this process, the questions that were not understood by the students were conveyed to the lecturer via e-mail, telephone, and instant messaging service.

Study settings and participants

We conducted a cross-sectional survey utilizing a self-administered survey. In the study participation is on a voluntary basis and no identifying information was requested. In the 2019-2020 Spring semester, 24 master of science students attended the Biostatistics course which is a compulsory course in the graduate program of the Institute of Health Sciences. One student did not attend the course during FTF education and four students did not continue WBA education after the transition to distance education. A total of five students were excluded from the study because they took this course for the second time and they preferred not to attend the whole education since attendance is not compulsory. After the exclusion, the study was conducted out on 19 students.

Studies conducted for the optimal number of students in distance education have been generally researched for online education in the literature. Although there is no study on the optimal number of students for WBA education, the optimal number of students in online education has been considered due to the interaction with the students via e-mail, telephone, instant messaging service, etc. Although various studies have been conducted on the optimal number of students for online education courses, there is no common consensus (16). Current literature suggests that the optimal number of students for online education should be between 15 and 30, manageable for a lecturer using average levels of interaction with students (17–19). Considering this information, it is seen that the number of students enrolled in the course is within the optimal limits.

Data collection and ethical considerations

Firstly, we asked overall 19 master of science students to fill in a demographic questionnaire. The demographic questionnaire consisted of age, gender, nationality, and job status. They were also asked whether they took this course for the first time and whether they have attended an online/WBA education before. Secondly, we generated questions from published scientific literature and other questionnaires. 17 questions were asked to get students' views on FTF and WBA education. All 17 questions in these surveys were on a 5-point Likert scale (1: strongly disagree to 5: strongly agree).

A 5-point Likert scale consisting of 22 items and 4 factors, developed by Özkaya et al., was applied to measure students' perceptions of distance education (20). The scale consisting of the factors "Students' perception", "Equipment facility", "Time Management" and "Facility and support of the institution" is a five-point Likert scale (between “1: strongly disagree” and “5: strongly agree”). The high scores obtained from the scale indicate a positive perception of distance education.

Finally, 3 open-ended questions were examined in order to receive feedback from the students about the education they received. These three open-ended questions were: “What are the advantages and disadvantages of FTF education?”, “What are the advantages and disadvantages of WBA education?”, and “What is required for a successful learning in biostatistics course?”.

Data analysis

The qualitative analysis was conducted by analyzing the open-ended responses using word cloud used as a tool to give focus on the written material. We performed a word-cloud analysis using the “tm”, “SnowballC”, “wordcloud”, and “RColorBrewer” packages for RStudio Version 1.4.1106 (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.). The frequencies of a specific word are reflected by the size of the text. The FTF and WBA words were excluded from the word-cloud analysis since opinions were requested about them.

Students' responses to 17 questions were statistically compared with the quantitative analysis. The data was examined by the Shapiro Wilk test to whether or not it presents normal distribution. The results were presented as mean ± standard deviation, median (interquartile range), or frequency. Mann Whitney U tests were used for nonnormally distributed data. The statistical significance level was considered as $p<0.05$. Statistical analyses were performed with IBM SPSS ver.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

Results

The mean age of the 19 students participating in the study was found to be 27.4 ± 3.5. Fifteen of the students (78.9%) were female and, 4 (21.1%) were male. Two students were foreign nationals. While 12 (63.2%) students took this course for the first time, the others took the course for the second time. Eleven students (57.9%) participated in any online / WBA education before the pandemic. Thirteen of the students (68.4%) stated that if they would take this course once again, would prefer WBA, five students (26.3%) FTF and only one student (5.3%) prefer online education (Table 1).

Table 1. Demographic variables n=19

Age (years)		27.4±3.5
Gender	Female	15 (78.9%)
	Male	4 (21.1%)
Nationality	National	17 (89.5%)
	International	2 (10.5%)
Do you have any jobs?	Yes	15 (78.9%)
	No	4 (21.1%)
Are you taking this lesson for the first time	Yes	12 (63.2%)
	No	7 (36.8%)
Have you attended any online/ web-based asynchronous education and/or courses before?	Yes	11 (57.9%)
	No	8 (42.1%)
If you want to take this course again, which one would you prefer?	FTF education	5 (26.3%)
	WBA education	13 (68.4%)
	Online education	1 (5.3%)

FTF: Face-to-face, WBA: Web-based asynchronous

Table 2 shows the results of the analysis of the answers given to the 17 questions of the students who chose the FTF education and the WBA education if they had taken this course again. There was no statistically significant difference between the FTF and WBA students in terms of internet access (p=0.117). WBA students were more motivated in WBA education than FTF students (p<0.001). WBA students found the course easier than FTF students in terms of the difficulty of the course (p=0.002). While students who preferred WBA education did not prefer to teach theoretical courses FTF (p=0.003), there was no significant difference between the two groups in terms of giving the practices in the form of WBA education (p=0.143). The score given to the question "It was easier to ask and discuss the incomprehensible issues in face-to-face education" was found to be higher in FTF students (p=0.019). There was no statistically significant difference between the two groups in terms of their answers to "In WBA education, I can ask questions to the lecturer at any time." (p=0.924) and "It is important for me to attend classes with my friends in face-to-face education." (p=0.289) questions. WBA students thought that the skill of the lecturer was important in choosing the type of education according to FTF students (p=0.002). WBA students agree more with the notion that "I can learn with WBA education without FTF education" (p=0.014). While FTF students were more satisfied with FTF training (p=0.014), WBA students were more satisfied with the WBA education (p=0.004). WBA students thought that WBA education provides more flexibility in terms of time (p=0.046) and resources (p=0.007). WBA students had more analytical thinking opportunities with WBA education (p=0.019). The WBA education influenced positively their opinions about this education (p=0.035), and it would be preferred more in the future (p=0.035).

Table 2. Comparison of the opinions of students who preferred FTF and WBA education*

If you want to take this course again, which one would you prefer?	FTF (n=5)	WBA (n=13)	p
1. I can easily provide internet access required for web-based asynchronous education.	3 (3-4)	5 (4-5)	0.117
2. I am more motivated in web-based asynchronous education than face-to-face education.	2 (1-2)	4 (4-5)	<0.001
3. Web-based asynchronous education was easier than face-to-face education in terms of the difficulty of the lesson.	2 (2-2)	4 (3-5)	0.002
4. I prefer face-to-face education in theoretical lectures.	4 (3-5)	2.5 (2-3)	0.003
5. I prefer web-based asynchronous education in applications.	2 (2-2)	4 (2-4)	0.143
6. It was easier to ask and discuss the incomprehensible issues in face-to-face education.	4 (3-4)	3.5 (3-4)	0.019
7. In web-based asynchronous education, I can ask questions to the lecturer at any time.	4 (4-4)	4 (4-4)	0.924
8. The lecture ability of the lecturer is important in choosing the education style.	4 (3-4)	4.5 (4-5)	0.002
9. I can learn with web-based asynchronous education without face-to-face education.	3 (2-3)	4 (3-4)	0.014
10. It is important for me to attend classes with my friends in face-to-face education.	3 (3-4)	2.5 (2-4)	0.289
11. I am satisfied with the face-to-face education.	4 (4-4)	3 (3-4)	0.014
12. I am satisfied with the web-based asynchronous education.	3 (3-3)	4.5 (4-5)	0.004
13. Compared to face-to-face education, web-based asynchronous education provides us with flexibility in terms of time usage.	4 (4-4)	5 (4-5)	0.046
14. Compared to face-to-face education, web-based asynchronous education provides us with flexibility in terms of resource use.	3 (2-3)	5 (4-5)	0.007
15. In the web-based asynchronous education environment, students get the opportunity to think analytically.	3 (3-4)	4 (3-4)	0.019
16. My experiences in web-based asynchronous education have positively changed my perspective on web-based asynchronous education.	3 (2-3)	4 (4-4)	0.035
17. I believe that in the future, web-based asynchronous education will be more preferred than traditional education.	2 (2-2)	4 (2-5)	0.035

*Descriptive statistics were given as median (interquartile range). FTF: Face-to-face, WBA: Web - based asynchronous

When the four factors and total score of the scale applied to the students were compared between the groups, "Students' perception" (p=0.027) and "Time Management" (p=0.019) were found to be statistically significant, while there was no statistically significant difference in terms of "Equipment facility", "Facility and support of the institution" and total score. Both "Students' perception" and "Time Management" factor scores of WBA students were found to be higher. Responses from students were taken in a written form, which was quantified based on the frequency of occurrence and visualized in Figure 2. As shown in figure 2, the students' thinking can be viewed as wildly divergent. Some of the answers given by students to open-ended questions about FTF and WBA were given below. The reasons for the positive and negative thoughts of the students against these educational strategies can be seen in the below sentences.

"…Although it provides usefulness for the employees in terms of the problem of creating time, I believe that WBA education is not likely to be as educational as FTF education..."
"…In WBA education, we had the chance to watch the lecture videos on the internet at any time and place with the opportunities provided by our teacher. We were able to access the lecture videos whenever we wanted and as many times as we wanted, without time constraints. At the same time, it provides advantages such as watching the lesson by dividing it, rewinding or advancing…"
"…Thanks to the WBA education, the time to go to university, the status of taking leave for the employees, the expenses, the problem of absenteeism, and missing the course are all gone…"
"…I think that in FTF education at school, there is a more disciplined study, more active listening to the lesson, and fewer distractions…"

Table 3. Comparison of distance education perception scale of students who preferred FTF and WBA education*

Distance education perception scale	Score Range	FTF (n=5)	WBA (n=13)	p
Students' perception	12-60	31 (26-32)	40 (34-45)	0.027
Equipment facility	5-25	16 (13-18)	15 (14-18)	1.000
Time Management	3-15	8 (7-8)	9 (9-10)	0.019
Facility and support of the institution	2-10	10 (9-10)	10 (6-11)	0.775
Total	22-110	65 (56-66)	70.5 (63-84)	0.328

*Descriptive statistics were given as median (interquartile range).

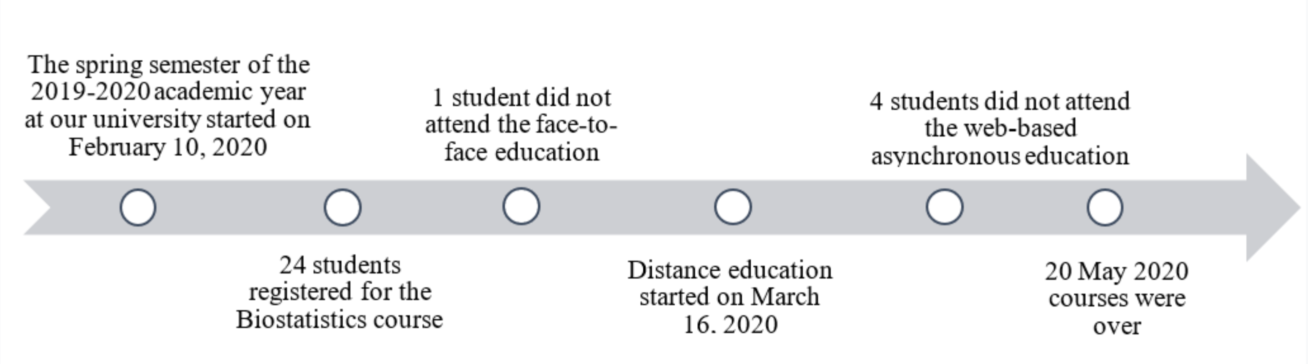


Figure 1. Timeline of the process

Figure 2. Word-cloud visualization of open-ended responses of post-graduate students

Discussion

The integration of technology in education offers qualitatively different learning experiences in higher education; provides a new way of collaborating among education stakeholders (student, lecturer, etc.) and diversity in access to resources, and improves information exchange (21). Also, Taft et al. presented the importance of 4 factors which consist of the information revolution, the competitiveness of higher education, changes in student lifestyles and characteristics, nationalities and geographical locations, and personal situations in the speed of transition to online education (16). Also, Allen and Seaman, and Seaman et al. emphasized the increase in online education in higher education in their studies (22,23). As a result, studies comparing it with traditional education to examine the effect and success of online education came to the fore (24–29).

With technological progress, students want to take distance education where they can choose alternative different programs that they can access without the constraints of time and space (30). With WBA education, students have opportunity to access more courses (31). Considering the working time, time spent with family, travel time and expenses,

students prefer a freer education program. They prefer the program where they can communicate online with their lecturers and friends, access course materials over the internet, and submit their homework. (32). In FTF education, students can instantly interact dynamically with their friends and instructor, while they must be in the classroom at a certain time. As a result of this, new and different questions can be asked by students in situations that are not understood. In FTF education, the discipline in learning is provided by the trainer, while in WBA education, it is the student's responsibility. Research shows that online students are more likely to drop out if they don't like the instructor, the format, or the feedback (33). Students' predisposition to technology can also be a factor in their education type preferences. Students who are prejudiced against technological innovations may prefer face-to-face education. As a result, both FTF and WBA education have pros and cons. Williams et al. suggested that FTF education should be considered as a supplement rather than a complete replacement due to poor performance in WBA education (34). In their study, Atchley et al. supported FTF education as online education students would drop out more easily and could lack feedback for both students and lecturer (35). On the other hand, Westhuis et al. stated in their study that students who received online education performed at least as much or even better than students who received FTF education (36).

As in higher education, the provision of distance education has increased also in the post-graduate education of universities. Biostatistics courses are not offered as standard at universities (i.e. they are frequently taught in undergraduate and post-graduate studies of health-related faculties and institutes. The "Biostatistics" course is a particularly attractive course to be offered via distance education. Various studies have also been conducted on the online delivery of biostatistics education. McGready and Brookmeyer compared the results of students who taught 16 weeks of biostatistics at a public health college in the online and on-campus format (37). Varghese et al. compared the academic performance of post-graduate dental students studying video-based learning and blended module-based learning in a biostatistics and research methodology course at a post-graduate program at a dental college in India (38).

The difference in our study is that after FTF education was interrupted due to the pandemic, the same students continued to study with WBA education. Of the 19 students in our study, 13 (68.4%) preferred WBA, five FTF (26.3%), and one online education (5.3%) if they would take this course once again. During the WBA education, Google Classroom was used for information, document, and video sharing. Google Classroom was chosen because of its prevalence, usefulness, and freebies. An element that seems to be a disadvantage due to the cost of distance education was provided to students free of charge. The video recorded at the time of the application, the data sets to be used in the applications, the analysis files related to the subjects were shared using this platform. There was no difference between the two student groups' thoughts on choosing WBA education in practice. The exercises made using the statistical package programs were recorded on video. It was seen that both groups preferred to use video while making the applications because there was no time and place constraints. When examined in terms of theoretical courses, students who prefer WBA education want to take courses with WBA, and students who prefer FTF education want to take courses with FTF. The students stated that they were more satisfied with the education system they preferred than the other group. WBA students stated that they have more flexible, motivated, and analytical thinking opportunities.

When the scale developed by Özkaya et al. was applied to measure the distance education perceptions of the students, the “students’ perception” and “time management” scores of the WBA students were found to be higher positively (39). There was no difference between WBA and FTF students in terms of “Equipment facility”, “Facility and support of the institution” and an overall score of the scale.

According to the open-ended questions, the most important emphasis of the students who prefer WBA education is flexible time, the possibility to watch it again and the cost of travel, etc. spending decreased. Employees shared that they do not have to worry about taking leave and absenteeism. They stated that if they could not understand the lecture videos, watching them again relieved them. In the answers given by the students who preferred FTF education, they stated that they made this choice because they had to be able to communicate with the lecturer instantly and they had to listen to the lesson.

Limitations

The limitations of the study are related to the student's skills/abilities and the student's predisposition to distance education. While comparison groups are divided according to students' preferences, students who prefer WBA education are likely to be more prone to computer, internet, and distance education than FTF students, and vice versa. There are also limitations for variables such as student intelligence, skill level, and gender. Considering the sample size obtained to provide education in the number of optimal students, these variables were not considered. Also, the ability of the lecturers to use technology is an important element of WBA education. Besides, the ability of the lecturers to use technology is an important element of WBA education. In this study, although the lecturer did not have any problems with the use of technology in WBA education, it is limited to generalizing these results according to the characteristics of the lecturers. Another important constraint is that the students' computers, mobile phones, and internet communication services are limited. Depending on the income level of the students, there may be students who cannot benefit from these opportunities.

Conclusion

In some cases, crises that occur further accelerate the current development. The emergence of the epidemic has accelerated the process of integrating technology into education. Educators, students, and educational institutions had to adapt to the new order. The question is, will there be a return to pre-pandemic in the education system from now on? WBA education is a very good alternative to courses similar to biostatistics courses where computer programs are frequently used. The transition to WBA education can be permanent if the state and universities provide internet, necessary hardware, and software support to students and faculty members and if a dynamic interaction is provided, which is the advantage of FTF education. Anxieties of students who prefer FTF education can be relieved by increasing instant interaction. WBA education is very attractive to graduate students and lecturers because of the advantages it

provides in terms of time, place and resources. It is seen as the first preferred method in cases where traditional education is interrupted. The progress in information and communication technologies works in favour of WBA education. It will most likely be shaped in the future, in line with the advancement in technology and the demands of students and lecturers.

Acknowledgments: none

Ethical Approval: *Ethical approval was taken from the Bursa Uludag University, Faculty of Medicine, Clinical Research Ethics Committee (Date 10 June 2020; Number: 2020-10/24). We conducted this study according to the principles of the Declaration of Helsinki. Informed consent was obtained from all the participants.*

Author Contributions: *Concept:GÖ, MOA. Literature Review:MOA. Design:GÖ, MOA. Data acquisition:GÖ. Analysis and interpretation: GÖ. Writing manuscript: GÖ, MOA. Critical revision of manuscript: GÖ, MOA.*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Pregowska A, Masztalerz K, Garlińska M, et al. A worldwide journey through distance education—from the post office to virtual, augmented and mixed realities, and education during the covid-19 pandemic. Vol. 11, Education Sciences. 2021.
2.Armstrong-Mensah E, Ramsey-White K, Yankey B, et al. COVID-19 and Distance Learning: Effects on Georgia State University School of Public Health Students. Vol. 8, Frontiers in Public Health. 2020.
3. Bao W. COVID -19 and online teaching in higher education: A case study of Peking University. Hum Behav Emerg Technol. 2020;2(2):113–5.
4. Humphrey EA, Wiles JR. Lessons learned through listening to biology students during a transition to online learning in the wake of the COVID-19 pandemic. Ecol Evol [Internet]. 2021 Mar 13 [cited 2021 Mar 19];00(1):ece3.7303. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ece3.7303
5. Sefriani R, Sepriana R, Wijaya I, et al. IJERE-Blended learning with Edmodo: The effectiveness of statistical learning during the COVID-19 pandemic. Int J Eval Res Educ. 2021;10(1):293–9.
6. Anshari M, Almunawar MN, Shahrill M, et al. Smartphones usage in the classrooms: Learning aid or interference? Educ Inf Technol. 2017;22(6):3063–79.
7. Griffiths ME, Graham CR. The potential of asynchronous video in online education. Distance Learn. 2009;6(2):13–22.
8. Chen T, Peng L, Jing B, et al. The impact of the COVID-19 pandemic on user experience with online education platforms in China. Sustain. 2020;12(18).
9. Borup J, West RE, Graham CR. Improving online social presence through asynchronous video. Internet High Educ. 2012 Jun 1;15(3):195–203.
10. Griffiths M, Graham C. Using asynchronous video in online classes: Results from a pilot study. Instr Technol Distance Learn. 2009;6(3):65–76.
11. Evans S, Wang R, Yeh T, et al. Evaluation of Distance Learning in an "Introduction to Biostatistics" Class: A Case Study. Stat Educ Res J. 2007; 6:59–77.
12. Abid Azhar K, Iqbal N. Effectiveness of Google Classroom: Teachers' Perceptions. Prizren Soc Sci J. 2018;2(2):52–66.
13. Al-Maroof RAS, Al-Emran M. Students acceptance of google classroom: An exploratory study using PLS-SEM approach. Int J Emerg Technol Learn. 2018;13(6):112–23.
14. Shaharanee INM, Jamil JM, Rodzi SSM. Google classroom as a tool for active learning. In: AIP Conference Proceedings. American Institute of Physics Inc.; 2016.
15. Zhang M. Teaching with Google Classroom. Packt Publishing Ltd. Birmingham; 2016.
16. Taft S, Perkowski T, Martin L. A Framework for Evaluating Class Size in Online Education. Q Rev Distance Educ. 2011;12(3):181.
17. Grandzol CJ, Grandzol JR. Interaction in Online Courses: More is NOT Always Better. Online J Distance Learn Adm. 2010;13.
18. Orellana A. Program Professor, Instructional Technology and Distance Education, Fischler School of Education and Human Services. 2006;33162(3):229–48.
19. Rovai AAP. A preliminary look at the structural differences of higher education classroom communities in traditional and ALN courses. J Asynchronous Learn Netw. 2002;6(1):41–56.
20. Özkaya G, Aydin MO, Alper Z. Distance education perception scale for medical students: a validity and reliability study. BMC Med Educ. 2021;21(1):1–8.
21. Alonso F, López G, Manrique D, et al. An instructional model for web-based e-learning education with a blended learning process approach. Br J Educ Technol. 2005;36(2):217–35.
22. Seaman JE, Allen IE, Seaman J. Grade Increase: Tracking Distance Education in the United States. Babson Survey Research Group. 2018;
23. Allen IE, Seaman J. Changing Course: Ten Years of Tracking Online Education in the United States. San Francisco, CA: Babson Survey Research Group and Quahog Research Group LLC, 2013.
24. Bir DD. Comparison of Academic Performance of Students in Online vs Traditional Engineering Course. J Open, Distance e-Learning. 2019;22(1):1–13.
25. Fish LA, Snodgrass CR. Business student perceptions of online versus face-to-face education: Student characteristics. Bus Educ Innov J. 2015;7(2):83–96.

26. Mather M, Sarkans A. Student Perceptions of Online and Face-to-Face Learning. Int J Curric Instr. 2018;10(2):61–76.
27. Smith GG. Teaching on-line versus face-to-face. 2002;30(4):337–64.
28. Sun A, Chen X. Online education and its effective practice: A research review. J Inf Technol Educ Res. 2016; 15:157–90.
29. Zacharias G, Yiannis G. Differences Between Regular and Distance Education in a Teacher's Training Program. Eur J Open, Distance E-Learning. 2017;20(2):17–29.
30. Wladis C, Conway KM, Hachey AC. The Online STEM Classroom—Who Succeeds? An Exploration of the Impact of Ethnicity, Gender, and Non-traditional Student Characteristics in the Community College Context. Community Coll Rev. 2015 Mar 12;43(2):142–64.
31. Salcedo CS. Comparative Analysis Of Learning Outcomes In Face-To-Face Foreign Language Classes Vs. Language Lab And Online. J Coll Teach Learn. 2010;7(2):43–54.
32. Richardson JC, Swan K. Examining social presence in online courses in relation to student's perceived learning and satisfaction. J Asynchr Learn. 2003;7(1):68–88.
33. Paul J, Jefferson F. A Comparative Analysis of Student Performance in an Online vs. Face-to-Face Environmental Science Course From 2009 to 2016. Front Comput Sci. 2019;1:7.
34. Williams A, Birch E, Hancock P. The impact of online lecture recordings on student performance. Australas J Educ Technol. 2012;28(2):199–213.
35. Atchley W, Wingenbach G, Akers C. Comparison of course completion and student performance through online and traditional courses. Int Rev Res Open Distance Learn. 2013;14(4):104–16.
36. Westhuis D, Ouellette PM, Pfahler CL. A comparative analysis of on-line and classroom-based instructional formats for teaching social work research. Adv Soc Work. 2006;7(2):74–88.
37. McGready J, Brookmeyer R. Evaluation of student outcomes in online vs. campus biostatistics education in a graduate school of public health. Prev Med (Baltim). 2013;56(2):142–4.
38. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. J Dent Educ. 2019 Apr 1;83(4):445–50.
39. Özkaya G, Aydin MO, Alper Z. Distance education perception scale for medical students: a validity and reliability study. BMC Med Educ. 2021;21(1):400.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

Determination of Obesity Awareness of University Students Who Are Studying in the Field of Health

Sağlık Alanında Öğrenim Gören Üniversite Öğrencilerinin Obezite Farkındalıklarının Belirlenmesi

Mustafa IKIZEK [1*] **Nükhet BAYER** [2]

[1] İç Hastalıkları Uzmanı, Lokman Hekim Üniversitesi, Sağlık Hizmetleri MYO, Ankara, TURKİYE

[2] Lokman Hekim Üniversitesi, Sağlık Bilimleri Fakültesi, Hemşirelik Bölümü, Ankara, Türkiye

Corresponding author:

Dr. Mustafa İKİZEK

Adress: Lokman Hekim Üniversitesi, Hemşirelik Fakültesi, Hemşirelik Yönetimi Bölümü, PK:06300, Çankaya, Ankara, Türkiye

email: mustafaikizektr@gmail.com

Received: 14.06.2022

Accepted: 04.07.2022

Cite as: IKIZEK.M, BAYER.N Determination of Obesity Awareness of University Students Who Are Studying in the Field of Health IJCMBS 2022;2(2):111-116 doi.org/ 10.5281/zenodo.6794217

Abstract

Background: Obesity has emerged as an important health problem affecting the whole world in recent years. In this study, it was aimed to examine the obesity awareness of university students studying in the field of health.

Materials and Methods: The study was conducted with 378 students, studying at the Faculty of Health Sciences of a foundation university, between April-May 2022. The participation rate for the questionnaire is 62%. Questionnaire method was used as data collection tool. The Obesity Awareness Scale (OAS) was used to determine students' awareness of obesity.

Results: The age range of the students is 18-35 years and the mean age is 20.71±2.13. 67% of the participants are under the age of 21 and 68.8% are women. 30.1% use cigarettes and 23.3% use alcohol. It was found that 68% of them do not exercise regularly, 80% of them sleep between 6-8 hours, half of them use the internet 4-6 hours a day. 12.2% of the participants were overweight and 5.1% were obese. It was found that the participants got points for obesity awareness (29.13±2.63), nutrition (20.40±2.31), physical activity (17.45±1.90) and overall scale (66.98±7.26).

Conclusions: Obesity awareness of the participants is sufficient. Awareness of women and those who exercise regularly is higher than men and those who do not exercise regularly. Trainings about obesity should be organized to increase the awareness of these students, who are the health workers of the future.

Keywords; Obesity, university, student, health, awareness.

ÖZ

Amaç: Obezite, son yıllarda tüm dünyayı etkileyen önemli bir sağlık sorunu olarak karşımıza çıkmaktadır. Bu çalışma ile sağlık alanında öğrenim gören üniversite öğrencilerinin obezite farkındalıklarının incelenmesi amaçlanmıştır.

Materyal ve Metod: Araştırma Nisan-Mayıs 2022 tarihleri arasında bir vakıf üniversitenin Sağlık Bilimleri Fakültesi'nde öğrenim gören 378 öğrenciyle yapılmıştır. Ankete katılım oranı %62'dir. Veri toplama aracı olarak anket yöntemi kullanılmıştır. Öğrencilerin obezite farkındalıklarını belirlemek için Obezite Farkındalık Ölçeği (OFÖ) kullanılmıştır.

Bulgular: Öğrencilerin yaş aralığı 18-35 yıl olup, yaş ortalaması 20.71±2.13'dür. %67'si 21 yaş ve altında, %68.8'i kadındır. %30.1'i sigara, %23.3'ü alkol kullanmaktadır. %68'inin düzenli egzersiz yapmadığı, %80'inin 6-8 saat arası uyuduğu, yarısının günde 4-6 saat internet kullandığı bulunmuştur. Katılımcıların %12.2'si fazla kilolu, %5.1'i obezdir. Katılımcıların obezite farkındalığı için (29.13±2.63), beslenme için (20.40±2.31), fiziksel aktivite için (17.45±1.90) ve ölçek geneli için (66.98±7.26) puan aldıkları bulunmuştur.

Sonuç: Katılımcıların obezite farkındalıkları yeterli düzeydedir. Kadınların erkeklere göre, düzenli egzersiz yapanların yapmayanlara göre farkındalıkları yüksektir. Geleceğin sağlık çalışanı olacak bu öğrencilerin, obezite hakkında farkındalıklarını artırmak için eğitimler düzenlenmelidir.

Anahtar kelimeler; Obezite, üniversite, öğrenci, sağlık, farkındalık.

Highlights

- Obesity awareness of the participants is sufficient.
- Awareness of women and those who exercise regularly is higher than men and those who do not exercise regularly.

Introduction

Obesity is a chronic metabolic disease characterized by an increase in body fat stores, resulting from the fact that the energy taken with food is more than the energy spent. It affects adolescents and children as well as adults (1,2). According to the World Health Organization (WHO) data, there are more than 650 million obese individuals worldwide, which is approximately 13% of the adult population. The prevalence of obesity increased approximately 3 times between 1975 and 2016. There were more than 124 million obese children and adolescents (6% of girls, 8% of boys) aged between 5-19 years worldwide in 2016 (3). Recent health studies in Turkey show that the prevalence of obesity is gradually increasing and the prevalence of obese individuals over the age of 15 was 16.9% in 2010, and increased to 21.1% in 2019 (4).

Occurrence of obesity varies depending on age, gender, education level, nutritional habits, economic situation, lack of physical activity, environmental factors, genetic and psychological factors (5). It is known that obesity can be associated with a wide variety of health problems, including, prediabetes, type 2 diabetes, cardiovascular diseases, hypertension, hyper/dyslipidemia, cerebrovascular disease, various cancers, obstructive sleep-apnea syndrome, non-alcoholic fatty liver disease, gastroesophageal reflux, biliary tract disease, polycystic ovary syndrome, infertility, osteoarthritis and depression (6-8).

The most commonly used measurement method in determining obesity is Body Mass Index (BMI), which is obtained by dividing the weight by the square of the height in meters (kg/m^2) (9). According to the classification of the WHO, a BMI below 18.5 is considered underweight, between 18.5-24.9 is considered normal weight, between 25-29.9 is considered overweight, and 30 and above is considered obese (10). It has been determined that the risk of diseases increase as the BMI value increases, and the increased BMI creates a basis for obesity and non-communicable diseases (11).

Obesity has been started to be seen frequently in developing countries as well as developed countries. Factors such as increased level of income, adopting a western lifestyle, changes in eating habits, and decreasing energy consumption due to increasing technology are some of the reasons for obesity (9,12). Obesity, which is responsible for 2% to 8% of health expenditures in developed countries, and even 15% in some countries, is now considered as a disease beyond a cosmetic problem (13). The fight against obesity has taken its place in the health policies of countries since the 2000s, and strategies and plans are being developed (14). In order to raise and expand the awareness of the society, the key role in this struggle belongs to the healthcare professionals (15).

University years are an important opportunity to develop health lifestyle behaviors. It is important to evaluate the awareness of university students about obesity, who represent the young adult population (16). It is expected that students who will work in the health sector and enlighten the society in the future should have a high awareness of obesity. In this study, it was aimed to examine the obesity awareness of university students studying in the field of health.

Materials and Methods

Samples

The population of the research consisted of 610 students studying at the Faculty of Health Sciences of a foundation university between April and May 2022. It was aimed to reach the entire universe in the determined time interval without going to the sample selection, and 378 students were reached. The participation rate in the questionnaire is 62%.

Data Collection Method

In this descriptive study, questionnaire method was used to collect data. The students were informed about the research and those who volunteered to participate were sent the online questionnaire via e-mail to fill the form. The questionnaire form consists of two parts. In the first part, there are 12 questions prepared by the researchers, including age, gender, income level, smoking and alcohol use, health problems, regular exercise, height and weight, presence of overweight in the family, daily internet use and sleep time. In the second part, the "Obesity Awareness Scale" (OAS), which was developed by Allen in 2011 and adapted into Turkish by Kafkas and Ozen in 2014, was used to determine students' awareness of obesity (17,18). The scale consists of 23 items. It consists of three sub-dimensions: obesity awareness (8 items), nutrition sub-dimension (7 items) and physical activity sub-dimension (8 items). The scale is in a 4-point likert structure from negative to positive. The internal consistency coefficient for the overall scale was reported as α=0.80 (17). As the total score obtained in the scale and the score obtained in the sub-dimensions increase, awareness increases. In the adaptation study of the scale, a three-dimensional structure consisting of 20 items was found and the internal consistency coefficient for the overall scale was determined as α=0.87 (18).

Statistical Analysis

Analysis of the research data was performed with SPSS (Version 21, Chicago IL, USA) statistical program. In the analysis of descriptive statistics, arithmetic mean, standard deviation and frequency analysis were used.

Whether the data showed a normal distribution or not was determined by the kurtosis and skewness values. It was observed that the Skewness and Kurtosis values were in the range of ±2, showing a normal distribution (19). Due to the normal distribution of the data, Independent Samples T Test was used to compare two independent groups, One Way ANOVA was used to compare three or more groups, and Pearson Correlation analysis was used to determine the relationships between variables. A p value of <0.05 was considered statistically significant.

Ethical Issues

The study was approved by Lokman Hekim University Non-Interventional Clinical Research Ethics Committee (Decision no: 2022/63 and Code no: 2022054). The research was conducted in line with the principles of the Declaration of Helsinki. Verbal consent was obtained from the patients participating in the study.

Limitations

The limitation of the study is that the study was conducted in a single center and data was not collected in a way to make comparisons according to departments.

RESULTS

The sociodemographic characteristics of the participants are given in Table 1. The age range of the students is 18-35 years and the mean age is 20.71±2.13. 67% of the participants are under the age of 21, 68.8% are women, and 52.6% of them have more family income than their expenses. 30.1% use cigarettes and 23.3% use alcohol.

Considering the mean score of the participants; It was found that they got 29.13±2.63 points for obesity awareness, 20.40±2.31 points for nutrition, 17.45±1.90 points for physical activity, and 66.98±7.26 points for OAS in general. In addition, the reliability coefficient for the total OAS was determined as 0.79 (Table 2).

When the OAS and its sub-dimensions were examined according to sociodemographic characteristics, it was found that women had a higher mean than men in all sub-dimensions and overall scale ($p<0.05$). When the participants were examined according to their regular exercise status, the physical activity sub-dimension mean score was found to be higher in those who do regular exercise compared to those who do not ($p=0.04$) (Table 3). No statistically significant difference was found in OAS and its sub-dimensions according to other sociodemographic characteristics (age, income level, smoking and alcohol use, health problems, BMI, presence of overweight in the family, daily internet use and sleep time) ($p>0.05$).

Table 1. Sociodemographic characteristics of the participants

Variables	n	%
Age groups (year)		
≤21	253	67.0
≥22	125	33.0
Sex		
Male	118	31.2
Female	260	68.8
Income level		
Income < expense	47	12.4
Income = expense	132	35.0
Income > expense	199	52.6
Smoking		
Yes	114	30.1
No	264	39.9
Alcohol use		
Yes	88	23.3
No	290	76.7
Having an overweight family member		
Yes	67	17.7
No	311	82.3
Having a health problem		
Yes	32	8.5
No	346	91.5
Regular exercise		
Yes	121	32.0
No	257	68.0
Daily sleep time (hours)		
< 6	44	11.7
6-8	302	79.9
≥9	32	8.4
BMI		

Weak	60	15.8
Normal	253	66.9
Overweight	46	12.2
Obese	19	5.1
Using internet (hours)		
≤3	152	40.2
4-6	188	49.8
≥7	38	10.0

Table 2. Descriptive statistics and reliability coefficients of the OAS and subdimesions

	Item numbers	Minimum	Maximum	Mean	SD	CA
Obesity awareness	9	9	36	29.13	2.63	0.73
Nutrition	6	6	24	20.40	2.31	0.75
Physical activity	5	5	20	17.45	1.90	0.80
Total OAS	20	20	80	66.98	7.26	0.79

(SD: Standard Deviation, CA: Cronbach Alfa)

Table 3. OAS mean scores according to sociodemographic characteristics of the students

	Obesity awareness		Nutrition		Physical activity		OAS Total	
Variables	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Sex								
Male	27.32	4.62	19.47	2.68	16.61	3.12	63.4	10.42
Female	29.51	3.66	21.03	2.36	18.86	2.30	69.4	8.32
p	0.001		0.001		0.001		0.03	
Regular exercise								
Yes	29.01	1.89	20.15	2.75	18.23	2.54	67.39	7.18
No	28.71	2.15	19.86	2.29	16.92	3.46	65.49	7.90
p	0.86		0.90		0.04		0.28	

(Independent Samples T Test) M:Mean, SD: Standard Deviation, OAS: Obesity Awareness Scale

DISCUSSION

With this study, it is aimed to examine the obesity awareness of university students who will serve the society as health workers in the future.

In our study, 12.2% of the students were overweighted and 5.1% were obese. When other studies conducted with university students were examined, the prevalence of overweight and obesity was found as follows: Saudi Arabia (18.6%-12.7%), Malaysia (15.9%-5.2%), India (26.8-10.7%), Egypt (36.9-12.5%) (20-23). The prevalence of overweight and obesity in studies conducted in Turkey was found as (26.1%-8.9%), (14-3.6%), (17%-9%) and (18.8%-4.9%), respectively (5, 24-26). It is thought that maintaining body weight at young ages and awareness of obesity will have an impact on preventing or reducing weight gain in later years (27). Our research is similar to previous studies and these results show that obesity should be considered as a public health problem and should be seriously addressed.

Decreased physical activity, increased time spent watching television and cell phone/internet in youth are reported to be a problem and it has been stated that these factors worsen weight gain and inactivity (20). In our study, it was found that half of the participants used the internet for 4-6 hours a day. When the studies conducted in Turkey are examined; It has been determined that 30% of university students use the internet for more than 3 hours (28), and in another study, 40% of students use the internet for up to 4 hours (29). In another study, it was found that the time spent in front of the screen (TV, computer, mobile phone) for students increased with the Covid-19 pandemic and was approximately 8 hours per day (30). In a study conducted in South Korea, it was found that the prevalence of overweight and obesity increased in those who used the Internet for more than 2 hours a day (31).

In the original OAS study; it was found that students scored obesity awareness (29.09±3.58), nutrition (19.41±97), physical activity (16.90±2.23) and the overall scale (65.40±8.84) (17). In the adaptation study to Turkish; obesity awareness (27.36±2.18), nutrition (20.27±2.54), physical activity (18.57±1.95), and overall scale (65.20±6.67) points were observed (18). When the mean scores of OAS and its sub-dimensions were examined in our study; (29.13±2.63), (20.40±2.31), (17.45±1.90), (66.98±7.26) points were found for obesity awareness, nutrition, physical activity and overall scale, respectively. The results of our study are similar to the results of other studies using the OAS scale (17,18,24,32,33).

Considering the OAS scale and its sub-dimensions, which include sociodemographic characteristics, women's awareness was found to be higher than men. These results are parallel with the findings of previous studies

(5,24,34). It is considered that the fact that women give more importance to external appearance and weight control than men is effective in high awareness for obesity. In addition, the physical activity sub-dimension scores of those who exercise regularly were found to be higher than those who do not. It is an expected result that those who exercise regularly give more importance to their health and have a higher awareness of obesity. In addition, there was no difference in the obesity awareness of the participants in terms of other sociodemographic characteristics. Obesity awareness does not change according to sociodemographic characteristics in some previous studies (32,35).

In our study, no statistically significant difference was found in OAS and its sub-dimensions according to other sociodemographic characteristics (age, income level, smoking and alcohol use, health problems, BMI, presence of overweight in the family, daily internet use and sleep time). In the study of Ozkan et al., no statistically significant difference was found between the mean OAS scores according to income level, the presence of obesity in the mother or father, smoking and alcohol use, regular meal consumption, and sleep time (32). Similarly, in the study of Alasmari et al., no statistically significant relationship was found between income level, nutritional habits, activity levels, smoking, alcohol use and awareness scores (35). Our research is similar to previous studies.

In conclusion, obesity awareness of students studying in the field of health is sufficient. Awareness of women and those who exercise regularly is higher than men and those who do not exercise regularly. Trainings should be organized to increase the awareness about obesity of these students, who are the health workers of the future. Obesity should be combated by ensuring that university students spend less time in front of the screen, do sports, exhibit healthy behaviors by avoiding harmful substances such as tobacco and alcohol, and creating a healthy eating culture. In future studies, it is recommended to examine obesity awareness according to faculties and departments.

Acknowledgements: We thank the students who participated in the research.

Ethical Approval: *The study was approved by Lokman Hekim University Non-Interventional Clinical Research Ethics Committee (Decision no: 2022/63 and Code no: 2022054)*

Author Contributions: *Concept: MI, NB. Literature Review: MI, NB. Design: MI. Data acquisition: MI, NB. Analysis and interpretation: MI, NB. Writing manuscript: MI, NB. . Critical revision of manuscript:MI*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Yumuk V, Tsigos C, Fried M, et al. European guidelines for obesity management in adults. Obesity Facts. 2015; 8:402–424.
2. Lim CS, Gowey MA, Silverstein J, et al. Depressive symptoms, ethnic identity, and health-related quality of life in obese youth. J Pediatr Psychol. 2015: 41; 441-452.
3. World Health Organization (WHO). Obesity and overweight. http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. (accessed 25 March 2022).
4. Turkish Statistical Institute. Türkiye Saglik Arastirmasi, 2019. Available from: https://data.tuik.gov.tr/Bulten/Index?p=Turkiye-Saglik-Arastirmasi-201933661 (accessed 25 March 2022).
5. Dulger H, Mayda AS. Nutritional habits and obesity prevalence of students at Bartin University Vocational School of Health Services. Journal of Duzce University Health Sciences Institute. 2017; 6(3): 173-177.
6. Turkey Endocrinology and Metabolism Society. Obezite tanı ve tedavi kılavuzu, 2019. Miki Matbaacılık, Ankara, 2019.
7. Guduk O, Selimoglu Namoglu S, Ozbek H, et al. Factors affecting obesity awareness of health care vocational school students. Cumhuriyet Univ Sag Bil Enst Derg. 2020;(5)3: 118-132.
8. Usalp S. The role of gender in heart diseases. International Journal of Current Medical and Biological Sciences. 2021; 1(1): 1–6.
9. Tekin F, Ozkocak V. Comparing anthropometric measurements in determining obesity in children: A systematic review. Journal of Adnan Menderes University Health Sciences Faculty. 2022: 6(1); 52-64.
10. World Health Organization Geneva SW. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation presented at the World Health Organization, June 3–5, Geneva, Switzerland, 1998.
11. Kilic K, Ozdogan Y. Obesity paradox. Gazi Saglik Bilimleri Dergisi. 2022:7(1):164-172.
12. Sengonul M, Ozay Arancioglu I, Yildirim Mavis C, et al. Obesity and psychology. Haliç University Journal of Health Sciences. 2019;2(3):1-12.
13. Canbay O, Dogru E, Katayifci N, et al. Investigation of obesity frequency and eating habits in a university hospital professionals. Medical Journal of Bakırköy. 2016;12:129-135.
14. Vallgårda S, Nielsen ME, Hartlev M, et al. Backward- and forward-looking responsibility for obesity: policies from WHO, the EU and England. Eur J Public Health. 2015;25(5):845-848.
15. Uzuntarla Y. Knowledge and attitudes of health personnel about organ donation: A tertiary hospital example, Turkey. Transplant Proc. 2018;50(10):2953-2960.

16. Agwu EM, Draper S, Croix MDS, et al. Health rating, obesity and hypertension among university students in Nigeria by gender and ethnicity. Public Health Int. 2017;2: 131-143.
17. Allen A. Effects of educational intervention on children's knowledge of obesity risk. Phd Thesis, Carroll College 2011.
18. Kafkas ME, Ozen G. The Turkish adaptation of the obesity awareness scale: a validity and reliability study. Inonu University Journal of Physical Education and Sport Sciences. 2014;1:1–15.
19. Pituch KA, Stevens JP. Applied multivariate statistics for the social sciences. Routledge, New York, 2012.
20. Aljefree NM, Shatwan IM, Almoraie NM. Impact of the intake of snacks and lifestyle behaviors on obesity among university students living in Jeddah, Saudi Arabia. Healthcare (Basel). 2022;10(2):400.
21. Gopalakrishnan S, Ganeshkumar P, Prakash MV, et al. Prevalence of overweight/obesity among the medical students, Malaysia. Med J Malaysia. 2012;67(4):442-444.
22. Pengpid S, Peltzer K. Prevalence of overweight/obesity and central obesity and its associated factors among a sample of university students in India. Obes Res Clin Pract. 2014;8(6):e558-570.
23. Bakr EM, Ismail NA, Mahaba HM. Impact of life style on the nutritional status of medical students at Ain Shams University. J Egypt Public Health Assoc. 2002;77(1-2):29-49.
24. Acaroglu T, Colakoglu T. Guidance of young people to activity, healthy nutrition and obesity awareness as a sport policy. Journal of Human Sciences. 2020; 17(1), 340-358.
25. Incedal Sonkaya Z, Gunay O. Healthy lifestyle behaviors and obesity in faculty and college students. Journal of Health Sciences. 2020; 29(3):161-167.
26. Uyanik G, Yilmaz M, Sahin A. Determination of obesity-related prejudice of health sciences students. Journal of General Health Sciences. 2020; 1(2):48-58.
27. Barışkan H, Karakoç Kumsar A. frequency of abdominal obesity and eating awareness levels in health science students. HEAD. 2020;17(2):162-169.
28. Kucuk EE. Assessment of the problematic internet use of university students and their opinions about its effects on their health. Tepecik Eğit. ve Araşt. Hast. Dergisi. 2017; 27(3):211-216.
29. Polat F, Karasu F. Evaluation of the relationship between obesity status and internet use of health school students at a university. Journal of Health Sciences. 2020;29 (1):34-41.
30. Zengin M, Duken ME, Yayan EH. A risk to consider in the pandemic: Weight gain in youths. Balıkesir Medical Journal. 2022;6(1):17-23.
31. Baek SI, So WY. Association between times spent on the internet and weight status in Korean adolescents. Iran J Public Health. 2011; 40:37-43.
32. Ozkan I, Adibelli D, Ilaslan E, Taylan S. Relationship between body mass index and obesity awareness of university students. ACU Sağlık Bil Derg 2020; 11(1):120-126.
33. Sancar B, Ozcanarslan F. Determining academicians' obesity awareness: The case of Toros University. Uluslararası İktisadi ve İdari Bilimler Dergisi. 2021; 7(2):5-14.
34. Dinccag N, Celik S, İdiz C, et al. Awareness of diabetes and obesity in Turkey. Turk J Endocrinol Metab. 2017; 21:31-36.
35. Alasmari HD, Al-Shehri AD, Aljuaid TA, et al. Relationship between body mass index and obesity awareness in school students. J Clin Med Res. 2017;9:520–524.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

The Relationship between SYNTAX Score and Resting/Post-exercise Ankle-Brachial index in Patients with Acute Coronary Syndrome

Akut Koroner Sendromlu Hastalarda SYNTAX Skoru ile Dinlenme/Egzersiz Sonrası Ayak Bileği-Kol İndeksi Arasındaki İlişki

Ayşenur Güllü[1], Muammer Karakayalı[2], Ali RızaDemir[1], Emre Yılmaz[3], Ertan Aydın[3] Mehmet Ertürk[1]

[1] Health Sciences University Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital

[2] Kafkas University Medical Fakulty, Department of Cardiology

[3] Giresun University Medical Faculty, Department of Cardiology

Correspondingauthor:

Dr. Emre YILMAZ

Adress:

Giresun University Medical Faculty, Department of Cardiology, Giresun, TURKİYE

email: dremreyilmaz@hotmail.com

Received: 15.06.2022

Accepted: 10.07.2022

Cite as: GULLU.A, KARAKAYALI. M, DEMİR. AR, YILMAZ. E, AYDIN.E, ERTURK.M The Relationship between SYNTAX Score and Resting/Post-exercise Ankle-Brachial index in Patients with Acute Coronary SyndromeIJCMBS 2022;2(2):117-126

doi.org/10.5281/zenodo.6810166

Highlights

We found that ABI, which is an easily applicable, noninvasive measurement technique in the diagnosis and follow-up of peripheral artery disease, is associated with the prevalence of coronary artery disease in patients with Acute Coronary Syndrome.

Abstract

Background: The aim of this study is to determine the relationship between the complexity of coronary artery disease (CAD), determined by the SYNTAX score, and the resting and post-exercise Ankle-Brachial Index (ABI).

Materials and Methods: Patients who were treated for Acute Coronary Syndrome (ACS) were evaluated in our study. The patients were divided into two groups according to their SYNTAX Score ≤22 or >22. In addition, patients were evaluated in two groups as <30 and ≥30 in the SYNTAX II (PCI) scoring. The measurements of the resting ABI and the post-exercise ABI were done in these patients.**Results**: The mean age of 118 patients was 57.50±11.19 years and 26 (22%) patients were female. In the group with SYNTAX Score>22, lower resting ABI (p <0.001) and post-exercise ABI (p <0.001) were observed, whereas the higher SYNTAX II PCI (p= 0.005) score was found. While lower resting ABI (p<0.001) and post-exercise ABI (p<0.001) were observed in the group with SYNTAX II PCI Score ≥30, TIMI (0.015), GRACE score (0.004) and SYNTAX score (p= 0.001) were higher. As a result of the ROC analysis: resting ABI cut-off value was detected as 0.935 with a sensitivity of 75% and a specificity of 75% [p<0.001; AUC(95% CI)= 0.786 (0.697-0.875)] and post-exercise ABI cut-off value was detected as 0.945 with a sensitivity of 80% and a specificity of 81% to predict SYNTAX score >22 [p <0.001; AUC (95% CI)= 0.836 (0.761-0.912)]. Diabetes mellitus, history of CAD, resting and post-exercise ABI variables were found to be independent predictors of the extent of CAD, expressed as SYNTAX score >22 and SYNTAX II PCI score ≥30.**Conclusion**: In ACS patients, post-exercise ABI measurements have a stronger diagnostic power than resting ABI measurements in predicting CAD complexity. ABI measures at resting and post-exercise are independent predictors of CAD complexity in ACS patients.

Keywords: Acute Coronary Syndrome, Ankle-Brachial Index, Coronary Artery Disease, SYNTAX Score

ÖZ

Amaç: Bu çalışmanın amacı, SYNTAX skoru ile belirlenen koroner arter hastalığı (KAH) yaygınlığı ile istirahat ve egzersiz sonrası Ayak Bileği-Kol İndeksi (ABKİ) arasındaki ilişkiyi belirlemektir.**Materyal ve Metod:** Çalışmamızda Akut Koroner Sendrom (AKS) nedeniyle tedavi edilen hastalar değerlendirildi. Hastalar SYNTAX Skoru ≤22 veya >22 olarak iki gruba ayrıldı. Ayrıca hastalar SYNTAX skoru II (PCI) puanlamasında <30 ve ≥30 olmak üzere iki grup olarak da değerlendirildi. Bu hastalarda istirahat ABKİ ve egzersiz sonrası ABKİ ölçümleri yapıldı.**Bulgular:** Çalışmaya dahil edilen118 hastanın yaş ortalaması 57.50±11.19 yıl ve 26 (%22) hasta kadındı. SYNTAX Skoru > 22 olan grupta daha düşük istirahat ABKİ (p<0.001) ve egzersiz sonrası ABKİ (p<0.001) görülürken, SYNTAX II PCI (p= 0.005) skoru daha yüksek bulundu. SYNTAX II PCI Skoru ≥ 30 olan grupta daha düşük istirahat ABKİ (p<0.001) ve egzersiz sonrası ABKİ (p<0.001) görülürken, TIMI (0.015), GRACE skoru (0.004) ve SYNTAX skoru (p= 0.001) daha yüksek bulundu. SYNTAX skorunu > 22 öngörüsü için yapılan ROC analizi sonucunda: %75 duyarlılık ve %75 özgüllük ile istirahat ABKİ kestirim değeri 0.935 [p<0.001; Eğri Altında kalan Alan (EAA)(95% Güven Aralığı)= 0.786 (0.697-0.875)] ve %80 duyarlılık ve %81 özgüllük ile egzersiz sonrası ABKİ kestirim değeri 0,945 olarak saptandı [p <0.001; EAA (%95 Güven Aralığı)= 0.836 (0.761-0.912)]. Diabetes mellitus, KAH öyküsü, istirahat ve egzersiz sonrası ABKİ değişkenleri SYNTAX skoru >22 ve SYNTAX II PCI skoru ≥ 30 ile ifade edilen KAH yaygınlığının bağımsız öngördürücüleri olarak bulundu.**Sonuç:** AKS hastalarında, egzersiz sonrası ABKİ ölçümleri, KAH yaygınlığını tahmin etmede istirahat ABKİ ölçümlerinden daha güçlü bir tanısal değere sahiptir. İstirahat ve egzersiz sonrası ABKİ ölçümleri, AKS hastalarında KAH yaygınlığının bağımsız öngörücüleridir.

Anahtar Kelimeler: Akut Koroner Sendrom, Ayak Bileği Kol İndeksi, Koroner Arter Hastalığı, SYNTAX skoru

Introduction

The extensity and complexity of Coronary Artery Diseases (CAD) have improtance in determining the treatment strategy, fully revascularization, follow-up during the hospitalization and for long term adverse cardiac events in the patients with Acute Coronary Syndrome (ACS). Synergy Between PCI With TAXUS and Cardiac Surgery (SYNTAX) Score is one of the most significant scales evaluating the extensity and complexity of the CAD (1, 2). SYNTAX Score is an anatomical scoring system which is projecting the severity of CAD according to the complexity, location and functional feature of the coronary lesion. However, the studies on this subject had been revealed the insufficiency of anatomical evaluation alone in determining the complexity of the CAD and SYNTAX II Score was developed by correlating clinical risk factors. SYNTAX Score can predict short and long term mortality and major cardiac events (MACE) in the patients undergone interventional treatment for CAD. In the present studies, it is stated that SYNTAX II Score have more predictive performance comparing to SYNTAX Score in terms of the prognostic relevence (3).

Ankle-Brachial Index (ABI) is a non-invasive, inexpensive and efficient method for diagnosing peripheral artery disease (PAD) (4). ABI has a good sensitivity and specificity comparing to doppler ultrasonography [sensitivity and specificity of 74.5% and 63.1%] and computed tomography [sensitivity and specificity of 65.5 and 68.8%] for diagnosis of PAD (5). As an indicator for PAD, ABI can be used for predicting the risk of cardiovascular disease (CVD). ABI is an independent risk factor with strong correlation for cardiovascular morbidity and mortality in the atherosclerotic diseases (6, 7). In the literature, it has been shown the relevance between the extensity and complexity of the CAD measured by SYNTAX score and resting ABI (8, 9). However, there is limited information on the effectiveness of such a comparison with SYNTAX II which has been shown to be more correlated with cardiac prognosis by considering clinical factors in addition to anatomical evaluation. Also, the post-exercise ABI has been shown to be more effective for predicting prognosis of ischemic diseases comparing to resting ABI by clinical studies (10, 11).

In our study, it is aimed to do more effective comparison by using the datas of post-exercise ABI, SYNTAX I and II scores. The effect on the power of decision of the afore mentioned scoring systems will be studying by searching the relevance among the complexity of CAD adjusted by the clinical risk factors, ABI at resting and after exercise. Thus, the treatment and follow-up strategies can be determined earlier by predicting the complexity of CAD of the patients with a pracitcal criterion applied at the bedside before diagnostic angiography.

Materials and Methods

The adult patiens subsequently admitted to our clinic with the diagnosis of ACS and executed coronary angiography/angioplasty were included to our prospective study. Ischemic electrophysiological changes in ECG and/or recently occured segmentar wall movement deficiency in left ventricule and/or elevation in troponin levels in addition to anginal complaints related to myocardial infarction were evaluated for the diagnosis of ACS. Exclusion criterias were: (i) clinical phenomenons mimicing ACS with high levels of cardiac biomarkers not originated from trombotic or aterosclerotic events (pulmonaryembolism, tachyarrythmias, coronary vasospasms, slow coronary flow…), (ii) mortality within the first 24 hours after hospitalization, (iii) the history of previous operation for coronary artery by-pass grafting, (iv) the history of previous intervention for revascularization percutanously and/or surgically for peripheral artery disease, (v) any obstacle for the ABI measurement such as: (a) extremity loss for any reason, (b) dialysis fistula in upper extremites, (c) atrial fibrilation, (d) the presence of any diseases preventing vascular compression like scleroderma, CREST syndrome and hypercalcemia, (e) the presence of edema or ulceration on the location of measurement, (f) arm circumferance below 24 cm or above 32 cm, (g) morbid obesity, and (vi) intolerance for ABI measurement. The criterion for terminating the study was failure to reach the adequate number of volunteers to continue the study. The patients were divided into two groups according to their SYNTAX Score $\leq$22 or >22 and SYNTAX II PCI score <30 or $\geq$30. The measurements of the resting ABI and the post-exercise ABI were done in these patients. Clinical features and laboratory test results at the admission of all patients were recorded. The study was executed with the permission of the local ethics comittee and by taking the infromed concent form from all of the patients in line with the principles of the Helsinki Declaration (Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training Research Hospital Clinical Research Ethics Commitee, Reference no:2018-50, Date: 28.05.2018).

The Ankle-Brachial Indeks

The ABI measurements of the patients were done by single vascular operator in seven days during hospitalization. Resting and post-exercise ABI measurements were recorded twice, after hospitalization and after angiography. Mean values of these dual measurements were used in the analsyses.

The resting ABI measurement

Systolic blood pressure were measured from bilateral brachial arteries by using blood pressure cuff and hand held doppler ultrasound(US) device after the patients resting for ten minutes in supine position. Same

measurements were done from bilateral posterior tibial or dorsalis pedis artery by covering the cuff around the ankle with hand held doppler US. ABI for each lower extremity was calculated by dividing the lower extremity systolic pressure to the highest brachial pressure obtained from the upper extremities. Calculated low ABI value was used in the analyses. The patients with ABI ≤ 0.9 was defined as PAD, the patients with 0.9 < ABI ≤ 1.4 as normal and ABI > 1.4 as vascular decompressed or vascular calcified (12).

The post-exercise ABI measurement

Active pedal-plantar flexion technique which is an alternative method for exercise treadmill technique for the patients with contraindication or gait disable was executed to measure the post-exercise ABI. To increase the work load and blood flow at the calves, the patients got raised upon the finger tips, stood in balance and stepped on the foot sole again for fifty times subsequently or as much as the patients could do if they can not reach the target repitition number. After the exercise, the patients were taken into the supine position and the ABI was measured. ABI measurement were repeated with an interval of five minutes until the baseline values were observed again. After recording all values for ABI, intra-observer agreements for resting ABI and post-exercise ABI measurements were calculated [Intra-observer agreement: kapa coefficient ($\varkappa$) for resting ABI measurement: 0.82 (p=0.011) (for the vascular operator); kapa coefficient ($\varkappa$) for post-exercise ABI measurement: 0.83 (p=0.014) (for the vascular operator)]

Coronary Angiography

The coronary angiographies were evaluated by two double-blinded invasive cardiologist who had not got any other role in the study design. The consensus was provided by taking the opinion of third double-blinded cardiologist in case of divergency. A stenosis rate of 50% or more in the epicardial arteries with a diameter of 1.5 mm or larger was considered as significant. The complexity of CAD was evaluated with the models of SYNTAX Score I or SYNTAX Score II (13) (http://www.syntaxscore.com). After recording all values for SYNTAX Score (SS) measurements, intra-observer and inter-observer agreements for SS measurements were calculated [Intra-observer agreement: kapa coefficient ($\varkappa$) for SS measurement: 0.85 (p:0.013) (for Cardiologist A) and 0.87 (p: 0.024) (for Cardiologist B); Inter-observer agreement (between Cardiologist A and B): $\varkappa$ for SS measurement: 0.84 (p:0.021).

The SYNTAX score I guides operators for percutaneous coronary intervention (PCI) and coronary by-pass graft surgery (CABG) with an anatomical assessment for the management of coronary artery disease. On the other hand, SYNTAX score II evaluates factors such as: age, creatinine clearance, left ventricular ejection fraction, left main coronary involvement, gender, chronic obstructive lung disease and peripheral artery disease in addition to the SYNTAX score I. As a result of the SYNTAX score II calculating, the 4-year mortality of the patients is determined and two separate scores are produced for PCI and CABG. Operators are directed to the intervention with the lowest risk of mortality.

The SYNTAX II PCI score was used to evaluate the study patients by grouping them in the analyzes, since PCI was applied to most of the patients included in the study and it was found to be more compatible with the SYNTAX score.

Definitons

Hypertension was defined as systolic and diastolic arterial blood pressure ≥140 and/or ≥90 mmHg or usage of spesific antihypertensive medication. Diabetes mellitus was defined as the fasting blood glucose level ≥126 mg/dl or post-prandial blood glucose level ≥200 mg/dl or HbA1C level ≥6.5% or usage of spesific antidiabetic medication. Smoking was considered as a definition for the patients with the history of active and regular smoking for six months or more. Chronic renal failure was considered for the patients with glomerular filtration rate (GFR) <60 ml/dk/1.73 m^2. The extensity and complexity of CAD was defined as SS over 22 after diagnostic coronary angiography. GRACE (Global Registery of Acute Coronary Events) risk score was calculated with the KILLIP class, systolic blood pressure, heart rate, age, creatinin (mg/dL) and highly sensitive troponin T levels (>24 ng/L). TIMI score was calculated with age (≥65years) and CAD risk factors (existing coronary artery stenosis over 50%, ST segment deviation in ECG at admission, at least two angina within the past 24 hours, acetylsalicylicacid usage within the past one week, increase in serum cardiac biomarkers). KILLIP class is a system used for classifing and predicting the risk of mortality in patients with acute myocardial infarction. Phyisical examination findings and development of cardiac failure is used to categorize the patients between class I and IV (asymptomatic, existing pulmonary congestion and rals, existing pulmonary edema or cardiogenic shock status).

Statistical Analysis

SPSS version 24.0 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis. Normality of distribution was evaluated with visual (histograms and probability curves) an analitic methods (Kolmogorov-Simirnov's and Shapiro-Wilk tests). Parametric variables were shown as mean ± Standard deviation (SD), non-parametric variables as median and categorical variables as percentage (%). Numeric variables such as the resting and post-exercise ABI values between the groups were analysed with student's T or Mann-Whitney U test and categorical variables with chi-square or Fischer exact test. Correlations between the resting or post-exercise ABI and SYNTAX I or II were studied with pearson or spearman analysis. Logistic regression test

was used to determine whether ABI measurements were an independent predictor in identifying ACS patients with a SYNTAX score above 22 and SYNTAX II PCI score ≥30. A p value <0.1 was considered as significant in univariate analysis anda p value <0.05 in multivariate analysis. ROC curve was used to determine the cut-off value of ABI measurements and it was considered as statistical significant if the area under the curve (AUC) larger than 0.5 and a p value <0.005.

Results

118 patients treated and followed up after diagnosis of ACS were included to our study. Of all patients, 92 (78%) were male and 26 (22%) were female. The mean age of the patients was 57.5±11.19 years. All patients divided into two groups according to whether SS calculated after coronary angiography was over 22 or not.32 patients (27.1%) had a SS over 22. In the group with SS>22, statistical significance was observed for higher rate of history of CAD and SBP measurement compared to the group with SS ≤22 (p=0.044 and p=0.048 respectively). All demographics and laboratory results were given inTable 1.

Table 1: Demographicsandlaboratory test results of allpatientsand SYNTAX Scoregroups.

Variables	Allpatients(n=118)	SS≤22 (n=86)	SS>22 (n=32)	*P*
Age (year)	57.50±11.19	56.85±11.39	59.25±10.60	0.302
Male Gender, n (%)	92 (78)	66 (76.7)	26 (81.3)	0.600
Smoking, n (%)	69 (58.5)	46 (53.5)	23 (71.9)	0.072
DM, n (%)	37 (31.4)	23 (26.7)	14 (43.8)	0.077
HT, n (%)	56 (47.5)	39 (45.3)	17 (53.1)	0.452
HL, n (%)	33 (28.0)	24 (27.9)	9 (28.1)	0.981
CAD, n (%)	32 (27.1)	19 (22.1)	13 (40.6)	0.044
COPD, n (%)	5 (4.2)	4 (4.7)	1 (3.1)	1.0
SBP	131.3 ± 20.9	128.9 ± 21.9	137.5 ± 16.6	0.048
DBP	77.8 ± 14.3	77.2 ± 15.8	79.4 ± 9.5	0.467
Heart Rate, beat/m	76.0 ± 12.7	74.8 ± 12.5	79.2 ± 12.9	0.097
Creatinine, mg/dL	0.89 ± 0.22	0.90 ± 0.22	0.85 ± 0.21	0.224
GFR, ml/m/1,73m^2	88.2 ± 22.1	86.7 ± 22.7	92.4 ± 20.1	0.217
Hemoglobin, gr/dL	13.51 ± 1.93	13.55 ± 1.89	13.38 ± 2.05	0.672
Platelet,10^3/uL	261.5 ± 80.0	258.5 ± 76.5	269.4 ± 89.5	0.514
MPV, fL	10.56 ± 1.17	10.61 ± 1.25	10.43 ± 0.89	0.453
RDW,%	13.32 ± 1.41	13.26 ± 1.37	13.49 ± 1.52	0.424
Neutrophyl, 10^3/uL	5.41 ± 1.68	5.55 ± 1.50	5.04 ± 2.09	0.147
Lymphyocyte, 10^3/uL	2.64 ± 1.04	2.67 ± 0.92	2.54 ± 1.31	0.552

SYNTAX: SynergyBetween PCI With TAXUS andCardiacSurgery, DM: DiabetesMellitus, HT:Hypertension, HL: Hyperlipidemia, CAD: CoronaryArteryDisease, COPD: ChronicObstructivePulmonaryDisease, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, GFR: GlomerularFiltration Rate.SS: SYNTAX score

ABI and risk scores of the groups were given in Table 2. Lower resting and post-exercise ABI scores and higher SYNTAX II PCI score were found in the group with SS>22 (p<0.001 and p=0.005 respectively). Statistical significance was not observed for other risk scores between the groups. While lower resting ABI (p<0.001) and post-exercise ABI (p<0.001) were observed in the group with SYNTAX II PCI Score ≥30, TIMI (0.015), GRACE score (0.004) and SYNTAX score (p= 0.001) were higher.

Table 2: Values of ABI and risk scores of allpatientsand SYNTAX Scoregroups.

	AllPatients (n=118)	**SS ≤22 (n=86)**	**SS >22 (n=32)**	**P**
Resting ABI	0.98±0.17	1.02± 0.16	0.87±0.12	<0.001
Post-exercise ABI	0.99±0.16	1.04±0.15	0.85±0.12	<0.001
TIMI Score	3.73±1.26	3.72±1.29	3.75±1.19	0.902
GRACE Score	98.77 ± 24.16	97.61 ± 23.57	101.84 ± 25.80	0.401
KILLIP Class	1 (0 - 2)	1 (0 - 2)	1 (0 - 2)	0.629
SYNTAX II PCI	23.2 (18.4 - 31.4)	22.0 (17.1 - 29.4)	28.4 (22.3 - 35.1)	0.005
SYNTAX II CABG	19.3 (13.8 - 25.8)	18.5 (12.0 - 26.3)	21.7 (14.9 - 25.1)	0.484
	AllPatients (n=118)	**SS II (for PCI) <30 (n=74)**	**SS II (for PCI) ≥30 (n=44)**	**P**
Resting ABI	0.98±0.17	1.03 ± 0.14	0.88 ± 0.15	<0.001
Post-exercise ABI	0.99±0.16	1.02 ± 0.17	0.83 ± 0.14	<0.001
TIMI Score	3.73±1.26	3.56 ± 1.14	4.62 ± 1.28	0.015
GRACE Score	98.77 ± 24.16	94.12 ± 20.72	114.25 ± 23.16	0.004
KILLIP Class	1 (0 - 2)	1 (0 - 2)	1 (0 - 2)	0.462
SYNTAX Score I	19.5 (14.7 – 24.3)	19.1 (15.8 – 26.1)	27.6 (21.7 – 34.8)	0.001

ABI: Ankle-BrachialIndeks, SYNTAX: SynergyBetween PCI With TAXUS andCardiacSurgery, GRACE: Global Registry of AcuteCoronaryEvents, TIMI: Thrombolysis in MyocardialInfarction.

It was found that the resting ABI had negative correlation significantly with diastolic blood pressure (p=0.028 and r=-0.202), systolic blood pressure (p<0.001 and r=-0.439), SYNTAX score (p=0.001 and r=-0.312) and SYNTAX II PCI score (p=0.016 andr=-0.222). Also post-exerice ABI was found to be correlated negatively with systolic blood pressure (p<0.001 and r=-0.421), SYNTAX score (p<0.001 and r=-0.513) and SYNTAX II PCI score (p<0.001 and r=-0.322). The negative correlation of SYNTAX score and resting or post-exercise ABI were demonstrated in Figure 1.

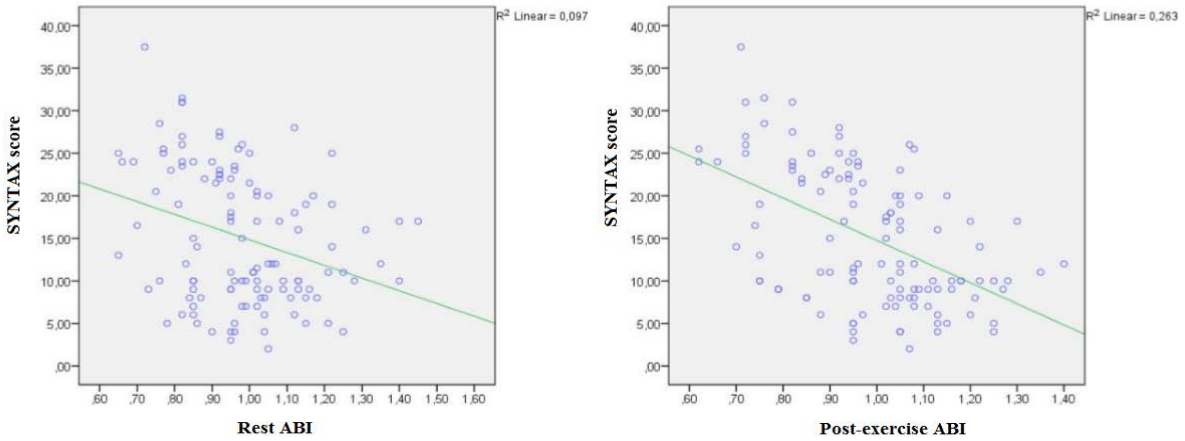


Figure 1: **Graphical presentation of the correlation between SYNTAX Score and resting ABI or post-exercise ABI**

ROC analysis was performed to determine the cut-off values of resting and post-exercise ABI scores in the detection of patients with SS >22. It was observerd that the resting ABI at a value of 0.935 could detect the patients with SS >22 with 75% sensitivity and 75% specificity [p<0.001, AUC (95%CI)= 0.786 (0.697-0.875)]. Also the post-exercise ABI at a value of 0.945 was found to detect the patients with SS >22 with 80% sensitivity and 81% specifity [p<0.001, AUC (95%CI)= 0.836 (0.761-0.912)] (Figure 2).

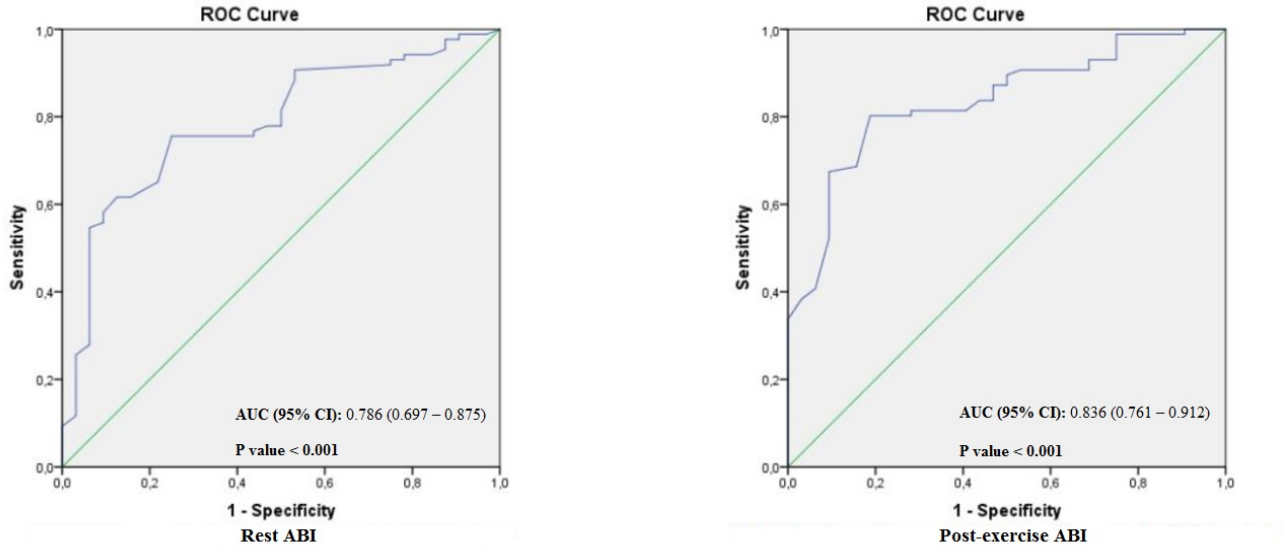


Figure 2: ROC analysis results of resting ABI and post-exercise ABI for SYNTAX Score>22 prediction
AUC: Area Under the Curve, CI: Confidence Interval, ABI: Ankle Brachial Index.

Based on the area under curve (AUC), sensitivity and specificity rates, the post-exercise ABI was considered more superior than the resting ABI in detection of the patients with SS>22.
Univariate logistic regression analysis was performed to determine the variables predicting SS>22 and SYNTAX II PCI score ≥30 in the patients with ACS. Smoking, diabetes mellitus (DM), the history of CAD, hypertension, resting and post-exercise ABI scores were the variables with statistical significance (p=0.075, p=0.08, p=0.047, p=0.052, p<0.001 and p<0.001 respectively) for SS>22. Multivariate regression analysis was performed after a model was built by using these detected variables. Diabetes mellitus [p=0.041, OR (95%CI)= 1.901 (0.691-5.233)], the resting ABI [p<0.001, OR (95%CI) = 2.702 (1.357-7.504)] and the post-

exercise ABI [p<0.001, OR (95%CI)= 2.968 (1.488-7.980)] were determined as independent predictors for SS>22 (Table 3). Age, male gender, GFR, TIMI score, GRACE score, DM, CAD history, rest ABI and post-exercise ABI scores were the variables with statistical significance (p=0.004, p=0.001, p=0.012, p=0.024, p=0.016, p<0.001, p= 0.005, p<0.001 and p<0.001 respectively) for SYNTAX II PCI score ≥30. Multivariate regression analysis was performed after a model was built by using these detected variables. Age [p=0.007, OR (95% CI)= 1.226 (1.028 – 1.402)], male gender [p=0.004, OR (95% CI)= 1.368 (1.206 – 1.544)], GFR [p=0.024, OR (95% CI)= 1.128 (1.066 – 1.296)], GRACE score [p=0.022, OR (95% CI)= 1.188 (1.069 – 1.343)], DM [p=0.001, OR (95% CI)= 1.874 (1.586 – 2.980)], CAD history [p=0.005, OR (95% CI)= 2.428 (1.455 – 4.204)], resting ABI [p<0.001, OR (95% CI)= 2.766 (1.512 – 6.806)] and post-exercise ABI [p<0.001, OR (95% CI)= 2.806 (1.498 – 7.024)] were determined as independent predictors for SYNTAX II PCI score ≥30 (Table 3).

Table 3. Multivariateregressionanalysisresultsfordeterminingtheindependentpredictorsfordetection of SYNTAX Score>22

	Univariate		Multivariate	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
Age	1.020 (0.983 - 1.058)	0.300	-	-
Male Gender	1.313 (0.474 - 3.638)	0.600	-	-
GFR	1.012(0.993-1.033)	0.217	-	-
TIMI Score	1.021(0.738-1.411)	0.901	-	-
GRACE Score	1.007(0.991-1.024)	0.398	-	-
KILLIP Class	1.237(0.521-2.935)	0.630	-	-
Smoking	2.222 (1.922 -5.354)	0.075	1.080 (0.052 - 4.815)	0.214
DM	2.130 (1.914 - 4.965)	0.080	1.901 (1.691 - 5.233)	0.041
CAD	2.413 (1.011 - 5.760)	0.047	2.687 (1.929 - 7.772)	0.068
HT	1.020 (1.000 - 1.040)	0.052	1.004 (0.979 - 1.029)	0.765
Resting ABI	2.844 (1.332 – 7.344)	<0.001	2.702 (1.357 – 7.504)	<0.001
Post-exercise ABI	2.924 (1.461 – 7.904)	<0.001	2.968 (1.488 – 7.980)	<0.001

OR: OddsRatio, CI: ConfidenceInterval, GFR: GlomerularFiltration Rate, TIMI: Thrombolysis in MyocardialInfarction, GRACE: Global Registry of AcuteCoronaryEvents, DM: DiabetesMellitus, HT: Hypertension, ABI: AnkleBrachial Index.

Table 4. Multivariateregressionanalysisresultsfordeterminingtheindependentpredictorsfordetection of SYNTAX Score II (for PCI) ≥30

	Univariate		Multivariate	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
Age	1.232 (1.045 – 1.608)	0.004	1.226 (1.028 – 1.402)	0.007
Male Gender	1.402 (1.006 – 1.860)	0.001	1.368 (1.206 – 1.544)	0.004
GFR	1.108 (1.006 – 1.312)	0.012	1.128 (1.066 – 1.296)	0.024
TIMI Score	1.098 (1.009 – 1.196)	0.024	1.096 (0.968 – 1.202)	0.088
GRACE Score	1.102 (1.044 – 1.284)	0.016	1.188 (1.069 – 1.343)	0.022
KILLIP Class	1.269 (0.945 – 1.962)	0.188	-	-
Smoking	1.986 (0.924 – 4.672)	0.246	-	-
DM	2.084 (1.882 – 3.994)	<0.001	1.874 (1.586 – 2.980)	0.001
CAD	2.406 (1.248 – 4.962)	0.005	2.428 (1.455 – 4.204)	0.005
HT	1.106 (0.902 – 1.342)	0.306	-	-
Resting ABI	2.793 (1.406 – 6.982)	<0.001	2.766 (1.512 – 6.806)	<0.001
Post-exercise ABI	2.892 (1.504 – 6.982)	<0.001	2.806 (1.498 – 7.024)	<0.001

OR: OddsRatio, CI: ConfidenceInterval, GFR: GlomerularFiltration Rate, TIMI: Thrombolysis in MyocardialInfarction, GRACE: Global Registry of AcuteCoronaryEvents, DM: DiabetesMellitus, HT: Hypertension, ABI: AnkleBrachial Index.

Discussion

To summerize our results of the patients with ACS, we observed that i) the CAD extensity can be easily determined by measuring ABI at bedside in the patients with ACS, ii) GRACE, TIMI and KILLIP scoring systems, which are important in the prognostic follow-up in CAD, do not present any significant difference in patients with higher SYNTAX scores, iii) the resting and post-exercise ABI are negatively correlated with blood pressure, SYNTAX score and SYNTAX II PCI score, iv) the post-exercise ABI is superior to the resting ABI in detection of the extensity and complexity of CAD and v) DM, the resting and post-exercise ABI were independent predictors for the CAD extensity.

As the percutaneous invasive interventions has become gold standard method for the treatment world wide, the scoring systems based on specifics of coronary lesions were needed to develope. GENSINI and SYNTAX

scores are the methods that shows the extensity of CAD by evaluating the findings of coronary angiography (14). Compared to other scoring systems, SYNTAX scoring system considers the lesion spesifics such as anatomical location, charactheristics, bifurcation or trifurcation features, trombosis and collateral vasculature in more detail. Various studies have stated that higher values of SYNTAX score indicate a higher risk of depression, MACE and mortality (15). Akgun et al showed that SYNTAX score was a independent predictor for MACE and mortality in the period of hospitalization and long term follow-up in their study performed with 2993 patients with STEMI (16).

Although SYNTAX score was firstly defined for stable CAD, recent guidelines have recognised it as available for ACS. Increasing long-term data on morbiditiy and mortality for ACS related to SYNTAX score has led to that score being widely preferred. Suggestions for management and follow-up also differ depending on whether the score calculated below 23, between 23 and 32 or above 32 (17). SYNTAX score is able to provide a prospective risk classification in patients with multiple CAD undergone percutaneous coronary intervention (PCI) and also demonstrates the technical difficulty of PCI (18). Furthermore, SYNTAX score is a guiding tool to determine coronary artery by-pass graft surgery or PCI for the patients with left coronary artery or multiple CAD. High SYNTAX values can predict the unwilling outcomes after PCI in patients undergone revascularization (19, 20). Wykrzykowska et al presented that SYNTAX score is an independent predictor for MACE and mortality in patients with CAD after percutaneous intervention (21). Moreover, SYNTAX score has a role to classify the risks in PCI performed patients with STEMI and is beneficial inproviding an additional risk classification on known MACE risk factors (22, 23). In the patients with high SYNTAX score, poor prognosis and increased MACEs can be explained by the differences on clinical, angiographic and interventional features. To sumup, its prognostic importance and efficacy have been demonstrated in many studies. However, it may delay effective planning of treatment and follow-up in the patients lately performed or not performed angiography due to its complexity of the application, need of multiple calculation process and including of anatomical evaluation. Therefore, a search for more practical method has been going on as an alternative to the SYNTAX score.

Given its prognostic predictive power for the CAD patients, SYNTAX II score is a new and superior to the SYNTAX score and it additionally considers age, left ventricular ejection fraction, glomerular filtration rate, peripheral artery disease, chronical obstructive pulmonary disease and left coronary artery disease (3). In our study, SYNTAX II score was evaluated for the correlation with the resting and post-exercise ABI and it revealed that a negative correlation exists between SYNTAX II score and resting or post-exercise ABI.

Nonaka M et al. reported that the SYNTAX II score was more successful in their study in which they evaluated the mortality and major adverse cardiovascular and cerebrovascular events of their patients after coronary bypass surgery. (3). We did not perform a prognostic follow-up or analysis in our study. According to the design of our study, the relationship between resting and post-exercise ABI indices and the prevalence of coronary artery disease was evaluated. In this context, ABI measurements at rest and after exercise, which were significantly related to the SYNTAX score in our analyzes, were also found to be significantly related to the SYNTAX II PCI score. ABI measurements at rest and after exercise were defined as independent predictors for SYNTAX score >22 and SYNTAX II PCI score ≥30, in which we expressed the prevalence of coronary artery disease.

Determining the mortality and morbidity risks are essential for clinicians after admission of a patient with ACS. Early identification the risks about mortality and morbidity based on objective criterias is important for determining the treatment and management. Various risk scoring systems have been aimed to develope to build a consensus on accurate management. For that purpose, TIMI and GRACE risk scores were defined by using clinical and demographic features of patients and these risk scores have been applying frequently (24, 25). In a study of Hammami et al on 238 patients with ACS, GRACE and TIMI scores were evaluated for the correlation with extensity of CAD measured by SYNTAX score. It was stated that both scoring systems showed a weak correlation with SYNTAX score. It was also revealed that GRACE and TIMI had a medium level predictive value with low rate of specificity and sensitivity but they can not predict the extensity of CAD (26). In our study, it was stated that both scoring systems did not have any role in differentiating the extensity of CAD and did not correlate with CAD extensity defined by SS>22. On the other hand, TIMI and GRACE scores were found to be correlated with the SYNTAX II PCI score. In addition, the GRACE score was found to be an independent predictor of the extent of CAD, expressed as SYNTAX II PCI ≥30.

Cordero et al stated that the pathological ABI is an equivalant to advancedage in terms of mortality and cardiovascular events after ACS. Also, they defined the subgroup of high-risk advanced age who can not be diagnosed as PAD because they do not generally reveal any symptoms after ACS. These results encourages ABI measurement to identify the high-risk patients who would need to be optimized medical interventions and probably have benefit from intense management (27). PAD is also related to significant increase in risk of fatal and nonfatal cardiovascular and cerebrovascular events (28-31). Chang et al studied the correlation between ABI and CAD complexity and extensity in the patients with possibility of CAD performed coronary angiography and angioplasty (32). When compared to the patients with ABI <0.9, the patients with ABI >0.9

had more complex and extend lesions. In another study, the authors reported the indipendent predictive worth of ABI to identify more number of obstructive coronary lesions in the ACS patients (33). In the study of Bertomeu V et al that presents the high prevalence of PAD in patients who had advanced age, smoking habit, diabetus mellitus or the history of cardiovascular event after the age of 40, high cardiovascular complication rates during the hospitalization for ACS were observed in the presence of PAD defined by ABI <0.9 (34). In their study designed with 200 patients over the age of 60 who were treated for ischemic heart disease, Amer et al presented the correlation between CAD complexity and extensity measured by SYNTAX score and ABI (35). In the literature, the relation between SYNTAX score and resting ABI was interpreted in various studies. The results obtained were compatible with our results. However, there have been insufficient data for the correlation between SYNTAX or SYNTAX II score and post-exercise ABI in which low values can be obtained even in patients obtained normal resting ABI measurements. This comparison distinguishes our study from resembling ones. Hyperaemia and other parameters showing an alteration in blood flow after exercise may indicate PAD in patients with hemodinamically in significant atherosclerotic lesions at rest (36). For that reason, it is recommended the post-exercise ABI to be measured in the symptomatic patients having normal resting ABI in order to distinguish arterial claudication and pseudoclaudication (5). Diehm et al evaluated the relation between mortality and cardiovascular event with resting and post-exercise ABI after 5-year follow-up of 6468 patients with advanced age (9). It was observed that the post-exercise ABI was not superior to the resting ABI in terms of more accurate prediction of mortality and cardiovascular event. However, the low sensitivity of ABI cut-off values in that study compromises the reliability of the comparison. In our study, the post-exercise ABI cut-off value for prediction of CAD complexity is 80%. Abnormal post-exercise ABI results are shown to be associated with an increased rate of lower extremity revascularizationin the patients with normal and abnormal resting ABI. Also, when compared to the patients with normal ABI measurements, the patients with abnormal resting or post-exercise ABI measurements was presented to have an increased rate of MACE and all-cause death in the study of Hammad TA et al. In this retrospective study, it is observed that the post-exercise ABI provides clinical and prognostic information beter than normal and abnormal resting ABI (10). The post-exercise ABI measurement has been compared to the resting ABI in various ways by several authors. Nonetheless, the number of studies investigating the correlation with CAD complexity is insufficient in the literature. In our study, the post-exercise ABI is found to be more efficient than the resting ABI in predicting CAD extensity.

Limitation of the study

Relatively lower number of patients and the single-center design of the study are the most important limiting factors of our study. Multi-centered, randomised studies designed with larger patient number are needed for these inferences to be pracitced to the general population. Also, it should be taken into consideration that the KILLIP class III and IV patients who can not tolerate the resting and post-exercise ABI measurements exluded from our study.

Conclusion

The extensity of CAD can be determined with ABI scorings which are able to be applied easily at the bedside in patients admitted to the hospital with the diagnosis of ACS. We found out that the post-exercise ABI is more superior than the resting ABI for determining of the extencity and complexity of CAD (SYNTAX Score >22). ABI at rest and after exercise is associated with the prevalence of CAD, expressed as a SYNTAX score >22 and a SYNTAX II PCI score >30. ABI at rest and after exercise are independent predictors of the prevalence of CAD.

Acknowledgements: We would like to thank our ABI measurement technician and all healthcare professionals who took an active part in the follow-up/treatment of our patients.

Ethical Approval: *Health Sciences University Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital Clinical Research Ethics Committee. 18/09/25*

Author Contributions: *Concept: A.G. , E.Y. , M.E. Literature Review: A.G. , M.K. , E.A. Design : A.G. , A.R.D. , E.Y. , M.E. Data acquisition: A.G. , M.K. , E.Y. Analysis and interpretation: A.R.D. , E.Y. Writing manuscript: A.G. , M.K. , E.Y. Critical revision of manuscript: E.A. , M.E.*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Sianos G, Morel MA, Kappetein AP, et al. The SYNTAX Score: an angiographic tool grading the complexity of coronary artery disease. EuroIntervention. 2005 Aug;1(2):219-27.

2. Serruys PW, Onuma Y, Garg S, et al. Assessment of the SYNTAX score in the Syntax study. EuroIntervention. 2009 May;5(1):50-6.

3. Nonaka M, Komiya T, Shimamoto T, et al. Comparison of clinical outcomes after coronary artery bypass grafting using stratified SYNTAX scores. Gen Thorac Cardiovasc Surg. 2020 Nov;68(11):1270-1277.
4. Anderson JL, Halperin JL, Albert NM, et al. Management of patients with peripheral artery disease (compilation of 2005 and 2011 ACCF/AHA guideline recommendations): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2013 Apr 2;127(13):1425-43.
5. Alagha M, Aherne TM, Hassanin A, Zafar AS, Joyce DP, Mahmood W, Tubassam M, Walsh SR. Diagnostic Performance of Ankle-Brachial Pressure Index in Lower Extremity Arterial Disease. Surg J (N Y). 2021 Jul 19;7(3):e132-e137.
6. Hirsch AT, Haskal ZJ, Hertzer NR, et al; American Association for Vascular Surgery; Society for Vascular Surgery; Society for Cardiovascular Angiography and Interventions; Society for Vascular Medicine and Biology; Society of Interventional Radiology; ACC/AHA Task Force on Practice Guidelines Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease; American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; Trans Atlantic Inter-Society Consensus; Vascular Disease Foundation. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (low erextremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; Trans Atlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation. 2006 Mar 21;113(11):e463-654.
7. Lamina C, Meisinger C, Heid IM, et al; Kora Study Group. Association of ankle-brachial index and plaques in the carotid and femoral arteries with cardiovascular events and total mortality in a population-based study with 13 years of follow-up. EurHeart J. 2006 Nov;27(21):2580-7.
8. Korkmaz L, Adar A, Erkan H, et al. Ankle-brachial index and coronary artery lesion complexity in patients with acute coronary syndromes. Angiology. 2012 Oct;63(7):495-9.
9. Núñez D, Morillas P, Quiles J, et al; PAMISCA studyresearchers. Usefulness of an abnormal ankle-brachial index for detecting multivessel coronary disease in patients with acute coronary syndrome. RevEspCardiol. 2010 Jan;63(1):54-9.
10. Diehm C, Darius H, Pittrow D, et al. Prognostic value of a low post-exercise ankle brachial index as assessed by primary care physicians. Atherosclerosis. 2011 Feb;214(2):364-72.
11. Hammad TA, Strefling JA, Zellers PR, et al. The Effect of Post-Exercise Ankle-Brachial Index on Lower Extremity Revascularization. JACC CardiovascInterv. 2015 Aug 17;8(9):1238-1244.
12. Xu D, Zou L, Xing Y, et al. Diagnostic value of ankle-brachial index in peripheral arterial disease: a meta-analysis. Can J Cardiol. 2013 Apr;29(4):492-8.
13. SYNTAX Working Group. SYNTAX score calculator. http:// www.syntaxscore.com.AccessedJanuary15,2011
14. Authors/Task Force members, Windecker S, Kolh P, Alfonso F, et al. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). EurHeart J. 2014 Oct 1;35(37):2541-619.
15. Usalp S, Gündüz R, Yaman B, et al. The Predictivevalue of the Patient Health Questionnaire-9 Score for Measuring the Severity of Coronary Artery Disease. Journal of Clinical and Analytical Medicine. 2021;12(10):1152-1156.
16. Akgun T, Oduncu V, Bitigen A, et al. Baseline SYNTAX score and long-term outcome in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Clin Appl Thromb Hemost. 2015 Nov;21(8):712-9.
17. Palmerini T, Genereux P, Caixeta A, et al. Prognostic value of the SYNTAX score in patients with acute coronary syndromes undergoing percutaneous coronary intervention: analysis from the ACUITY (Acute Catheterization and Urgent Intervention Triage StrategY) trial. J AmCollCardiol. 2011 Jun 14;57(24):2389-97.
18. van Gaal WJ, Ponnuthurai FA, Selvanayagam J, et al. The Syntax score predicts peri-procedural myocardial necrosis during percutaneous coronary intervention. Int J Cardiol. 2009 Jun 12;135(1):60-5.
19. Serruys PW, Morice MC, Kappetein AP, et al; SYNTAX Investigators. Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. N Engl J Med. 2009 Mar 5;360(10):961-72.
20. Serruys PW, Onuma Y, Garg S, et al. Assessment of the SYNTAX score in theSyntax study. EuroIntervention. 2009 May;5(1):50-6.
21. Wykrzykowska JJ, Garg S, Girasis C, et al. Value of the SYNTAX scorefor risk assessment in the all-comers population of the randomized multicenter LEADERS (Limus Eluted from A Durable versus ERodable Stent coating) trial. J AmCollCardiol. 2010 Jul 20;56(4):272-7.
22. Magro M, Nauta S, Simsek C, et al. Value of the SYNTAX score in patients treated by primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: The MI SYNTAX score study. AmHeart J. 2011 Apr;161(4):771-81.
23. Garg S, Sarno G, Serruys PW, et al; STRATEGY and MULTISTRATEGY Investigators. Prediction of 1-year clinical outcomes using the SYNTAX score in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a substudy of the STRATEGY (Single High-Dose BolusTirofiban and Sirolimus-Eluting Stent Versus Abciximab and Bare-Metal Stent in Acute Myocardial Infarction) and MULTISTRATEGY (Multicenter Evaluation of Single High-Dose Bolus Tirofiban Versus Abciximab With Sirolimus-Eluting Stent or Bare-Metal Stent in Acute Myocardial Infarction Study) trials. JACC CardiovascInterv. 2011 Jan;4(1):66-75.

24. Fox KA, Anderson FA Jr, Dabbous OH, et al; GRACE investigators. Intervention in acute coronary syndromes: do patients undergo intervention on the basis of their risk characteristics? The Global Registry of Acute Coronary Events (GRACE). Heart. 2007 Feb;93(2):177-82.
25. Bradshaw PJ, Ko DT, Newman AM, et al. Validation of the Thrombolysis In Myocardial Infarction (TIMI) risk index for predicting early mortality in a population-based cohort of STEMI and non-STEMI patients. Can J Cardiol. 2007 Jan; 23(1):51-6.
26. Hammami R, Jdidi J, Mroua F, et al. Accuracy of the TIMI and GRACE scores in predicting coronary disease in patients with non-ST-elevation acute coronary syndrome. Rev Port Cardiol (EnglEd). 2018 Jan;37(1):41-49.
27. Cordero A, Morillas P, Bertomeu-González V, et al; en nombre de losinvestigadores del estudio PAMISCA. Pathologicalankle-brachialindex is equivalent of advancedage in acute coronary syndromes. Eur J ClinInvest. 2011 Dec;41(12):1268-74.
28. Agnelli G, Cimminiello C, Meneghetti G, et al; Poly vascular Atherothrombosis Observational Survey (PATHOS) Investigators. Low ankle-brachial index predicts an adverse 1-year outcome after acute coronary and cerebrovascular events. J ThrombHaemost. 2006 Dec;4(12):2599-606.
29. Lee AJ, Price JF, Russell MJ, et al. Improved prediction of fatal myocardial infarction using the ankle brachial index in addition to conventional risk factors: the Edinburgh Artery Study. Circulation. 2004 Nov 9;110(19):3075-80.
30. Tsai AW, Folsom AR, Rosamond WD, et al. Ankle-brachialindexand 7-year ischemic stroke incidence: the ARIC study. Stroke. 2001 Aug;32(8):1721-4.
31. Froehlich JB, Mukherjee D, Avezum A, et al; GRACE Investigators. Association of peripheral artery disease with treatment and outcomes in acute coronary syndromes. The Global Registry of Acute Coronary Events (GRACE). Am Heart J. 2006 May;151(5):1123-8.
32. Chang ST, Chen CL, Chu CM, et al. Ankle-arm index as a predictor of lesion morphology and risk classification for coronary artery disease undergoing angioplasty. Int J Cardiol. 2006 Nov 18;113(3):385-90.
33. Chen CC, Hung KC, Hsieh IC, et al. Association between peripheral vascular disease indexes and the numbers of vessels obstructed in patients with coronary artery disease. Am J MedSci. 2012 Jan;343(1):52-5.
34. Bertomeu V, Morillas P, Gonzalez-Juanatey JR, et al; “The Prevalence of Peripheral Arterial Disease in Patients with Acute Coronary Syndrome” (PAMISCA) Investigators. Prevalence and prognostic influence of peripheral arterial disease in patients >or= 40 years old admitted into hospital following an acute coronary event. Eur J VascEndovascSurg. 2008 Aug;36(2):189-196.
35. Amer MS, Tawfik HM, Elmoteleb AM, et al. Correlation between ankle brachial index and coronary artery disease severity in elderly Egyptians. Angiology. 2014 Nov; 65(10):891-5.
36. Ouriel K, McDonnell AE, Metz CE, et al. Critical evaluation of stresstesting in the diagnosis of peripheral vascular disease. Surgery. 1982 Jun;91(6):686-93.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISN: 2791-8815

Original Article

New Biomarkers in the Diagnosis of COVID-19: Amino Acids

COVİD-19 Tanısında Yeni Biyobelirteçler: Amino Asitler

Sukru AKMESE[1*], Ismail KOYUNCU[2], E. Sabri SEYHANLI[3]

[1] Pharmacy Services Program, Vocational School of Health Services, Harran University, Sanlıurfa, Türkiye
2.Departments of Medical Biochemistry, Faculty of Medicine, Harran University, Sanliurfa, Türkiye
3.Departments of Emergency Medicine, Mehmet Akif Inan Training and Research Hospital, Health Science University, Sanliurfa, Türkiye

***Corresponding author:**
Sukru AKMESE
Adress:
Gökberk Family Healthcare Center, Samsun/TURKİYE
email:
akmesesukru@harran.edu.tr
Received: 15.05.2022
Accepted: 22.07.2022
Cite as: AKMESE. S, KOYUNCU:I,SEYHANLI.ES New Biomarkers in the Diagnosis of COVID-19: Amino Acids IJCMBS 2022;2(2):127-135
doi.org/ 10.5281/zenodo.6869301

Abstract

Background: Coronavirus disease 2019 (COVID-19) is still spreading rapidly around the world, and early detection before its severe symptoms begin is important, but challenges in early detection remain. There is also very little data on the effects of COVID-19 on amino acid metabolism. The aim of this study is to analyze the free amino acid profile in the blood serum of COVID-19 patients and to determine their biomarker potential for diagnosis.

Materials and Methods: In this study, blood samples were taken from 30 COVID-19 patients and 30 healthy individuals, and their amino acid profile was analyzed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Biomarker and pathway analyzes from multivariate statistical analyzes were performed with the MetaboAnalyst program.

Results: Among the amino acids we analyzed, 6 of them were amino acids with strong potential to be used in the diagnosis of COVID-19. According to the AUC value (>0.800), these amino acids are alpha-aminoadipic acid, arginine, proline, alanine, sarcosine and citrulline, respectively. In addition, according to the pathway analysis, it was found that arginine and proline metabolisms were significantly impaired in COVID-19 disease (p<0.001 and impact value: 0.356).

Conclusions: This study showed that changes in the amino acid profile of COVID-19 patients may be important in diagnosis, treatment, and prognosis. According to our results, alpha-aminoadipic acid may be an excellent candidate for diagnosis.

Keywords: Amino Acid Profiling, Biomarker, COVID-19, MetaboAnalyst, Metabolomics

ÖZ

Amaç: Koronavirus hastalığı 2019 (COVİD-19) hala dünya çapında hızla yayılıyor ve şiddetli semptomları başlamadan önce erken teşhis önemlidir ancak erken teşhisteki zorluklar devam etmektedir. Ayrıca COVİD-19'un amino asit metabolizması üzerindeki etkileri hakkında çok az veri bulunmaktadır. Bu çalışmanın amacı, COVİD-19 hastalarının kan serumundaki serbest amino asit profilini analiz etmek ve tanı için biyobelirteç potansiyellerini belirlemektir.

Gereç ve Yöntem: Bu çalışmada 30 COVİD-19 hastası ve 30 sağlıklı bireyden kan örnekleri alındı ve amino asit profilleri sıvı kromatografi-kütle spektrometresi/kütle spektrometresi (LC-MS/MS) ile incelendi. MetaboAnalyst programı ile çok değişkenli istatistiksel analizlerden biyobelirteç ve yol analizleri yapıldı.

Bulgular: Analiz ettiğimiz amino asitlerden 6 tanesi COVİD-19 tanısında kullanılabilecek güçlü potansiyele sahipti. AUC değerine göre (>0.800), bu amino asitler sırasıyla alfa-aminoadipik asit, arginin, prolin, alanin, sarkosin ve sitrülindir. Ayrıca yolak analizine göre COVİD-19 hastalığında arginin ve prolin metabolizmalarının anlamlı düzeyde bozulduğu bulundu (p<0,001 ve etki değeri: 0.356).**Sonuç:** Bu çalışma, COVİD-19 hastalarının amino asit profilinde meydana gelen değişikliklerin teşhis, tedavi ve prognozda önemli olabileceklerini gösterdi. Bizim sonuçlarımıza göre alfa-aminoadipik asit, tanı için mükemmel bir aday olabilir.

Anahtar kelimeler: Amino Asit Profili, Biyobelirteç, COVİD-19, MetaboAnalyst,

Highlights

- Amino acid metabolism changes significantly in COVID-19 disease
- Amino acids may be new biomarkers in the diagnosis of COVID-19.

Introduction

COVID-19, caused by infection with the Human Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), is expected to spread rapidly around the world and new global waves are expected (1). According to the data reported to the World Health Organization, 483.556.595 cases and 6.132.461 deaths have been reported worldwide. In addition, 11.054.362.790 doses of vaccine were administered (2). In many studies, it has been reported that people with obesity, diabetes, old age, male gender, cardiovascular disease and chronic disease show hypersensitivity to COVID-19, and their risk of death increases if they are infected with SARS-CoV-2. In addition, it has been observed that the symptoms (asymptomatic, mild, moderate or severe symptoms) of this disease show great variability (3, 4). The number of cases and deaths continues to increase worldwide as a result of the development of new variants or the decrease in the effect of drugs and vaccines on the virus. There is still a need for prognostic tests that can illuminate the effect of COVID-19 on host metabolism, stage the disease or predict its severity, and be used in early diagnosis (5, 6).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is still the main diagnostic method for COVID-19. However, under the current pandemic conditions, the sensitivity of RT-PCR is not high to keep the pandemic under control. Therefore, missing a case of COVID-19 in RT-PCR tests is a public health risk and will cause the pandemic to spread to wider areas (7). Computed Tomography (CT), another method used in the detection of COVID-19 pneumonia, causes the escape of infected people due to its failure in early diagnosis, cost, insufficient radiologists and time (7, 8). These shortcomings of diagnostic methods necessitated the discovery of new biomarkers. If the disease is diagnosed early, treatment can be planned early and deaths can be reduced. In this context, the metabolomics approach is recognized as a promising technique in the discovery of new biomarkers (1, 6, 9).

The metabolomics approach, which belongs to the science of omics, studies numerous endogenous or exogenous metabolites such as carbohydrates, lipids, nucleic acids, organic acids and amino acids (10). Various invasive and non-invasive biological samples such as blood, urine, saliva and cerebrospinal fluid are used in metabolomic analysis. However, blood better reflects the metabolomic profile in disease states (11). Identification of the metabolic pathway in the cell provides an understanding of the physiology of the cell and the general state of the organism (12). In addition, among the goals of metabolomics is to develop biomarkers that can be used in the evaluation of diagnosis, prognosis and therapeutic response (13). Amino acids are metabolites with high potential that can be used both in the explanation of the pathophysiology and in the diagnosis of diseases, since they are the building blocks of proteins, relationship with organ systems, and metabolic regulators of physiological states (14, 15).

The current study aimed to improve our understanding of the pathogenesis of the disease by analyzing the serum amino acid profile of COVID-19 patients with LC-MS/MS, and also to discover new biomarkers for early diagnosis of the disease before severe symptoms of the disease appear.

Material and Method

Study Design

This study, which complies with The Code of Ethics of the World Medical Association (Declaration of Helsinki), was approved by the local ethics of Harran University Faculty of Medicine (01 June 2020, session 10). All individuals participating in this study were given detailed information and written informed consent was obtained from all patients.

30 adult patients with positive SARS-CoV-2 RT-PCR test hospitalized in Harran University Medical Faculty Training and Research Hospital were included in the COVID-19 group. The control group was selected from healthy volunteers. Exclusion criteria were COVID-19 disease, acute and chronic diseases, contact with COVID-19 positive patients, and drug use.

Sample Collection and Analysis of Amino Acid Profiles

Blood samples placed in anticoagulant tubes were centrifuged at 4000 rpm for 10 minutes at 4^0C and, the supernatants (plasma) were transferred to new tubes and frozen in liquid nitrogen and stored at -80^0C until analyzed. The plasma amino acid amount was determined by LC-MS/MS according to the Jasem brand kit protocol. 50 µl of supernatant was added to a new tube, 50 µl of internal standard mix and 700 µl of Reagent-1 were added and vortexed for 10 seconds. It was centrifuged at 4000 rpm for 5 min. The obtained supernatant was transferred to HPLC vial and 32 amino acid species were analyzed in LC-MS/MS (Shimadzu 8045, Japan) device.

Statistical Analysis

Data were statistically analyzed using IBM SPSS 25.0 and presented as mean ± standard deviation. Normality was evaluated with the Shapiro-Wilk test. The differences between the two groups were analyzed using Student's T-test and Mann-Withney U Test. A *p* value less than 0.05 was considered statistically significant.

Metabolite data obtained as a result of LC-MS/MS analysis were uploaded to MetaboAnalyst 5.0 server. First, partial least squares discriminant analysis (PLS-DA) was performed to determine the trends and clustering of individuals in the COVID-19 and control groups. Amino acids contributing to differentiation and clustering between groups were scored with variable significance in projection (VIP) analysis. A heatmap was created through hierarchical clustering, which allows visualization of the concentrations in groups of the 32 amino acids we analyzed. MetaboAnalyst's 'Biomarker Analysis' module based on the receiver operating characteristic (ROC) curve was used to identify potential biomarkers associated with COVID-19. In addition, pathway analysis was performed using MetaboAnalyst 5.0 and the Homo Sapiens-Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results

In our study, the COVID-19 group consisted of 11 males and 19 females, and the control group consisted of 16 males and 14 females volunteers. The mean age in the covid-19 group was found to be statistically significantly higher than the control group (p<0.05) **(Table 1).**

Tablo 1. Demographics of participant.

Demographic Data	**COVID-19**	**CONTROL**	***P***
Gender (Male/Female)	11/19	16/14	
Age (Years)	34.6 ± 5.95	30.05 ± 4.43	0.01

32 amino acids were analyzed in the serum of patients and healthy controls. The mean concentrations and standard deviations of amino acids are shown in **Table 2**. According to the analysis results, the decrease in alanine, arginine, beta-aminoisobutyric acid, citrulline, cystine, ethanolamine, glycine, histidine, proline and sarcosine amino acids in the COVID-19 group compared to the control group was found to be statistically significant (p<0.05). In addition, the increase in alloisoleucine, alpha-aminoadipic acid, alpha-aminobutyric acid, isoleucine and ornithine amino acids was statistically significant in the COVID-19 group compared to the control group (p<0.05). However, the changes in the remaining 17 amino acids (asparagine, glutamic acid, lysine, methionine, serine, threonine, tryptophan, valine, aspartic Acid, beta-alanine, 1-Methylhistidine, gamma aminobutyric acid, glutamine, hydroxyproline, leucine, tyrosine, phenylanalanine) were not statistically significant (p>0.05).

COVID-19 Patients Differentiated by PLS-DA

PLS-DA was performed to visualize the distribution of COVID-19 patients and healthy individuals in groups, and results were shown as two-dimensional (2D) and three-dimensional (3D) **(Figure 1).** According to the results of the analysis, it was seen that there was evident separation and clustering. The analyzed amino acids were considered successful in separating the two groups.

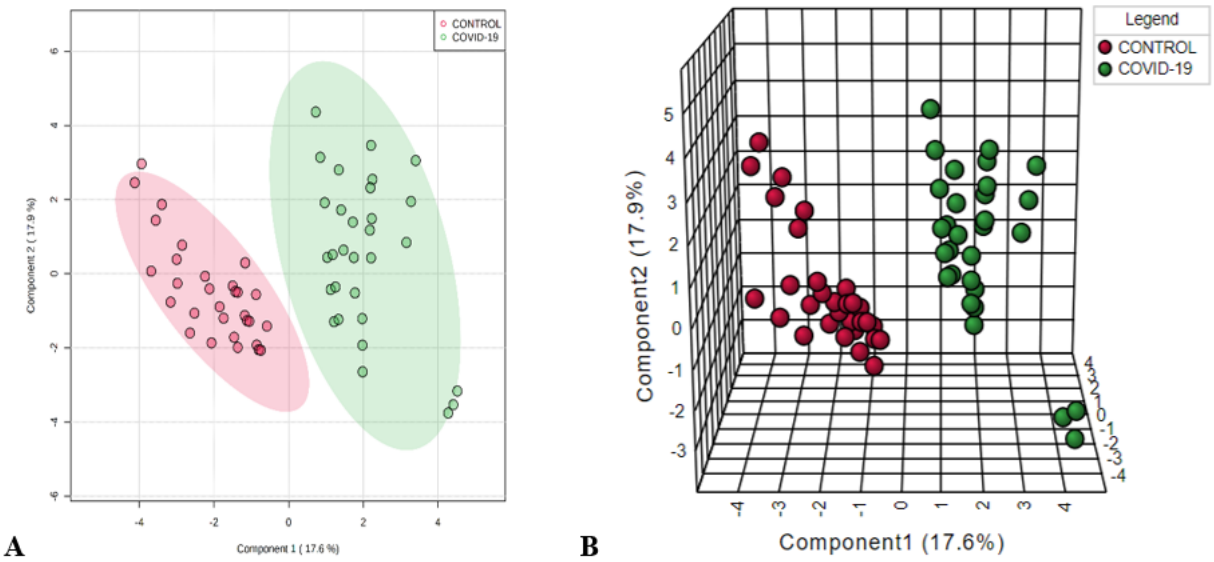


Figure 1. 2D (A) and 3D (B) score plots of PLS-DA analysis. Screening of Differential Amino Acids

Table 2. Quantitative analysis of serum amino acids composition of the groups.

Amino Acid (µmol/L)	Abbreviation	COVID-19	CONTROL	*p*
Alanine[a]	Ala	339.65±89.47	478.90±110.49	<0.001
Alloisoleucine[a]	Allo-Ile	0.68±0.13	0.62±0.08	0.027
Arginine[a]	Arg	66.80±19.85	101.88±15.66	<0.001
Asparagine[a]	Asn	44.78±11.94	46.60±8.49	0.498
Citrulline[a]	Cit	24.47±10.44	39.45±14.54	<0.001
Glutamic Acid[a]	Glu	143.28±68.20	127.56±40.01	0.281
Histidine[a]	His	76.43±17.92	93.45±17.47	<0.001
Isoleucine[a]	Ile	80.49±16.17	72.61±10.69	0.030
Lysine[a]	Lys	185.23±33.67	169.45±38.28	0.095
Methionine[a]	Met	27.04±6.25	24.70±4.59	0.104
Proline[a]	Pro	172.84±53.57	276.44±75.23	<0.001
Sarcosine[a]	Sar	27.15±7.77	36.83±7.28	<0.001
Serine[a]	Ser	171.61±44.65	168.96±29.66	0.787
Threonine[a]	Thr	130.52±32.76	128.87±31.13	0.841
Tryptophan[a]	Trp	59.63±9.02	53.68±14.29	0.059
Valine[a]	Val	228.92±41.61	223.64±37.32	0.607
1-Metilhistidine[b]	1-Mhs	1.75±0.60	2.26±1.14	0.188
Alpha-Aminoadipic Acid[b]	A-Aaa	1.38±0.68	0.26±0.08	<0.001
Alpha-Aminobutyric Acid[b]	A-Aba	20.13±7.69	12.89±3.80	<0.001
Aspartic Acid[b]	Asp	41.46±11.98	45.85±15.01	0.478
Beta-Alanine[b]	B-Ala	3.25±1.30	3.36±1.38	0.201
Beta-Aminoisobutyric Acid[b]	B-Aiba	0.14±0.13	0.83±0.65	0.001
Cystine[b]	Cys	12.12±6.60	19.86±7.00	<0.001
Ethanolamine[b]	Eta	13.93±8.97	19.44±8.67	0.022
Gamma-Aminobutyric Acid[b]	GABA	6.70±5.42	4.85±4.88	0.243
Glutamine[b]	Gln	600.26±180.33	586.94±164.08	0.701
Glycine[b]	Gly	341.82±80.36	410.33±118.05	0.031
Hydroxyproline[b]	Hyp	59.55±20.51	56.62±10.64	0.255
Leucine[b]	Leu	159.09±42.50	149.22±18.65	0.124
Ornithine[b]	Orn	79.26±31.34	60.67±23.24	0.008
Phenylanalanine[b]	Phe	106.20±27.96	111.36±16.91	0.101
Tyrosine[b]	Tyr	68.86±15.57	64.24±14.45	0.280

[a]Student's T-Test, [b]Mann-Withney U Test

VIP analysis ranks the amino acids that contributed to the differentiation of the control group and the COVID-19 group according to their contribution power (Figure 2). Alpha-aminoadipic acid, arginine and proline are the three amino acids with the highest VIP scores (>1.7). The higher the VIP score, the higher the contribution to separation. In addition, a heat map was drawn to visualize the densities of amino acids in both the COVID-19 and Control groups (Figure 3). In the heatmap, columns represent individuals in groups and rows represent amino acids. The brown and blue bands indicate the up- and down-regulation of amino acids, respectively. In addition, the increase and decrease of the depth of these colors express the increase and decrease of the regulation. Looking at the heat map, it is seen that the density of 17 amino acids increased and the density of 15 amino acids decreased relatively in the COVID-19 group.

Biomarker Candidates for COVID-19

ROC analysis was performed to identify potential biomarkers for COVID-19. As a result of the analysis, the AUC, cut-off value, sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), and p values of the 6 biomarkers with the highest AUC values are shown in Table 3. Our analyzes showed that alpha-aminoadipic acid, arginine and proline could be an biomarker for COVID-19 in the light of this information and the ROC curve is shown in Figure 4. Alpha-aminoadipic acid value higher than 0.694 µmol/L, arginine value lower than 81.6 µmol/L and proline value lower than 225 µmol/L showed that the person may have covid 19 disease.

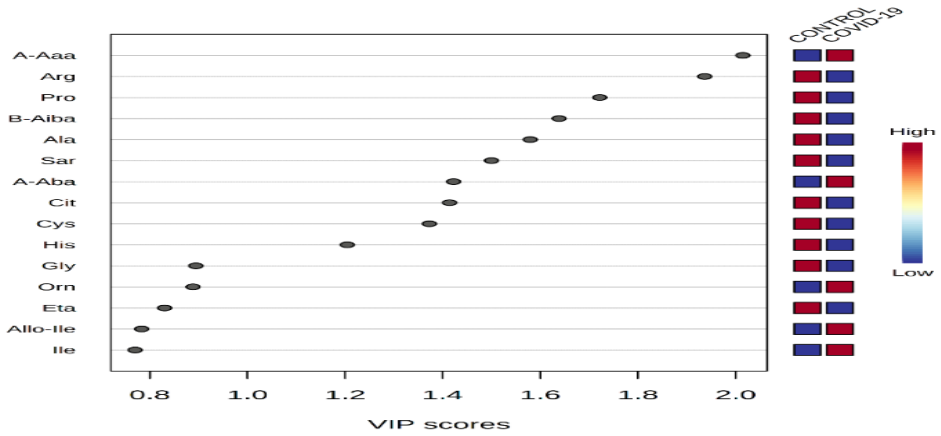


Figure 2. VIP score graph of serum amino acids.

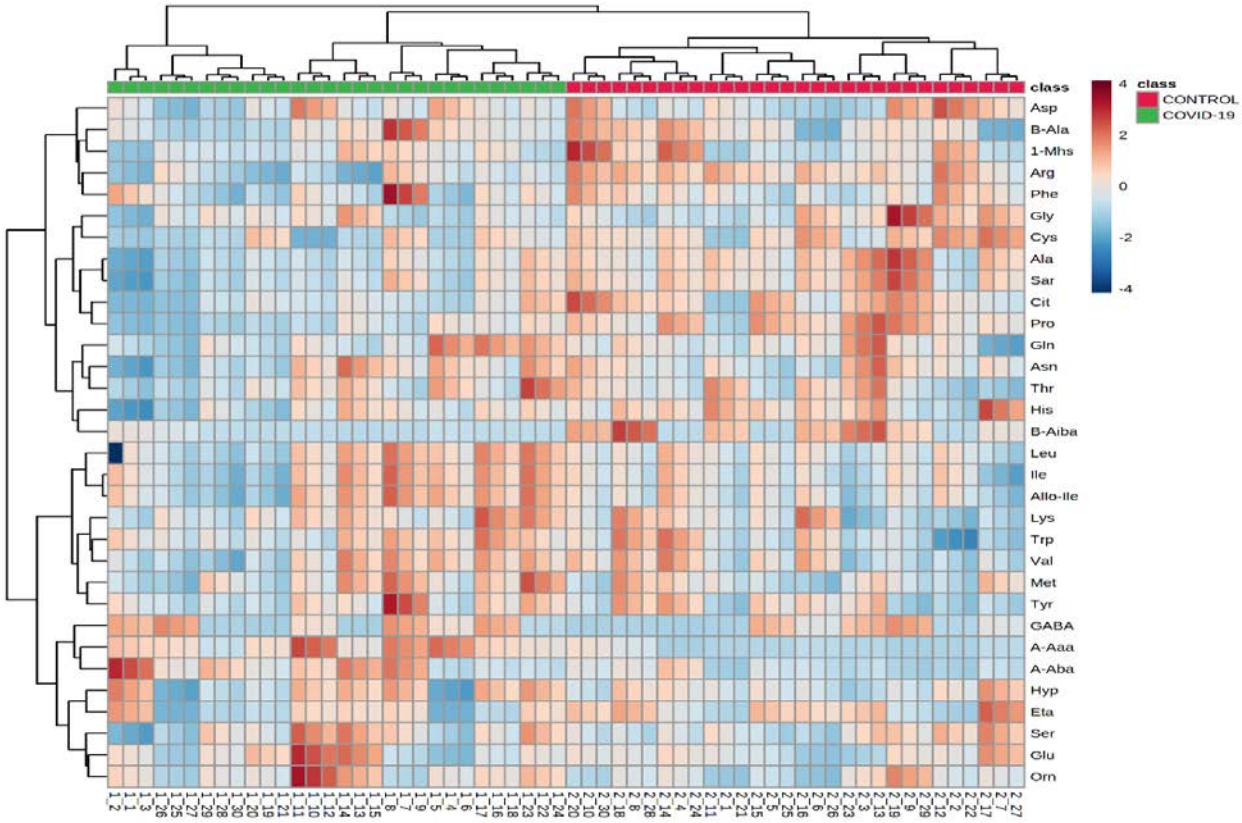


Figure 3. Heatmap: Amino acid concentration differences in groups.

Table 3. ROC analysis results

Amino Acids	Cut-off	AUC	Sensitivity	Specificity	LR (+)	LR (-)	*p*
Alpha-Aminoadipic Acid	0.694	0.964	0.90	0.9	9.00	0.111	<0.001
Arginine	81.6	0.923	0.80	0.93	12.0	0.214	<0.001
Proline	225	0.867	0.80	0.76	3.42	0.260	<0.001
Alanine	417	0.841	0.80	0.76	3.48	0.260	<0.001
Sarcosine	31.3	0.817	0.73	0.83	4.40	0.320	<0.001
Citrulline	33.7	0.802	0.90	0.66	2.70	0.150	<0.001

Table 4. Impaired metabolic pathway in the COVID-19.

Pathway Name	Match Status	*P*	-log(*p*)	Impact
Arginine and proline metabolism	5/38	<0.001	17.352	0.356
Aminoacyl-tRNA biosynthesis	20/48	<0.001	4.2154	0.166
Beta-Alanine metabolism	3/21	<0.001	3.4011	0.399
Alanine, aspartate and glutamate metabolism	6/28	0.002	2.6597	0.621
Glutathione metabolism	4/28	0.009	2.0148	0.111
Glycine, serine and threonine metabolism	5/33	0.012	1.8998	0.555

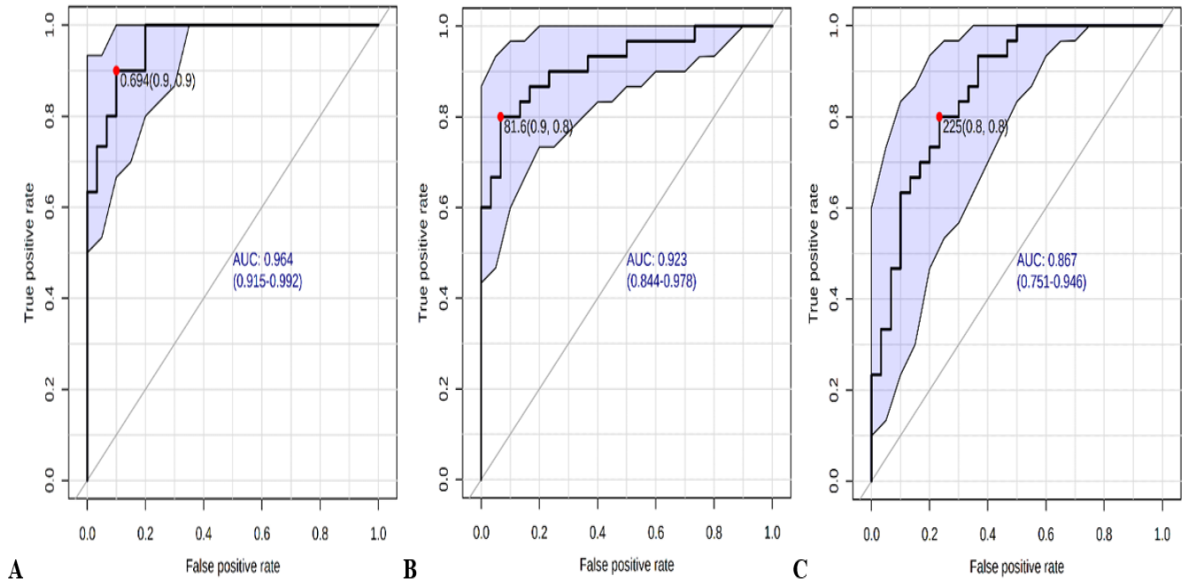


Figure 4. ROC curves of Alpha-aminoadipic acid (A), Arginine (B) and Proline (C). (Red dots show cut-off, sensitivity and specificity values)

Pathway Analysis

The discovery of biological pathways may contribute to elucidating the altered metabolomic metabolism of humans infected with SARS-CoV-2. Analysis results showed the presence of significantly altered metabolites and biological pathways in COVID-19 patients. Disruption in 34 metabolic pathways has been identified. However, $p < 0.05$ or pathway impact > 0.1 indicates significantly altered pathway. Match Status, *p*-value, -log(*p*) and impact values of altered metabolic pathway ($p < 0.05$) are given in **Table 4.**

Pathway analysis (Impact>0.1 and $p<0.05$) showed 6 different pathways that changed significantly in COVID-19 (**Table 4 and Figure 5).** These; arginine and proline metabolism, aminoacyl-tRNA biosynthesis, beta-alanine metabolism, alanine, aspartate and glutamate metabolism, glutathione metabolism and glycine, serine and threonine metabolism. In addition, metabolic pathway with impact value > 0.1 and - log (*p*) > 10 are the pathway with the highest impared. According to this information, our analysis result showed that Arginine and proline metabolism was the most disrupted metabolic pathway.

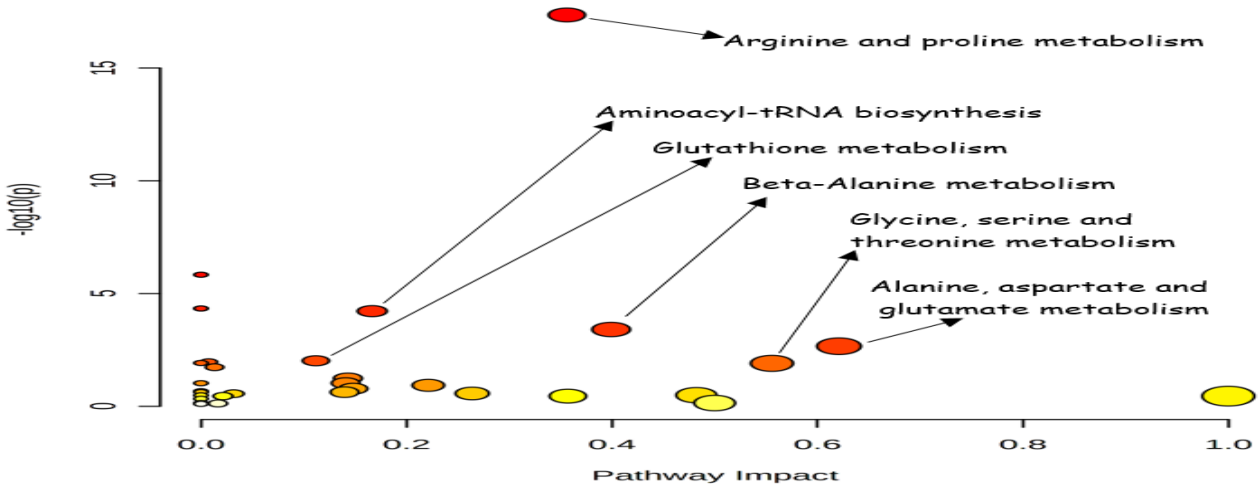


Figure 5. Summary of pathway analysis.

Discussion

Metabolomics is a promising "omics" field in systems biology; Its purpose is the comprehensive analysis of low molecular weight endogenous and exogenous metabolites. Amino acids, organic acids, fatty acids and carbohydrates are important metabolites in the biological system. In addition, they are attractive candidates for understanding disease phenotypes, as they are end products of transcription and translation (16). Metabolomics approach could enable mapping of disruptions of early biochemical changes in diseases therefore, it may provide an opportunity to develop predictive biomarkers that could result in earlier intervention and provide valuable insights into the mechanisms of diseases (17). Despite difficulties in the selection of the mass spectrometry method and the evaluation of metabolomic data, it offers excellent potential in the discovery of diagnostic biomarkers for COVID-19 (18).

It is known that changes in amino acid concentrations in biological fluids provide information about many types of diseases such as diabetes mellitus, oncologic diseases (such as colorectal cancer, breast cancer, lung cancer, esophageal cancer, pancreatic cancer and prostate cancer), Alzheimer's disease, cardiovascular disease, bowel disease and obesity (19). In addition, the importance of the use of foods with high amino acid/protein content in the treatment was reported in order to strengthen immunity against COVID-19 and reduce the emerging symptoms (20). As a result of the data obtained, it has been suggested that amino acids can be used as a biomarker and treatment target in terms of diagnosing diseases and examining the effectiveness of treatment (21). In our study, three amino acids (Alpha-aminoadipic acid, Arginine ve Proline) were identified that change significantly in the serum of the participants, contribute the most to the separation of sick and healthy individuals (VIP scores > 1.7), and also have high potential (AUC>850) as diagnostic markers that can be used in the diagnosis of the disease.

First, Alpha-aminoadipic acid, also known as 2-aminoadipic acid, is a derivative of the amino acid lysine. Lysine, which is found in the structures of proteins, forms intermediate allysin by oxidation and deamination. However, if it undergoes further oxidation, alpha-aminoadipic acid is formed and passes from the tissues to the plasma as a result of the breakdown of proteins, as in other amino acids (22). Alpha-aminoadipic acid has been reported to be a biomarker with high potential for diseases with high morbidity and mortality in humans such as skin aging, renal failure, crystalline sclerosis, aging, and sepsis (23, 24). In a study by Borchers et al. in which biomarkers that can be used to determine the severity of COVID-19 disease were investigated, alpha-aminoadipic acid was reported to be a good biomarker. In our study, it was found to be significantly higher in the COVID-19 group than in the control group. It contributed the most to the separation between the groups (VIP score >2.0), and according to the ROC analysis result, we concluded that it could be a good biomarker for the diagnosis of COVID-19 disease.

The second, arginine, a semi-essential amino acid, is used as a substrate in several enzymatic reactions and is metabolized to nitric oxide (NO), ornithine, urea, citrulline, agmatine, glutamate, creatine, proline, and polyamines (25, 26). NO and citrulline are formed from arginine in the reaction catalyzed by nitric oxide synthase (NOS). The many physiological functions of NO include neurotransmission, smooth muscle relaxation, inhibition of platelet activation and immune response (27). NO inactivates viruses by modifying the proteins and nucleic acids required for viral replication. It can also reduce in vitro viral replication of various viruses (Herpes virus, Coxsackie virus, Rhino virus, Japanese Encephalitis virus, Hanta virus, Vaccinia virus, Retro virus and the SARS virus). An increase in the level of nasally produced NO is associated with a reduction in cold symptoms. Therefore, NO constitutes a defense mechanism for viruses in the respiratory tract (28). In a recent study, the plasma Arginine level of patients infected with COVID-19 was found to be significantly lower than in healthy controls (29). In the study of Atila et al., it was reported that arginine may be the gold standard in the diagnosis of COVID-19 (21). In addition, another study showed that plasma arginine level is inversely proportional to the severity of COVID-19 (30). In our study, it levels were found to be significantly lower in the COVID-19 group compared to the control group. This decrease supports other studies and may be due to the increase in the use of arginine to increase or compensate the level of NO produced by the body's defense against SARS-CoV-2. According to our results, arginine made a high contribution to the separation of healthy and sick groups (VIP>1.8), and it may be a biomarker with a very high potential to be used in the diagnosis of COVID-19.

Third, proline is an imino acid that is synthesized in humans both through food and endogenously in the body (31). It has important roles in protein synthesis, formation of metabolites (arginine, polyamine and glutamate), oxidative stress reactions, wound healing and body defense (32). Six monogenic congenital defects associated with disruptions in proline metabolism have been identified. It has also been reported to be associated with the neuropsychiatric disorder schizophrenia (33). In the study conducted to evaluate the proline metabolism of people affected by COVID-19, the proline level was found to be significantly lower in the covid group compared to the control group (34). In the metabolomic study by Troisi et al., COVID-19 patients were grouped according to disease severity (asymptomatic, moderately symptomatic, severely symptomatic) and compared with a healthy control group. As a result of the study, proline levels decreased in the control and asymptomatic groups compared to the moderate symptomatic and severe symptomatic groups. The presence of COVID-19 or an increase in the severity of the disease decreased the proline level (35). In the study of Atila et al., COVID-19 patients were divided into four groups (Asymptomatic patients, mildly affected, moderately affected, severely/critically affected) and compared with the healthy control group. As a result of the study, it was reported that the proline level decreased as the severity of the disease increased (21). According to our results, the proline level was found to be significantly lower in the COVID-19 group compared to the control group, which supports other studies. In addition, it has made an important contribution to the separation of sick and healthy individuals and has become the third biomarker with a high potential to be used in the diagnosis of COVID-19 patients.

The relatively small sample size of this study is an important limitation. In addition, metabolomics has been used clinically for the past few years and amino acids have become important metabolites in this field. It is known that factors such as age, gender, lifestyle, diet, measurement techniques and disease severity can affect

the amino acids profile. Therefore, expanding the sample, stratifying patients by disease severity and sex, and analyzing more amino acids (32 amino acids in this study) may help confirm the study results.

Conclusion

Consequently, COVID-19 viral infection alters and/or affects cellular metabolic pathway, thus causing significant changes in the levels of many amino acids. Identification of changed amino acids can be biomarkers for diagnosis. The spread of metabolic science will make important contributions to a better understanding of the pathophysiology of many diseases, including COVID-19, and to the discovery of diagnostic biomarkers and therapeutic agents.

Acknowledgements: None

Ethical Approval: *The local ethics committee of Harran University Faculty of Medicine (01 June 2020, session 10) approved the study.*

Author Contributions: *Author Contributions: Concept: S.A. Literature Review: S.A., I.K.. Design: S.A., I.K.. Data acquisition: S.A., I.K., E.S.S.. Analysis and interpretation: S.A., I.K.. Writing manuscript: S.A., I.K.. Critical revision of manuscript: S.A., I.K..*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. El-Shabasy RM, Nayel MA, Taher MM, et al. Three wave changes, new variant strains, and vaccination effect against COVID-19 pandemic. Int J Biol Macromol. 2022; 204:161-168.
2. Covid19.who.int. WHO Coronavirus (COVID-19) Dashboard 2022 [updated 15 May 2022. Available from: https://covid19.who.int/.
3. Pang Z, Zhou G, Chong J, et al. Comprehensive meta-analysis of COVID-19 global metabolomics datasets. Metabolites. 2021;11(1):44.
4. Saccon E, Bandera A, Sciumè M, et al. Distinct Metabolic Profile Associated with a Fatal Outcome in COVID-19 Patients during the Early Epidemic in Italy. Microbiol Spectr. 2021;9(2):e00549-21.
5. Bailey M, Spick MP, Longman K, et al. Untargeted saliva metabolomics reveals COVID-19 severity: Saliva Metabolomics for SARS-COV-2 Prognosis. MedRxiv. 2021.
6. López-Hernández Y, Monárrez-Espino J, Oostdam ASHV, et al. Targeted metabolomics identifies high performing diagnostic and prognostic biomarkers for COVID-19. Sci Rep. 2021;11(1):1-13.
7. Wu YH, Gao SH, Mei J, et al. Jcs: An explainable covid-19 diagnosis system by joint classification and segmentation. IEEE Trans Image Process. 2021;30:3113-26.
8. Poortahmasebi V, Zandi M, Soltani S, et al. Clinical performance of RT-PCR and chest CT scan for COVID-19 diagnosis; a systematic review. Front Emerg Med. 2020;4(2):e57.
9. Fraser DD, Slessarev M, Martin CM, et al. Metabolomics profiling of critically ill coronavirus disease 2019 patients: identification of diagnostic and prognostic biomarkers. Crit Care Explor. 2020;2(10):e0272.
10. Ussher JR, Elmariah S, Gerszten RE, et al. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. J Am Coll Cardiol. 2016;68(25):2850-70.
11. Doğan HO, Şenol O, Bolat S, et al. Understanding the pathophysiological changes via untargeted metabolomics in COVID-19 patients. J Med Virol. 2021;93(4):2340-49.
12. Pallares-Méndez R, Aguilar-Salinas CA, Cruz-Bautista I, et al. Metabolomics in diabetes, a review. Ann Med. 2016;48(1-2):89-102.
13. Serkova NJ, Standiford TJ, Stringer KA. The emerging field of quantitative blood metabolomics for biomarker discovery in critical illnesses. Am J Respir Crit Care Med. 2011;184(6):647-55.
14. Kume S, Araki Sİ, Ono N, et al. Predictive properties of plasma amino acid profile for cardiovascular disease in patients with type 2 diabetes. PloS one. 2014;9(6):e101219.
15. Liu X, Jin F, Wang C, et al. Targeted metabolomics analysis of serum amino acid profiles in patients with Moyamoya disease. Amino Acids. 2022;54(1):137-46.
16. Leichtle AB, Nuoffer JM, Ceglarek U, et al. Serum amino acid profiles and their alterations in colorectal cancer. Metabolomics. 2012;8(4):643-53.
17. Zhang A, Sun H, Wang X. Serum metabolomics as a novel diagnostic approach for disease: a systematic review. Anal Bioanal Chem. 2012;404(4):1239-45.
18. Asim M, Sathian B, Banerjee I, et al. A contemporary insight of metabolomics approach for COVID-19: Potential for novel therapeutic and diagnostic targets. Nepal J Epidemiol. 2020;10(4):923-27.
19. Song Y, Xu C, Kuroki H, et al. Recent trends in analytical methods for the determination of amino acids in biological samples. J Pharm Biomed Anal. 2018;147:35-49.
20. Karaogul E, Hayoğlu İ, Cicek A, et al. Foods That Improving the Immune System against Covid-19 and Its Infectiousness. International Journal of Current Medical and Biological Sciences. 2021;1(2):58-68.

21. Atila A, Alay H, Yaman ME, et al. The serum amino acid profile in COVID-19. Amino acids. 2021;53(10):1569-88.
22. Wang TJ, Ngo D, Psychogios N, et al. 2-Aminoadipic acid is a biomarker for diabetes risk. J Clin İnvestig. 2013;123(10):4309-17.
23. Estaras M, Ameur FZ, Estévez M, et al. The lysine derivative aminoadipic acid, a biomarker of protein oxidation and diabetes-risk, induces production of reactive oxygen species and impairs trypsin secretion in mouse pancreatic acinar cells. Food Chem Toxicol. 2020;145:111594.
24. Sell DR, Strauch CM, Shen W, et al. 2-aminoadipic acid is a marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal failure and sepsis. Biochem J. 2007;404(2):269-77.
25. Adebayo A, Varzideh F, Wilson S, et al. l-Arginine and COVID-19: An Update. Nutrients. 2021;13(11):3951.
26. Wu G, Morris Jr SM. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336(1):1-17.
27. Davis KL, Martin E, Turko IV, et al. Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol. 2001;41(1):203-36.
28. Martel J, Ko Y-F, Young JD, et al. Could nasal nitric oxide help to mitigate the severity of COVID-19?. Microbes Infect. 2020;22(4-5):168-71.
29. Rees CA, Rostad CA, Mantus G, et al. Altered amino acid profile in patients with SARS-CoV-2 infection. Proc Natl Acad Sci. 2021;118(25):e2101708118.
30. Sacchi A, Grassi G, Notari S, et al. Expansion of myeloid derived suppressor cells contributes to platelet activation by L-arginine deprivation during SARS-CoV-2 infection. Cells. 2021;10(8):2111.
31. Vettore LA, Westbrook RL, Tennant DA. Proline metabolism and redox; maintaining a balance in health and disease. Amino Acids. 2021;53(12):1779-88.
32. Wu G, Bazer FW, Burghardt RC, et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino acids. 2011;40(4):1053-63.
33. Hu C-aA, Phang JM, Valle D. Proline metabolism in health and disease. Amino Acids. 2008;35(4):651-2.
34. Ergin Tuncay M, Neselioglu S, Asfuroglu Kalkan E, et al. Modified Proline Metabolism and Prolidase Enzyme in COVID-19. Lab Med. 2022.
35. Troisi J, Cavallo P, Masarone M, et al. Serum metabolomic profile of symptomatic and asymptomatic SARS-CoV-2 infected patients. Res Sq. 2020.

IJCMBS
THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Original Article

The effects of Cisplatin on Embrionic Renal Cell (Hek-293) Amino Acid Metabolism

Cisplatinin Embriyonik Renal Hücre (Hek-293) Amino Asit Metabolizması Üzerine Etkileri

Erkan ARSLAN[1*], Ismail KOYUNCU[2], Sukru AKMESE[3]

[1]Department of Urology, Usak University, Usak, TÜRKİYE
[2]Departments of Medical Biochemistry, Faculty of Medicine, Harran University, Sanlıurfa, TÜRKİYE
[3] Pharmacy Services Program, Vocational School of Health Services, Harran University, Sanlıurfa,TÜRKİYE

***Corresponding author:**

Dr. Erkan ARSLAN
Adress:
Department of Urology, Usak University, Usak, TÜRKİYE
email: drurology.erkan@gmail.com
Received: 21.06.2022
Accepted: 22.07.2022
Cite as:ERKAN.A, KOYUNCU.İ,AKMRSE The effects of Cisplatin on Embrionic Renal Cell (Hek-293) Amino Acid Metabolism IJCMBS 2022;2(2):136-142 doi.org/ 10.5281/zenodo.6870680

Abstract

Background: Cisplatin is one of the most widely used and most potent chemotherapy drug, due to its side effects in normal tissues and organs, especially its nephrotoxic effects in the kidneys, its use was limited. Therefore, new studies are being conducted to elucidate the mechanism of nephrotoxicity and to identify new protective agents in order to reduce the side effects of cisplatin. This study was conducted to investigate the effects of cisplatin on amino acid metabolism of embryonic kidney cells (HEK-293). **Materials and Methods:** In the study, HEK-293 were incubated with cisplatin at different doses (1-20µg) in appropriate medium for 24 hours, and then the cells were homogenized. The intracellular free amino acid profile in the samples was analyzed by LC-MS/MS method. **Results:** Compared to the control group, a significant decrease was observed in 1-methylhistidine, 3-methylhistidine, aspartic acid and cystine levels, while a significant increase was found in other amino acids. As a result of conducting advanced bioinformatic analyzes through Principal Component Analysis (PCA) and Variable Importance in Projection (VIP), it was determined that the most significant difference that was realized between the control and cisplatin applied groups was in glutamine, glutamic acid, glycine, arginine and lysine amino acids.
Conclusions: It was observed that high doses of cisplatin can inhibit protein synthesis in kidney cells and increase the amount of free amino acids by increasing the digestion of intracellular damaged proteins.
Keywords: Amino Acid, LC-MS/MS, Metabolomic, Prostate Cancer

ÖZ

Amaç: Sisplatin en yaygın kullanılan ve en güçlü kemoterapi ilaçlarından biridir, normal doku ve organlardaki yan etkileri, özellikle böbreklerdeki nefrotoksik etkileri nedeniyle kullanımı sınırlı kalmıştır. Bu nedenle, sisplatinin yan etkilerini azaltmak için nefrotoksisite mekanizmasını aydınlatmak ve yeni koruyucu ajanları belirlemek için yeni çalışmalar yürütülmektedir. Bu çalışma, sisplatinin embriyonik böbrek hücrelerinin (HEK-293) amino asit metabolizması üzerindeki etkilerini araştırmak amacıyla yapılmıştır.
Gereç ve Yöntem: Çalışmada HEK-293, sisplatin ile farklı dozlarda (1-20µg) uygun ortamda 24 saat inkübe edildikten sonra hücreler homojenize edildi. Numunelerdeki hücre içi serbest amino asit profili LC-MS/MS yöntemi ile analiz edildi.
Bulgular: Kontrol grubu ile karşılaştırıldığında, 1-metilhistidin, 3-metilhistidin, aspartik asit ve sistin düzeylerinde önemli bir düşüş gözlenirken, diğer amino asitlerde önemli bir artış tespit edildi. Temel Bileşen Analizi (PCA) ve Projeksiyonda Değişken Önem (VIP) ile ileri biyoinformatik analizler sonucunda, kontrol ve sisplatin uygulan gruplar arasında en önemli farkın glutamin, glutamik asit, glisin, arginin ve lisin amino asitlerinde olduğu belirlendi.
Sonuç: Yüksek dozlarda sisplatinin böbrek hücrelerinde protein sentezini engelleyebileceği ve hücre içi hasarlanan proteinlerin sindirimini artırarak serbest aminoasit miktarını artırabileceği görüldü.
Anahtar Kelimeler: Amino Asit, LC-MS/MS, Metabolomik, Prostat Kanseri

Highlits

- High doses of cisplatin alter amino acid and protein metabolism in kidney cells.
- Free amino acids significantly reflect nephrotoxicity.

Introduction

Cisplatin is one of the most remarkable achievements in the 'battle against cancer'. Although it was accidentally discovered forty years ago, cisplatin showed very highly effect as a chemotherapy treatment. Cisplatin nowadays, it is used in the treatment of many types of cancer such as non-small cell lung cancer, ovarian, cervical, testicular, head and neck cancer (1-5). It has a high cure rate of over 90% in testicular cancers. Although many of the drugs used in treatment of cancer are complex organic compounds, meanwhile, cisplatin is considered to be an inorganic and simple molecule. Cisplatin's anticancer activity and its mechanisimare not fully understood, but a widely accepted view of points declared that cisplatin binds to DNA, leading to the formation of interchain crosslinks. The cross-linking causes defective DNA templates and cessation of DNA synthesis and replication. This mechanism of action may cause cross-linking and DNA damage in rapidly dividing cells as well as in cancer cells (6-10). The success of cisplatin in the treatment of cancer has led to an increase in studies aimed at investigating such side effects. Although cisplatin is a mainstay for the treatment of cancer, its use is mainly limited to two factors: severe side effects in normal tissues and acquired resistance to cisplatin (2-5). The cisplatin's resistance mechanism has been studied; this mechanism may include increased out flowor decreased uptake of cisplatin, increased DNA repair, neutralization of cisplatin by glutathione and other sulfur-containing molecules, and defective apoptotic signaling in response to DNA damage (7-10). Another important limiting factor in the use of cisplatin is that its side effects on normal tissues, such as ototoxicity, nephrotoxicity, vomiting and nausea, and neurotoxicity. Various approaches and studies have been done for many years to reduce these side effects. One strategy was done to synthesize and screen for new cisplatin analogues with lower toxicity in normal tissues. In this way, several analogues of cisplatin, such as carboplatin, have been identified with less serious side effects (11-14). Another approach that has been successfully used to hydrate patients during cisplatin therapy. Although many efforts was made, the nephrotoxicity side effect of cisplatin, in particular, remain an important limiting factor in the use of cisplatin in cancer treatment. Understanding the cisplatin's nephrotoxicity mechanism may lead to new renoprotective interventions (15-17). In this study, the effects of cisplatin on the amino acid metabolism of normal kidney cells were examined for the first time.

Material and Method

Cell Culture Conditions

Normal human embryonic kidney epithelial (HEK-293) cells, that obtanied and stocked from American Type Culture Collection(ATCC), were used in the study. DMEM-F12 (Sigma-Aldrich Cat No: D9785,USA) fattening media, 10% FBS (Sigma-Aldrich Cat No: F7524, USA) and 1% L-glutamine (Sigma-Aldrich Cat No: 59202C, USA), 1% penicillin/streptomycin (Sigma-Aldrich Cat No: P4333, USA) were used for the nutrition and growth of the cells. The cells were taken from -80 °C and dissolved in a water bath at 37°C.The thawed cell solution was carefully transferred to the nutrient medium taken into the falcon tube and centrifuged at 5000 rpm for 5 minutes, 2 ml of medium was added to the formed pelleted cells, suspended with a pipette and homogenized, 8 ml of medium was added to a 25 cm^2 flask and the cell solution was added. It was incubated at 37 °C, 5% CO_2 and 95% humidity for 24 hours and cell proliferation was observed. After the cells reached 80% in 25 cm^2 flasksthe study was started. After the sample was taken from the medium after incubation, the remaining medium was removed. In order to remove the remaining medium waste, the cells were washed 2 times with cold 1x Dulbecco Phosphatebuffered saline (dPBS, Sigma-Aldrich Cat No: D8537, USA) and scraped with a cell scraper. After being taken into 1 ml cold 1x dPBS medium, the cell suspension was taken into a 15 ml sterile falcon tube. The pellet was obtained by centrifuging at 1200 rpm for 5 minutes and removing the supernatants. After the obtained pellet was washed 2 times with 1xdPBS, it was lysed with cold lysis buffer and homogenizer (Qiagen tissue lyser, Germany) at 4^{+}°C for 10 minutes. The resulting lysate was centrifuged at 14,000 rpm. Analyzes were made with the obtained supernatant.

Investigation of free amino acid profile by LC-MS/MS

Intracellular free amino acid profiling analysis was performed using LC-MS/MS (Shimadzu-8045) and applying commercial kit protocol (Bome Trivitron, Trimaris-BR130030, Turkey)(21). In this kit method, a derivative method is used in the analysis of free amino acids, after 100 µL of sample is taken, it is mixed with an internal standard mixture consisting of 20 amino acids with C13 and N15 labeled atoms prepared in 0.1 M HCl. In the second step, basic organic buffer components prepared in propanol are added tothe pH balance toenable the derivative reactionis taking place more efficiently. At this stage, the precipitation of the proteins in the sample also takes place. Then, a chloroform/isooctane mixture containing 5% alkyl chloroformate as an active ingredient is added to the mixture and left at room temperature for 3 minutes. Then, by centrifugation the derivatized amino acids are taken to the upper phase that contains organic solvents. 1 µL of this phase is injected into the LC-MS/MS system. Since the molecular weightafter the extraction and derivatization process of the esterified amino acids increases and becomes more volatile accordinglly, the signal given by the MS device also increases. The separation of chromatographic was performed on Trimaris Amino Acid LC-MS/MS column (250 mm x 2 mm, 3 µM) containing C18 reversed phase filler. Mobile phase A content was determined as Water: MeOH:1M Ammonium formate (85:14:1) and Mobile phase B content was determined as MeOH. Amino acid molecules were analyzed in MRM mode by ESI (+) ionization method.

Statistical Analysis

Data were statistically analyzed using SPSS version 22.0 (SPSS Inc.). Results were given as mean ± standard deviation. Differences between groups were analyzed using the Kruskal-Wallis test followed by the Tamhane test, and $p<0.05$ was considered significant. MetaboAnalyst 5.0 was used for multivariate statistical analysis. Segregation and clustering between groups were determined by Principal Component Analysis (PCA). Variable Importance in Projection (VIP) scores were found to detect amino acids that contributed to this dissociation and aggregation. In addition, a heat map was created to visualize the densities of amino acidsthat varied between groups.

RESULTS

Effects of cisplatin on the profile of the free amino acid of kidney cells

Different doses effects of cisplatin on the amino acid profile (40 amino acid) of HEK-293 cells, compared with the control group, were statistically examined by the classical SPSS method, and the results are given in Table-1. Compared with the control, it was observed that cisplatin caused a significant change in other amino acid except anserine and alpha-aminopimelic acid in kidney cells. When comparing with the control group, a significant decrease was observed in 1-methylhistidine, 3-methylhistidine, aspartic acid and cystine levels, while a significant increase was observed in other amino acids.

Table 1. Quantitative analysis of inside cell of amino acids composition of the groups.

Amino Acids Name	Group Means (µm)					*p*
	Control	Cisplatin 1µm	Cisplatin 5µm	Cisplatin 10µm	Cisplatin 20µm	
1-Methylhistidine	0.017	0.008	0.013	0.031	0.065	<0.01
3-Methylhistidine	0.008	0.007	0.002	0.004	0.018	<0.01
Alanine	1.523	3.205	3.025	4.318	11.682	<0.01
Alloisoleucine	0.001	0.004	0.004	0.013	0.021	<0.01
Alpha-Aminoadipic Acid	0.003	0.064	0.065	0.038	0.185	<0.01
Alpha-Aminobutyric Acid	0.003	0.004	0.008	0.004	0.114	<0.01
Arginine	0.517	1.459	1.282	5.756	9.911	<0.01
Argininosuccinic Acid	0.032	0.061	0.195	0.025	0.182	<0.01
Asparagine	0.353	0.711	0.351	1.216	2.583	<0.01
Aspartic Acid	3.729	4.222	2.277	2.757	3.700	<0.01
Beta-Alanine	0.136	0.235	0.176	0.163	0.707	<0.01
Beta-Aminoisobutyric Acid	0.025	0.058	0.071	0.217	0.436	<0.01
Citrulline	0.134	0.479	0.448	0.572	0.483	<0.01
Cystathionine	0.813	0.006	0.422	0.012	1.886	<0.01
Cystine	0.013	0.007	0.010	0.526	0.022	<0.01
Gamma-Aminobutyric Acid	0.017	0.150	0.096	0.313	0.554	<0.01
Glutamic Acid	11.454	17.025	12.837	11.655	34.274	<0.01
Glutamine	0.810	2.013	14.013	29.007	57.784	<0.01
Glycine	4.491	7.585	6.217	7.681	17.938	<0.01
Histamine	0.011	0.028	0.034	0.002	0.023	<0.01
Histidine	0.277	0.599	0.604	1.460	2.322	<0.01
Hydroxylysine	0.012	0.021	0.022	0.007	0.007	<0.01
Hydroxyproline	0.073	0.168	0.181	0.533	0.927	<0.01
Isoleucine	0.527	1.032	1.052	3.481	5.965	<0.01
Leucine	0.827	1.790	1.580	4.515	7.714	<0.01
Lysine	0.758	1.960	1.731	5.425	9.215	<0.01
Methionine	0.171	0.345	0.292	0.774	1.500	<0.01
Ornithine	1.097	2.570	2.101	3.959	4.510	<0.01
Phenylalanine	0.516	1.141	1.094	2.972	5.029	<0.01
Proline	1.632	3.338	2.640	3.481	11.900	<0.01
Serine	1.498	2.933	2.524	4.040	5.409	<0.01
Serotonin	0.007	0.004	0.008	0.025	0.004	<0.01
Taurine	12.792	17.293	19.488	14.290	22.729	<0.01
Thiaproline	0.011	0.004	0.005	0.031	0.066	<0.01
Threonine	1.259	2.591	2.608	5.168	11.150	<0.01
Tryptophan	0.086	0.180	0.162	0.440	0.811	<0.01
Tyrosine	0.379	0.862	0.848	2.456	4.326	<0.01
Valine	0.549	1.230	1.268	3.666	6.023	<0.01
Anserine	17.511	19.709	18.478	18.231	18.619	>0.05
Alpha-Aminopimelic Acid	0.857	0.863	0.907	0.854	0.891	>0.05

Different doses of cisplatin distinguished by PCA Analysis

PCA was performed to visualize the distribution of samples in the cisplatin-administered and control groups. Two-dimensional and three-dimensional score graphs of PCA analysis are shown in figure 1A and figure 1B, respectively. PC1, PC2 and PC3 accounted for 93.7% and 5% of the variation in the data, respectively. PCA analysis In the amino acid profile of different doses of cisplatin applied to HEK-293 cells, a significant clustering and separation was observed between the groups.

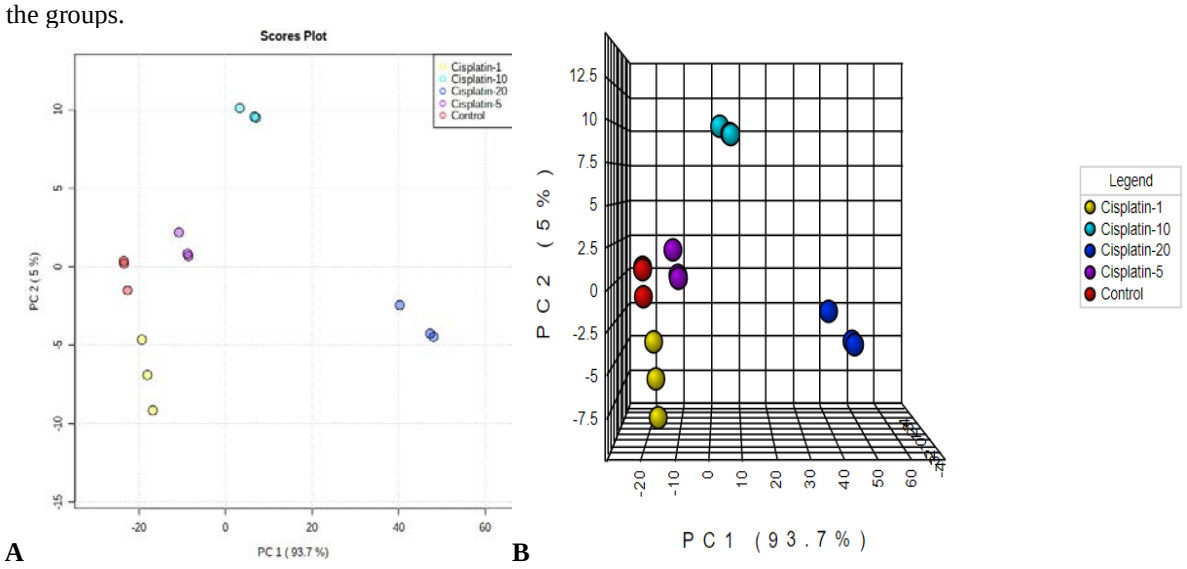


Figure 1. The amino acid profile of the Cisplatin groups applied at different doses and the control group. 2D (A) and 3D (B) score graphs of PCA.

Screening of Differential Metabolites

VIP analysis was performed to determine which amino acids changed significantly compared to the control group in HEK-293 cells treated with different doses of cisplatin (Figure 2.). The higher the VIP score (more than 1), the higher the probability of separation. Conssequantly, it was showed that the first three amino acids with the highest VIP score were glutamine, glutamic acid and glycine. An increase in the score of amino acids indicates that their contribution to the separation between the groups increases.

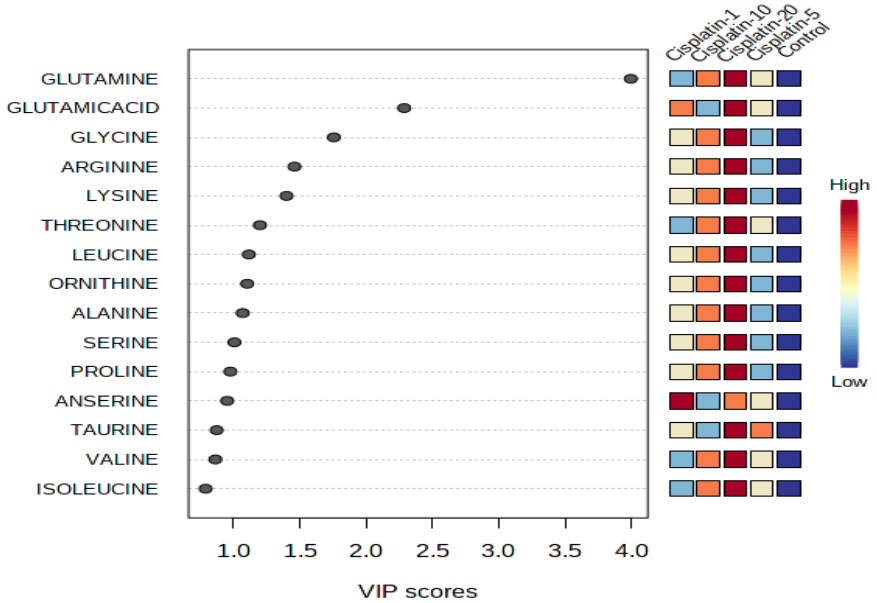


Figure 2. VIP score analysis graph.

A heat map was drawn to show the concentrations of the 40 amino acids analyzed in groups (Figure 3). In the heatmap, the columns represent cellular samples and the rows represent amino acids. Blue bands indicate that amino acids are down-regulated and brown bands are up-regulated. In addition, the increase in the depth of blue and brown colors indicates that the difference in amino acids between the groups increases. Dose increase was generally positively correlated with amino acid density. There is a significant difference in density compared to the control group, especially in the 20µm and 10µm Cisplatin applied groups.

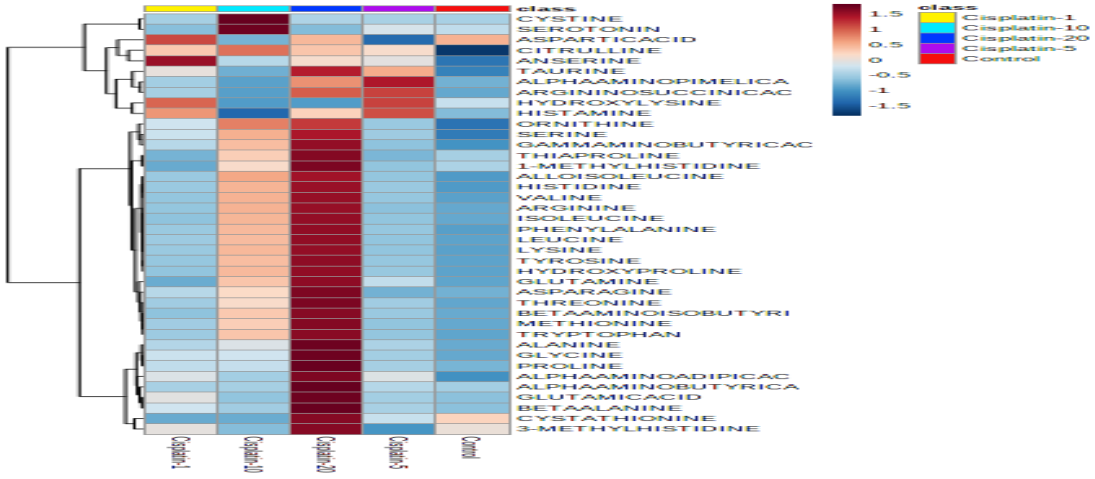


Figure 3. Hierarchical clustering heatmap of amino acids. Each group was shown in a different color code. Dendrograms were generated with Ward's clustering method and Euclidean distance measure.

Metabolic Pathway Analysis

To identify potential amino acid metabolism pathways affected by cisplatin, the pathway analyzes of amino acids identified by comparisons of groups (Cisplatin-1µm/Control, Cisplatin-5µm/Control, Cisplatin-10µm/Control and Cisplatin-20µm/Control) were investigated using MetaboAnalyst 5.0. The metabolic pathway analysis results in four comparisons of differential amino acids are shown in Figure 4. A total of 31 pathways were affected in all comparisons. Affected paths are common to all comparisons and have the same impact value. These pathways, Cisplatin-1µm/Control;11, for Cisplatin-5µm/Control; 10, for Cisplatin-10µm/Control; 9, for Cisplatin-20µm/Control; The effect value of 11 ways is >0.2 and p<0.05. Pathways with an effect value greater than 0.2 and $p < 0.05$ are considered to vary significantly. The most affected metabolic pathways that were detected are the phenylalanine, tyrosine and tryptophan biosynthesis pathway; aspartate, alanine and glutamate metabolism; D-glutamine, D-glutamate metabolism and arginine biosynthesis. In the comparisons shown in Figure 4, the match status, impact, *p*-value and -log(p) values of the altered metabolic pathways indicated by the arrow are shown in Table 2.

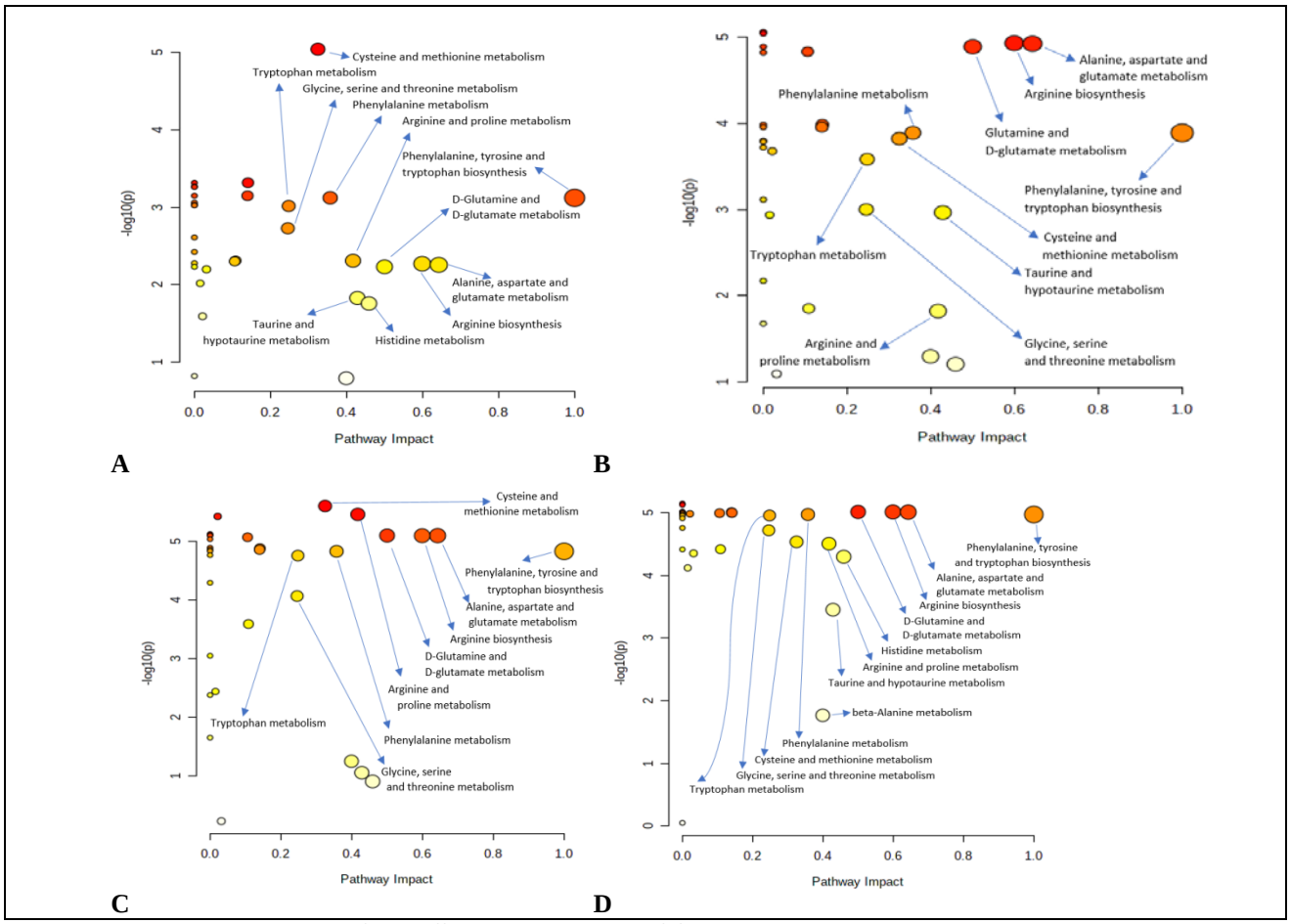


Figure 4. Metabolic pathway results from differential amino acids of four group comparisons, (A) Cisplatin-1µm/Control**, (B)** Cisplatin-5µm/Control, **(C)** Cisplatin-10µm/Control, and **(D)** Cisplatin-20µm/Control**. The color and size of the sphere depend on the p-value and the effect value, respectively. Shown with arrows are those with an effect value > 0.2 and p < 0.05.**

Discussion

Although cisplatin is an effective cancer drug that is frequently used in the treatment of various types of cancer, its various side effects, especially nephrotoxicity, limit its use. Acute kidney injury occurs in 20-35% of patients after cisplatin administration. This damage is dose-related and often results in dose reduction or discontinuation of therapy. In addition to DNA damage in the kidney proximal tubules, cisplatin causes nephrotoxicity through different cytotoxic mechanisms such as oxidative stress and inflammation (17, 18). As a result of studies carried out to date, it has been determined that increased apoptosis as a result of increased oxidative stress and DNA damage plays a fundamental role in the pathophysiology of cisplatin. However, it is still not clear how different cisplatin-induced pathways integrate and lead to kidney damage (17).

The protective efficacy of different antioxidant substances (curcumin, naringenin, Vit-E, etc.) to reduce nephrotoxicity caused by cisplatin has been investigated by many researchers. As a result of these studies, it was concluded that these substances, which have antioxidant and anti-inflammatory properties, can be used as supplements to alleviate cisplatin-induced nephrotoxicity (19-20). However, it is unclear whether such natural compounds with antioxidant activity interfere with cancer treatment while preventing cisplatin-induced kidney damage. Therefore, besides the protective effect of such natural compounds, it is necessary to investigate whether they have effects on tumor growth. Such studies may help to break the limited clinical use of cisplatin (21). Despite the studies on such protective agents, the pathogenesis of the toxic effect of cisplatin on kidney cells is still not clear. Therefore, new studies are needed. In this study, the effects of cisplatin on amino acid metabolism of embryonic kidney cells were investigated for the first time. In this way, it was aimed to predict the possible efficacy of amino acids both in the pathophysiology and in the treatment of nephrotoxicity.

Amino acids are the building blocks of proteins and are known to play a fundamental role in mediating epigenetic regulation and post-transcriptional modification as well as regulating the energetic regulation, biosynthetic support and homeostatic balance, as well as the regulation of redox balance. An example is the regulation of the methionine cycle, which plays an important role in DNA and histone methylation, by methionine, serine and glycine, and the derivation of acetyl-CoA, which plays an important role in tumor growth, from branched-chain amino acids and lysine (21-22).

Amino acids are also critical for the growth of normal and cancer cells as a building material of nucleotides biosynthesis, the regulation of lipid biosynthesis, methylation and acetylation in the regulation of ROS homeostasis by providing specific metastasis and tumor cells through epigenetic regulation. In addition, certain metabolic intermediates of certain amino acids may contribute to both tumorigenic and anti-tumorigenic activities. For example; While nitric oxide (NO), a product of arginine metabolism, promotes tumor growth by promoting angiogenesis, it can also act as a tumor suppressor by upregulating p53 (22-24).

In this study, the free amino acid profile of different doses of cisplatin in normal kidney cells was investigated. As a result of the SPSS analysis of the data obtained, it was observed that there was an increase in almost all free amino acids in the cisplatin administered groups. As a result of PCA, PLS-DA and VIP analyzes performed using the metaboanalyst program, it was determined that the most significant difference between the control and cisplatin applied groups was in glutamine, glutamic acid, glycine, arginine and lysine amino acids. It was observed that these amino acids increased with the increasing in the dose in the cisplatin administered groups, while the greatest increase was observed in the highest dose.

Conclusion

Cisplatin, a chemotherapeutic agent, is used in many types of cancer. However, the most important dose-dependent limitation of cancer treatment is that it causes nephrotoxicity. The science of metabolomics has become important in elucidating this pathophysiology. The spread of this science, which examines metabolites such as amino acids, may make it clear by which cellular mechanisms cisplatin causes nephrotoxicity. According to our data, it can be concluded that high dose cisplatin significantly inhibits intracellular protein synthesis and causes protein degradation. More scientific studies are needed to understand the effect on amino acid and protein metabolism. In this way, successful results can be obtained in the treatment by providing the use of cisplatin in the required doses to people with cancer.

Acknowledgements: None

Ethical Approval: None

Author Contributions: *Author Contributions: Concept: E.A., I.K., S.A. Literature Review: E.A. Design: E.A., I.K. Data acquisition: E.A., I.K. S.A. Analysis and interpretation: E.A., I.K. Writing manuscript: E.A., I.K., S.A. Critical revision of manuscript:E.A., I.K.*

Conflict of Interest: *The authors report no conflict of interest.*

Financial Disclosure: *Authors have no financial interests in the research.*

References

1. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014; 740:364-78.
2. Rosenberg B, Vancamp L, Krigas T. Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature. 1965;205(4972):698-99.
3. Cohen SM, Lippard SJ. Cisplatin: from DNA damage to cancer chemotherapy. Prog Nucleic Acid Res Mol Biol. 2001; 67:93-130.

4. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol 2003;23(5):460-64.
5. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003; 22(47):7265-79.
6. Singh L, Aldosary S, Saeedan AS, et al. Prolyl hydroxylase-2a promising target to inhibit hypoxia-induced cellular metabolism in cancer cells. Drug Discov Today. 2018;23(11):1873-82.
7. Brozovic A, Ambriovic-Ristov A, Osmak M. The relationship between cisplatin-induced reactive oxygen species, glutathione and BCL-2 and resistance to cisplatin. *Crit Rev Toxicol.* 2010;40(4):347-59.
8. Hilgers W, Faivre S, Chieze S, et al. A phase I and pharmacokinetic study of irofulven and cisplatin administered in a 30-min infusion every two weeks to patients with advanced solid tumors. Invest New Drugs. 2006;24(4):311-9.
9. Sasaki T. Isayama H, Nakai Y, et al. Feasibility study of gemcitabine and cisplatin combination chemotherapy for patients with refractory biliary tract cancer. Invest New Drugs. 2011;29(6):1488-93.
10. Koyuncu I. Ratlarda Sisplatin Kaynaklı Nefrotoksisite Üzerine Naringeninin Koruyucu Etkisinin İncelenmesi. Harran Üniversitesi Veteriner Fakültesi Dergisi. 2018;7(1):119-25.
11. Koyuncu I, Kocyigit A, Gonel A, et al. The protective effect of naringenin-oxime on cisplatin-induced toxicity in rats. Biochem Res Int. 2017; 2017:1-9.
12. Kocyigit A, Koyuncu I, Taskin A, et al. Antigenotoxic and antioxidant potentials of newly derivatized compound naringenin-oxime relative to naringenin on human mononuclear cells. Drug Chem Toxicol. 2016;39(1):66-73.
13. Özkol H, Musa D, Tuluce Y, et al. Ameliorative influence of Urtica dioica L against cisplatin-induced toxicity in mice bearing Ehrlich ascites carcinoma. Drug Chem Toxicol. 2012;35(3):251-7.
14. Cornelison TL, Reed E. Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. Gynecol Oncol. 1993;50(2):147–158.
15. Eastman A. The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes. Pharmacol Ther. 1987;34(2):155–166.
16. Wernyj RP, Morin PJ. Molecular mechanisms of platinum resistance: still searching for the Achilles' heel. Drug Resist Updat. 2004;7(4-5):227–32.
17. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int. 2008;73(9):994–1007.
18. Fang, Cy, Lou Dy, Zhou Lq, et al. Natural products: potential treatments for cisplatin-induced nephrotoxicity. Acta Pharmacol Sin. 2021; 42:1951-1969.
19. Ridzuan NRA, Rashid NA, Othman F, et al. Protective role of natural products in cisplatin-induced nephrotoxicity. Mini Rev Med Chem. 2019;19(4):1134–43.
20. Mathew JL, Kabi BC, Rath B. Anti-oxidant vitamins and steroid responsive nephrotic syndrome in Indian children. J Paediatr Child Health. 2002;38(5):450-54.
21. Vučetić M, Cormerais Y, Parks SK, et al. The Central Role of Amino Acids in Cancer Redox Homeostasis: Vulnerability Points of the Cancer Redox Code. Front Oncol. 2017; 7:319
22. Li Z, Zhang H. Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. Cell Mol Life Sci. 2016;73(2):377–92.
23. Menegon S, Columbano A, Giordano S. The dual roles of NRF2 in cancer. Trends Mol Med. 2016;22(7):578–93.
24. Lieu EL, Nguyen T, Rhyne S, et al. Amino acids in cancer. Exp Mol Med. 2020;52(1):15-30.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Review

A New Perspective on the Adaptation and Proliferation Mechanism of Cancer Cells: Atypical kinase eEF-2K

Kanser Hücrelerinin Adaptasyon ve Proliferasyon Mekanizmasına Yeni Bakış: Atipik kinaz eEF-2K

Seyhan Taskin[1*]

[1] Department of Physiology, Faculty of Medicine, Harran University, Sanliurfa, TURKİYE

Corresponding author:
Seyhan Taskin
Adress:
Department of Physiology, Faculty of Medicine, Harran University, Sanliurfa, TURKİYE
email: seyhan_taskin@yahoo.com
Received: 29.03.2022
Accepted: 22.04.2022
Cite as: TASKIN.S A New Perspective on the Adaptation and Proliferation Mechanism of Cancer Cells: Atypical kinase eEF-2K IJCMBS 2022;2(2):143-149
doi.org/ 10.5281/zenodo.6555602

Abstract

Eukaryotic elongation factor 2 kinase (eEF-2K), also known as calcium/calmodulin-dependent protein kinase III, is a member of the group of atypical α-kinases. eEF-2K is an essential regulatory molecule in cellular protein synthesis. It can control the protein chain elongation rate by phosphorylating/inactivating eukaryotic elongation factor 2 (eEF-2). The activity of eEF-2K is dependent on calcium ions and calmodulin and is extensively regulated by posttranslational phosphorylation. Recent data show that eEF-2K helps protect cancer cells against nutrient restriction and energy depletion. It also exerts a cytoprotective effect in many conditions, including oxidative stress and hypoxia. In light of the foregoing, eEF-2K may be a new potential pharmacological target for cancer therapy.

Keywords: eEF-2, eEF-2K, Protein synthesis, Cancer, mTORC1, AMPK

ÖZ

Ökaryotik uzama faktörü 2 kinaz (eEF-2K), atipik α-kinazlar grubunun bir üyesidir. Kalsiyum/kalmodüline bağımlı protein kinaz III olarak da bilinen eEF-2K, hücresel protein sentezinde önemli bir düzenleyici moleküldür. eEF-2K, ökaryotik uzama faktörü 2'yi (eEF-2) fosforile/inaktive ederek protein zinciri uzama oranını kontrol etme işlevi görür. eEF-2K'nın aktivitesi kalsiyum iyonlarına ve kalmoduline bağlıdır. Buna ek olarak, posttranslasyonel fosforilasyon ile aktivitesi kapsamlı bir şekilde düzenlenir. Son veriler, eEF-2K'nın kanser hücrelerini besin açlığı ve enerji tükenmesine karşı korumaya yardımcı olduğunu ve ayrıca oksidatif stres, hipoksi dahil pek çok koşulda sitoprotektif etkili olduğunu göstermektedir. eEF-2K'nın kanser tedavisi için yeni bir potansiyel farmakolojik hedef olabileceği düşünülmektedir.

Anahtar Kelimeler: eEF-2, eEF-2K, Protein sentezi, Kanser, mTORC1, AMPK

Introduction

Cells constantly need protein synthesis to perform their metabolic activities. Notably, this synthesis process entails high rates of energy consumption. Protein synthesis is a tightly controlled biochemical process with many control steps (1). The eukaryotic elongation factor-2 kinase (eEF-2K), which functions as modulator of protein synthesis, is a calcium/calmodulin (Ca^{2+}/CaM)-dependent protein kinase. eEF-2K phosphorylates eukaryotic elongation factor-2 (eEF-2) (2). The phosphorylation of eEF-2 by eEF-2K generally reduces protein translation. Especially under stress conditions, such as nutrient and energy deprivation, oxidative stress, and hypoxia, cells increase their eEF-2K activity to slow down protein synthesis, thus saving energy and adapting to new conditions (3). This activation–inactivation mechanism is an important intersection of the cellular adaptation and differentiation processes.

Especially in the uncontrolled proliferation mechanism specific to cancer cells, eEF-2K is selectively activated. In vitro experiments have shown that cell proliferation and eEF-2K activity increase in the presence of epidermal growth factor and insulin-like growth factor 1. When eEF-2K activity is inhibited by Rottlerin (eEF-2K inhibitor), cell proliferation induced by epidermal growth factor and insulin-like growth factor 1 is blocked. It has been shown that decreased expression of eEF-2K causes downregulation of signaling pathways involved in the cell cycle and proliferation (4–6). Inhibition of eEF-2K activity further highlighted the importance of inhibitory mechanisms in chemotherapy. Inhibitors with different chemical origins can be effective in energy metabolism, cell proliferation, signaling pathways and protein synthesis stages. Catalytic inhibition of eEF-2 (competitive inhibition), inhibition of ATP production, downregulation of mRNA levels, induction of protein degradation and inhibition of cell permeability are the prominent inhibition mechanisms of inhibitor substances (6–9).

Increasing evidence indicates that the eEF-2K/eEF-2 pathway is involved in crucial physiological and pathological processes, such as cancer, cardiovascular diseases, and neurodegenerative diseases (10). In this review, we will summarize the main evidence supporting the defined role of eEF-2K activity in cancer.

Structures of eEF-2 and eEF-2K

The eEF-2 molecule is a protein of approximately 95.2 kDa. It is located in the cytoplasm. Protein translation includes initiation, elongation, and termination phases. The eEF-2 molecule is involved in the elongation phase and acts as the translocase responsible for the movement of newly formed peptidyl-tRNAs (11). The addition of newly synthesized amino acids to the peptide chain is an energy-requiring process. The energy required for translation is obtained from the hydrolysis of GTP, the ATP analog, by eEF-2 (12,13). The phosphorylated/dephosphorylated control of eEF-2 activity allows rapid and transient modulation of protein synthesis. The phosphorylation of eEF-2 through the Threonine-56 (Thr-56) residue reduces its affinity for the ribosome, which inhibits protein chain elongation (14). eEF-2 is catalyzed by a highly specific protein kinase, eEF-2K (2).
The eEF-2K enzyme is a member of group called "α-kinases," which differ from the eukaryotic protein kinase superfamily. It contains 725 amino acid residues and has a mass of about 82.1 kDa (15,16). Notably, the catalytic domain and the CaM binding site are found in the N-terminus of the primary structure. Meanwhile, the SEL1 domain can be found in the C-terminal part; protein-protein interactions usually take place in this region. At the end of the C-terminal is a key region for interactions with eEF-2 (Figure 1). The catalytic domain and SEL1 domains are linked by a so-called "binding domain," which contains several phosphorylation sites that can regulate the activity of eEF-2K (2,17,18).

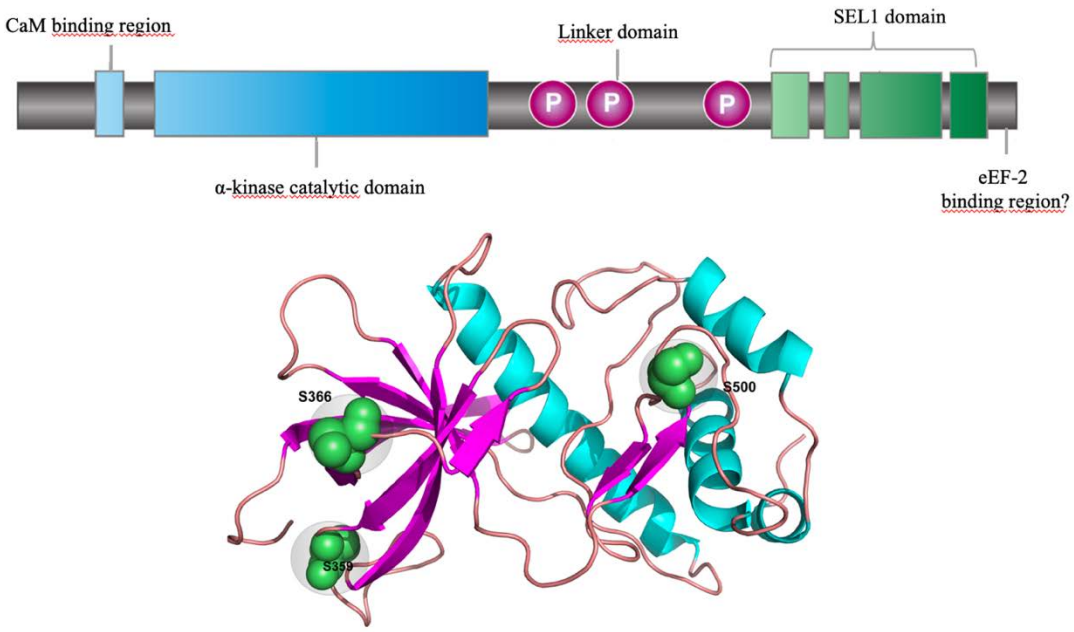


Figure 1: The structure of the eEF-2K. eEF-2K is a protein kinase with a CaM binding site at its N-terminus, α-kinase catalytic domains, and a SEL1 domain at its C-terminal region (2).

Activation of eEF-2K

The activation of eEF-2K using a two-step allosteric activation mechanism is regulated by autophosphorylation in the presence of Ca^{2+} and CaM. In the first step of eEF-2K activation, high-affinity Ca^{2+}/CaM binding results in autophosphorylation at the Thr348 residue, giving rise to a conformational change. The binding of phosphorylated Thr348 to a phosphate-binding site in the kinase domain results in a second conformational change. Consequently, this change increases eEF-2K activity by increasing its affinity for eEF-2 and its intrinsic catalytic activity (19,20).
eEF-2K activity can be controlled by several other signals in addition to autophosphorylation reactions. It is affected by intracellular pH, hypoxia, stress, and nutrient restriction. AMP-activated protein kinase (AMPK) and glycogen synthase kinase 3 have been identified as positive regulators of eEF-2K activity (3,21,22) AMPK is the main sensor of cellular energy levels that is activated when ATP levels change (23). AMPK phosphorylates several substrates to maintain energy balance. The activation of AMPK induces the phosphorylation of eEF-2K and inhibits protein synthesis, potentially conserving energy (22). This probably includes the inhibition of the mammalian target of rapamycin complex 1 (mTORC1), a negative regulator of eEF-2K. AMPK may also contribute to its activation by directly phosphorylating eEF-2K (24).
Hypoxia causes the activation of eEF-2K. eEF-2K undergoes hydroxylation on the Proline-98 residue located between the CaM binding site and its catalytic domain. Proline hydroxylation is catalyzed by the oxygen-dependent enzyme proline

hydroxylase. Hydroxylation under normoxemic conditions partially impairs CaM binding to eEF-2K and significantly limits the CaM-induced activity of eEF-2K. At low oxygen levels, where proline hydroxylase function is impaired, this residue cannot be hydroxylated, and eEF-2K is more strongly activated by Ca^{2+}/CaM. This will slow down protein synthesis and save energy under conditions where oxidative metabolism cannot keep up with cellular energy demands and oxygen is insufficient (15,25).

Another factor affecting the activation of eEF-2K is pH. Low pH values (approximately pH 6.8-6.6) lead to eEF-2K activation and increased phosphorylation of eEF-2. Xie et al. argued that the histidine residues detected in the structure of eEF-2K play a key role in the formation of this effect. Due to their structural properties, histidines can be positively charged by taking protons at acidic pH values. Ultimately, the positive charge at the CaM binding site increases Ca^{2+} binding and positively regulates eEF-2K activation (26).

It has been reported that eEF-2K is negatively regulated by the mTORC1 signaling pathway via direct or indirect phosphorylation (21). The mTORC1 signaling pathway is activated by amino acids, hormones, or growth factors; it has been identified as a key cellular sensor of the nutrient or energy state of cells (27). There are several interactions described between mTORC1 and eEF-2K. By directly phosphorylating eEF-2K via Ser78/Ser396, mTORC1 can disrupt Ca^{2+}/CaM activity. In addition, an indirect phosphorylation reaction occurs when the p70S6 kinase activated by mTORC1 phosphorylates eEF-2K, resulting in the inhibition of eEF-2K activity. ERK signaling, which is an oncogenic signaling pathway, also causes the inactivation of the eEF-2K enzyme by phosphorylating it over serine residues (21,28).

In summary, eEF-2K supports cell survival by providing adaptation to hypoxia, nutrient, and energy deprivation, and by blocking translation. Even more remarkable, cancer cells use eEF-2K activity to adapt to a nutrient-restricted state (2,29).

Cancer and eEF-2K

Cancer cells are typically characterized by uncontrolled cell division and proliferation. In this state of uncontrolled growth and proliferation, protein synthesis is unregulated. The question of how the known role of eEF-2K in protein synthesis changes in cancer cells has drawn the interest of cancer researchers. Studies have reported that eEF-2K plays a critical role in the cell cycle, autophagy, and apoptosis. eEF-2K has been shown to be overexpressed in breast cancer, glioma, esophageal squamous cell carcinoma, and pancreatic cancer (7,12,30–32).

Breast cancer: In a study with MDA-MB-231 and MCF-7 breast cancer cell lines, the silencing of eEF-2K with the help of siRNA has been shown to significantly reduce cell proliferation and colony formation. However, *in vivo* targeting of eEF-2K expression by systemic liposomal siRNA in a mouse breast cancer model showed that it inhibited tumor growth, induced apoptosis, and increased sensitivity to the chemotherapy agent doxorubicin. It was concluded that the disruption of eEF-2K expression in breast cancer cells results in the downregulation of signaling pathways that affect the growth, survival, and resistance to chemotherapy agents (5). Similar results were also found evaluating the effects of silencing eEF-2K on autophagy, cell viability, tumorigenesis, and invasion in the breast cancer cell line. It has been shown that silencing eEF-2K significantly suppresses the tumorigenesis, invasion, and chemotherapy resistance of chemotherapy-resistant cancer cell lines while reducing the accumulation of LC3 protein, an autophagic marker, and suppressing autophagy (33).

Esophageal squamous cell carcinoma: Examining the biological function of eEF-2K in esophageal squamous cell carcinoma, Zhu et al. showed that eEF-2K expression is higher in esophageal squamous cell carcinoma tissues compared with non-tumor tissues. It was also found that the overexpression of eEF-2K had higher rates of migration and invasion and promoted tumor growth. Cells overexpressing eEF-2K had increased proliferation compared with the controls, while silencing eEF-2K decreased cell proliferation (30).

Pancreatic cancer: Pancreatic cancers with low survival rates constitute one of most aggressive types of cancer. Ashour et al. showed that eEF-2K is overexpressed in pancreatic cancer cells (PANC-1 and MIAPaCa-2). It has been shown that the inhibition of eEF-2K induces intrinsic and extrinsic factors in pancreatic cancer cells and contributes to the sensitivity of tumor cells to the chemotherapy agent doxorubicin (7).

Lung cancer: Bircan et al. reported that eEF-2K is overexpressed in lung cancer cell lines (A549, H1299, H322, H292, and H226) and is associated with shorter patient survival in lung cancer. The inhibition of eEF-2K expression by siRNA or a chemical inhibitor (Rottlerin) has been shown to significantly suppress lung cancer cell proliferation, colony formation, cellular survival, and tumorigenesis by inhibiting cyclin D1, Src, and MAPK/ERK signaling. In addition, the *in vivo* targeting of eEF-2K expression systemically by nanoliposomal siRNA in a lung cancer xenograft tumor model was found to inhibit tumor growth. Finally, it has been reported that the expression of eEF-2K promotes proliferation, invasion, and tumor growth of lung cancer (34).

Glioma: In human glioma cells, the inhibition of eEF-2K by siRNA or NH125 down-regulates the expression of the anti-apoptotic protein Bcl-xL. This inhibition reportedly increases TRAIL (tumor the necrosis factor-related apoptosis inducing ligand)-induced apoptosis (31). Since TRAIL has the ability to trigger rapid apoptosis in malignant cells, it is accepted as an anticancer agent candidate (35). It has been found that the inhibition of eEF-2K can sensitize glioma cells to TRAIL treatment, and this effect is mediated through the regulation of Bcl-xL. These results show that eEF-2K can regulate tumor cell apoptosis to protect tumor cells and increase drug resistance in cancer (31).

Nasopharyngeal Carcinoma: Zhao et al. investigated the combined antitumor effects of eEF-2K inhibitor NH125 and AKT inhibitor MK-2206 in nasopharyngeal carcinoma cell lines. eEF-2K-mediated autophagy induced by AKT inhibition played a protective role in nasopharyngeal carcinoma cells. The NH125-MK-2206 inhibitor combination showed a synergistic effect. This effect was found to further enhance the growth inhibitory effect of MK-2206 on nasopharyngeal carcinoma cells in *in vitro* and *in vivo* experiments. Based on these results, it was concluded that the inhibition of eEF-2K-mediated autophagy could increase the effects of chemotherapy agents (36).

Hepatocellular Carcinoma It has been found that eEF-2K is overexpressed, and its activity is high in hepatocellular carcinoma. Researchers have stated that this may be a prognostic marker (37). In another study, the suppression of eEF-2K expression in hepatocellular carcinoma cells led to decreased PI3K/Akt and STAT3 activity, which promoted cancer cell growth, survival, and migration. It has been posited that a high level of eEF-2K expression is associated with a high rate of metastatic and worsening liver cancer type, and eEF-2K promotes angiogenesis (38).

Colon cancer: It has been demonstrated that the atypical kinase eEF-2K does not have the same effect on all cancer cells. Cancer cell proliferation, growth, autophagy, and other related mechanisms were investigated by silencing and overexpressing the eEF-2K gene in HT-29 and HCT-116 colon cancer cell lines. Contrary to its previously described role, siRNA-mediated silencing of the eEF-2K gene was found to promote cancer cell growth and proliferation and result in increased autophagy mediated by the AMPK-ULK1 pathway (39).
It should be noted that although increased eEF-2K expression in many cancer types promotes tumor development by increasing proliferation, invasion, and metastatic activities of cancer cells, there are also studies that prove the opposite (39,40).

eEF-2K-mediated survival strategies of cancer cells

Rapidly growing cancer cells require protein synthesis at high rates for uncontrolled proliferation. However, cancer cells have a limited supply of nutrients (41). Therefore, it seems contradictory that proliferating cells overexpress eEF-2K, which negatively regulates protein synthesis. Several possible mechanisms have been proposed to explain how increased eEF-2K activation and reduced protein synthesis confer a survival advantage for cancer cells (Figure 2). First, considering that protein synthesis consumes a large proportion of cellular energy, eEF-2K may help cells conserve such resources, especially under nutrient-restricted conditions (22). Second, the activation of eEF-2K can inhibit the production of apoptotic and pro-apoptotic proteins that will induce apoptosis in the cell, which may promote the synthesis of proteins that are particularly important for cell survival. In addition, since the inhibition of eEF-2K sensitizes cells to chemotherapy, its overexpression may contribute to increased survival against chemotherapeutic agents (3,5). Another possible mechanism is that eEF-2K may mediate the induction of autophagy, which is one of the cell survival strategies, especially in nutrient deprivation (42). Apart from the above-mentioned mechanisms, there may also be unexplored interactions. The discovery of the eEF-2K molecule's effects on cell survival and proliferation has made it a potential target for cancer therapy. It should not be overlooked that the benefit of targeting eEF-2K in cancer treatment may vary depending on the type and stage of cancer.

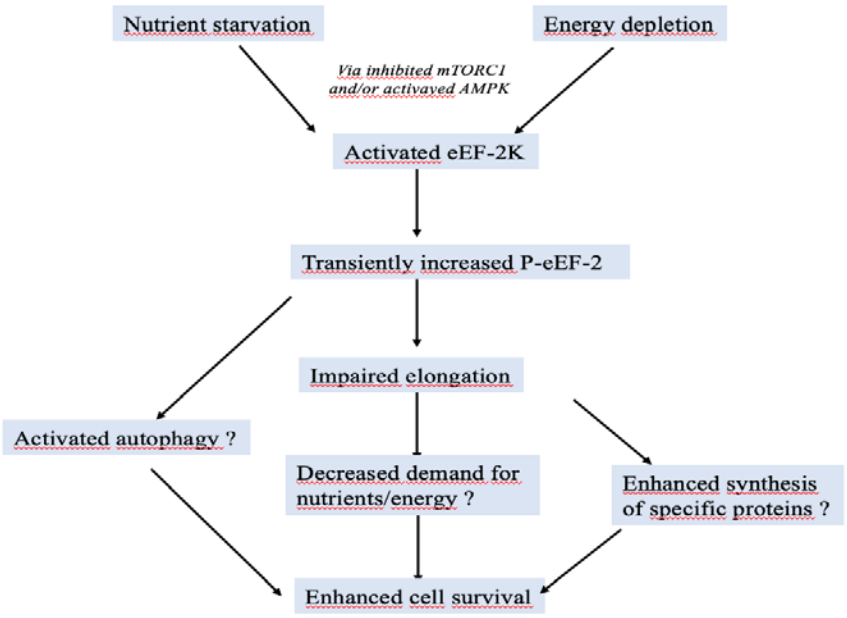


Figure 2: Regulation of eEF-2K activation through nutrient and energy balance (51)

eEF-2K inhibitors

miRNAs and siRNAs carried by nanoliposomes have a very important role in the targeted treatment of cancer for therapeutic purposes. Using these molecules, target genes can be silenced. Moreover, their related protein levels can be controlled by gene-level inhibition. In cancer research, the targeting of eEF-2K by siRNAs or miRNAs is frequently used in *in vivo* and *in vitro* studies (5,39). In addition, there are natural or synthetic compounds that are used to inhibit eEF-2K activity by targeting oncogenic pathways. (Figure 3) (3). Single or dual signal pathway targeted drugs and combination drugs (eEF2K inhibitor NH-125 and Akt inhibitor MK-2206 combination) are compounds used for this purpose (43). There are studies to inhibit eEF2K activity in cancer using re-purposed drugs (e.g. fluoxetine, pemetrexed) (44,45) The pharmacological agents exert their inhibitory effects through different mechanisms, such as cell proliferation inhibition, gene and protein expression inhibition, and ATP/CaM competitive inhibition. Rottlerin (7), NH125 (36,46), TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) and TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) (9), A-484954 (8), cefatrizine (47), β-phenylalanine derivative inhibitor 211 (48), DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile) (49), Thymoquinone (50) are among the eEF-2K inhibitors identified as a result of studies.

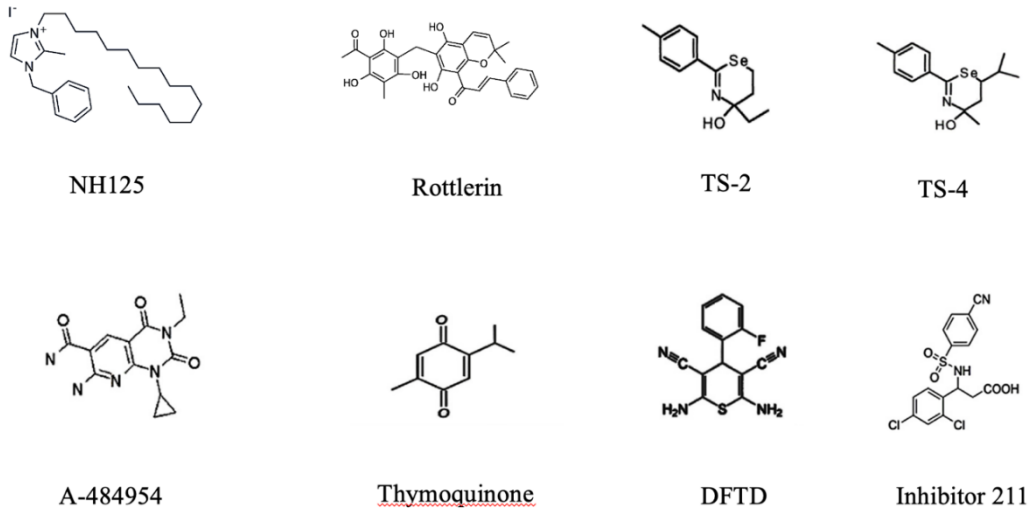


Figure 3: eEF-2K inhibitors. Rottlerin, NH125, TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), A-484954, β-phenylalanine derivative inhibitor 211, DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile), Thymoquinone are among the eEF-2K inhibitors.

CONCLUSION

In this review, we summarize the main evidence describing the function of eEF-2K, the mechanisms regulating its activity, and its role in cancer cells. It is clear that eEF-2K protects cancer cells under stress conditions, such as nutrient and energy deprivation. In addition, it has been reported that eEF-2K plays a critical role in signaling pathways, such as the cell cycle, proliferation, autophagy, and apoptosis. Notably, it is overexpressed in many cancer types. These findings suggest that eEF-2K may be a new potential pharmacological target for cancer therapy. Moreover, the sensitization of cells to chemotherapy through the inhibition of eEF-2K may provide a new perspective on the combined treatment of chemotherapeutics. More studies are needed to evaluate the usefulness of eEF-2K as a therapeutic target in oncology.

Ethical Approval: *None*

Author Contributions: *Concept: S.T. Literature Review: S.T. Design : S.T. Data acquisition: S.T. Analysis and interpretation S.T. Writing manuscript: S.T. Critical revision of manuscript: S.T.*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Ryazanov AG, Pavur KS, Dorovkov M V. Alpha-kinases: A new class of protein kinases with a novel catalytic domain. Curr Biol. 1999;9(2):R43-5.
2. Fu LL, Xie T, Zhang SY, Liu B. Eukaryotic elongation factor-2 kinase (eEF2K): A potential therapeutic target in cancer. Apoptosis. 2014;19(10):1527–31.
3. Karakas D, Ozpolat B. Eukaryotic elongation factor-2 kinase (eEF2K) signaling in tumor and microenvironment as a novel molecular target. J Mol Med. 2020;98(6):775–87.
4. Parmer TG, Ward MD, Yurkow EJ, et al. Activity and regulation by growth factors of calmodulin-dependent protein kinase III (elongation factor 2-kinase) in human breast cancer. Br J Cancer. 1999;79(1):59–64.
5. Tekedereli I, Alpay SN, Tavares CDJ, et al. Targeted silencing of elongation factor 2 kinase suppresses growth and sensitizes tumors to doxorubicin in an orthotopic model of breast cancer. PLoS One. 2012;7(7).
6. Li S, Li Y, Bai Y. What is the impact of eukaryotic elongation factor 2 kinase on cancer: A systematic review. Eur J Pharmacol. 2019;857(June):172470.
7. Ashour AA, Abdel-Aziz AAH, Mansour AM, et al. Targeting elongation factor-2 kinase (eEF-2K) induces apoptosis in human pancreatic cancer cells. Apoptosis. 2014;19(1):241–58.
8. Chen Z, Gopalakrishnan SM, Bui MH, et al. 1-benzyl-3-cetyl-2-methylimidazolium iodide (NH125) induces phosphorylation of eukaryotic elongation factor-2 (eEF2): A cautionary note on the anticancer mechanism of an eEF2 kinase inhibitor. J Biol Chem. 2011;286(51):43951–8.
9. Cho SI, Koketsu M, Ishihara H, et al. Novel compounds, '1,3-selenazine derivatives' as specific inhibitors of eukaryotic elongation factor-2 kinase. Biochim Biophys Acta - Gen Subj. 2000;1475(3):207–15.
10. Beretta S, Gritti L, Verpelli C, et al. Eukaryotic Elongation Factor 2 Kinase a Pharmacological Target to Regulate Protein Translation Dysfunction in Neurological Diseases. Neuroscience. 2020;445:42–9.
11. Merrick WC. Eukaryotic protein synthesis: Still a mystery. J Biol Chem. 2010;285(28):21197–201.
12. Zhu H, Yang X, Liu J, et al. Eukaryotic elongation factor 2 kinase confers tolerance to stress conditions in cancer cells. Cell Stress Chaperones. 2015;20(2):217–20.
13. Kaul G, Pattan G, Rafeequi T. Eukaryotic elongation factor-2 (eEF2): Its regulation and peptide chain elongation. Cell Biochem Funct. 2011;29(3):227–34.
14. Ryazanov AG, Shestakova EA, Natapov PG. Phosphorylation of elongation factor 2 by EF-2 kinase affects rate of translation. Nature. 1988;334(6178):170–3.
15. Wang X, Xie J, Proud CG. Eukaryotic elongation factor 2 kinase (eEF2K) in cancer. Cancers (Basel). 2017;9(12):1–15.
16. Human eEF-2K. [cited 10 May 2022] Available from:https://www.proteinatlas.org/ENSG00000103319-EEF2K.
17. Pigott CR, Mikolajek H, Moore CE, et al. Insights into the regulation of eukaryotic elongation factor 2 kinase and the interplay between its domains. Biochem J. 2012;442(1):105–18.
18. Pavur KS, Petrov AN, Ryazanov AG. Mapping the functional domains of elongation factor-2 kinase. Biochemistry. 2000;39(40):12216–24.
19. Tavares CDJ, Obrien JP, Abramczyk O, et al. Calcium/calmodulin stimulates the autophosphorylation of elongation factor 2 kinase on Thr-348 and Ser-500 to regulate its activity and calcium dependence. Biochemistry. 2012;51(11):2232–45.
20. Tavares CDJ, Ferguson SB, Giles DH, et al. The molecular mechanism of eukaryotic elongation factor 2 kinase activation. J Biol Chem. 2014;289(34):23901–16.
21. Wang X, Regufe da Mota S, Liu R, et al. Eukaryotic Elongation Factor 2 Kinase Activity Is Controlled by Multiple Inputs from Oncogenic Signaling. Mol Cell Biol. 2014;34(22):4088–103.
22. Browne GJ, Finn SG, Proud CG. Stimulation of the AMP-activated Protein Kinase Leads to Activation of Eukaryotic Elongation Factor 2 Kinase and to Its Phosphorylation at a Novel Site, Serine 398. J Biol Chem. 2004;279(13):12220–31.
23. Hardie DG, Ross FA, Hawley SA. AMPK: A nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251–62.
24. Liu R, Proud CG. Eukaryotic elongation factor 2 kinase as a drug target in cancer, and in cardiovascular and neurodegenerative diseases. Acta Pharmacol Sin. 2016;37(3):285–94.
25. Moore CEJ, Mikolajek H, Regufe da Mota S, et al. Elongation Factor 2 Kinase Is Regulated by Proline Hydroxylation and Protects Cells during Hypoxia. Mol Cell Biol. 2015;35(10):1788–804.
26. Xie J, Mikolajek H, Pigott CR, et al. Molecular Mechanism for the Control of Eukaryotic Elongation Factor 2 Kinase by pH: Role in Cancer Cell Survival. Mol Cell Biol. 2015;35(10):1805–24.
27. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. T. 168, Cell. Cell Press; 2017. p. 960–76.
28. Wang X, Li W, Williams M, et al. Regulation of elongation factor 2 kinase by p90RSK1 and p70 S6 kinase. EMBO J. 2001;20(16):4370–4379.
29. Heise C, Gardoni F, Culotta L, et al. Elongation factor-2 phosphorylation in dendrites and the regulation of dendritic mRNA translation in neurons. Front Cell Neurosci. 2014;8:1–8.
30. Zhu H, Song H, Chen G, et al. eEF2K promotes progression and radioresistance of esophageal squamous cell carcinoma. Radiother Oncol. 2017;124(3):439–47.
31. Zhang Y, Cheng Y, Zhang L, et al. Inhibition of eEF-2 kinase sensitizes human glioma cells to TRAIL and down-

regulates Bcl-xL expression. Biochem Biophys Res Commun. 2011;414(1):129–34.
32. Meric-Bernstam F, Chen H, Akcakanat A, et al. Aberrations in translational regulation are associated with poor prognosis in hormone receptor-positive breast cancer. Breast Cancer Res. 2012;14:R138.
33. Wang R-X, Xu X-E, Huang L, et al. eEF2 kinase mediated autophagy as a potential therapeutic target for paclitaxel-resistant triple-negative breast cancer. Ann Transl Med. 2019;7(23):783–783.
34. Bircan HA, Gurbuz N, Pataer A, et al. Elongation factor-2 kinase (eEF-2K) expression is associated with poor patient survival and promotes proliferation, invasion and tumor growth of lung cancer. Lung Cancer. 2018;124(July):31–9.
35. Bellail A, Qi L, Mulligan P, et al. TRAIL Agonists on Clinical Trials for Cancer Therapy: The Promises and the Challenges. Rev Recent Clin Trials. 2009;4(1):34–41.
36. Zhao YY, Tian Y, Liu L, et al. Inhibiting eEF-2 kinase-mediated autophagy enhanced the cytocidal effect of AKT inhibitor on human nasopharyngeal carcinoma. Drug Des Devel Ther. 2018;12:2655–63.
37. Pott LL, Hagemann S, Reis H, et al. Eukaryotic elongation factor 2 is a prognostic marker and its kinase a potential therapeutic target in HCC. Oncotarget. 2017;8:11950–62.
38. Zhou Y, Li Y, Xu S, et al. Eukaryotic elongation factor 2 kinase promotes angiogenesis in hepatocellular carcinoma via PI3K/Akt and STAT3. Int J Cancer. 2020;146(5):1383–95.
39. Xie CM, Liu XY, Sham KWY, et al. Silencing of EEF2K (eukaryotic elongation factor-2 kinase) reveals AMPK-ULK1-dependent autophagy in colon cancer cells. Autophagy. 2014;10(9):1495–508.
40. De Gassart A, Demaria O, Panes R, et al. Pharmacological eEF 2K activation promotes cell death and inhibits cancer progression. EMBO Rep. 2016;17(10):1471–84.
41. Garcia-Bermudez J, Williams RT, Guarecuco R, et al. Targeting extracellular nutrient dependencies of cancer cells. Mol Metab. 2020;33:67–82.
42. Wu H, Zhu H, Liu DX, et al. Silencing of elongation factor-2 kinase potentiates the effect of 2-deoxy-D-glucose against human glioma cells through blunting of autophagy. Cancer Res. 2009;69(6):2453–60.
43. Zhu S, Liao M, Tan H, et al. Inhibiting Eukaryotic Elongation Factor 2 Kinase: An Update on Pharmacological Small-Molecule Compounds in Cancer. J Med Chem. 2021;64(13):8870–83.
44. Sun D, Zhu L, Zhao Y, et al. Fluoxetine induces autophagic cell death via eEF2K-AMPK-mTOR-ULK complex axis in triple negative breast cancer. Cell Prolif. 2018;51(2):1–10.
45. Ye WL, Zhang LX, Guan Y Di, et al. Virtual screening and experimental validation of eEF2K inhibitors by combining homology modeling, QSAR and molecular docking from FDA approved drugs. New J Chem. 2019;43(48):19097–106.
46. Devkota AK, Tavares CDJ, Warthaka M, et al. Investigating the kinetic mechanism of inhibition of elongation factor 2 kinase by NH125: Evidence of a common in vitro artifact. Biochemistry. 2012;51(10):2100–12.
47. Yao Z, Li J, Liu Z, et al. Integrative bioinformatics and proteomics-based discovery of an eEF2K inhibitor (cefatrizine) with ER stress modulation in breast cancer cells. Mol Biosyst. 2016;12(3):729–36.
48. Guo Y, Zhao Y, Wang G, et al. Design, synthesis and structure-activity relationship of a focused library of β-phenylalanine derivatives as novel eEF2K inhibitors with apoptosis-inducing mechanisms in breast cancer. Eur J Med Chem. 2018;143:402–18.
49. Devkota AK, Edupuganti R, Yan C, et al. Reversible covalent inhibition of eEF-2K by carbonitriles. ChemBioChem. 2014;15(16):2435–42.
50. Kabil N, Bayraktar R, Kahraman N, et al. Thymoquinone inhibits cell proliferation, migration, and invasion by regulating the elongation factor 2 kinase (eEF-2K) signaling axis in triple-negative breast cancer. Breast Cancer Res Treat. 2018;171(3):593–605.
51. Kenney JW, Moore CE, Wang X, et al. Eukaryotic elongation factor 2 kinase, an unusual enzyme with multiple roles. Adv Biol Regul. 2014;55:15–27.

Increasing evidence indicates that the eEF-2K/eEF-2 pathway is involved in crucial physiological and pathological processes, such as cancer, cardiovascular diseases, and neurodegenerative diseases (10). In this review, we will summarize the main evidence supporting the defined role of eEF-2K activity in cancer.

Structures of eEF-2 and eEF-2K

The eEF-2 molecule is a protein of approximately 95.2 kDa. It is located in the cytoplasm. Protein translation includes initiation, elongation, and termination phases. The eEF-2 molecule is involved in the elongation phase and acts as the translocase responsible for the movement of newly formed peptidyl-tRNAs (11). The addition of newly synthesized amino acids to the peptide chain is an energy-requiring process. The energy required for translation is obtained from the hydrolysis of GTP, the ATP analog, by eEF-2 (12,13). The phosphorylated/dephosphorylated control of eEF-2 activity allows rapid and transient modulation of protein synthesis. The phosphorylation of eEF-2 through the Threonine-56 (Thr-56) residue reduces its affinity for the ribosome, which inhibits protein chain elongation (14). eEF-2 is catalyzed by a highly specific protein kinase, eEF-2K (2).
The eEF-2K enzyme is a member of group called "α-kinases," which differ from the eukaryotic protein kinase superfamily. It contains 725 amino acid residues and has a mass of about 82.1 kDa (15,16). Notably, the catalytic domain and the CaM binding site are found in the N-terminus of the primary structure. Meanwhile, the SEL1 domain can be found in the C-terminal part; protein-protein interactions usually take place in this region. At the end of the C-terminal is a key region for interactions with eEF-2 (Figure 1). The catalytic domain and SEL1 domains are linked by a so-called "binding domain," which contains several phosphorylation sites that can regulate the activity of eEF-2K (2,17,18).

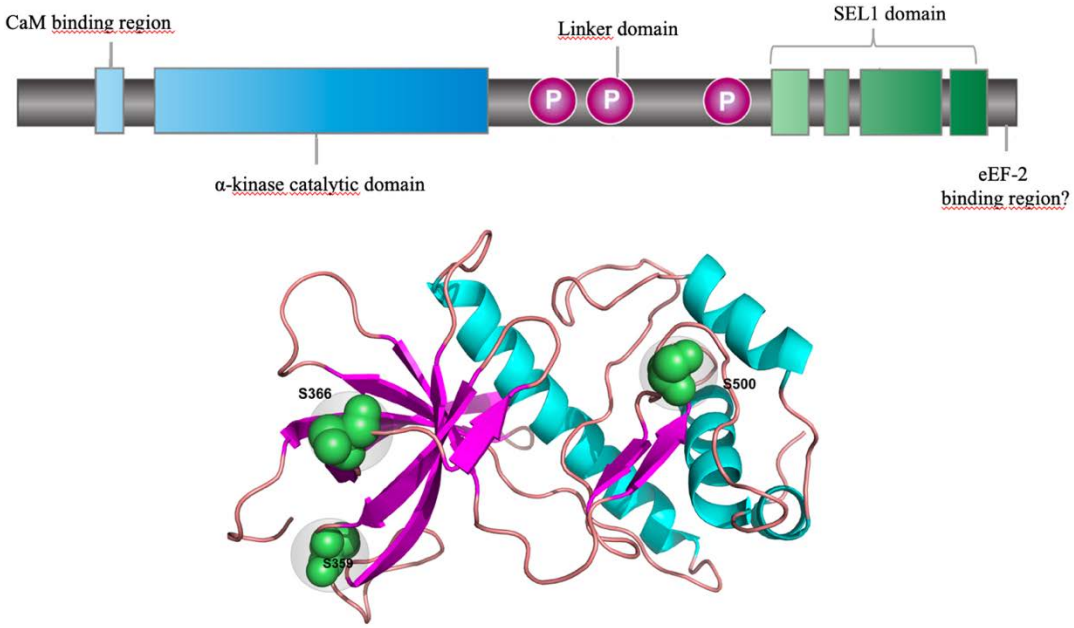


Figure 1: The structure of the eEF-2K. eEF-2K is a protein kinase with a CaM binding site at its N-terminus, α-kinase catalytic domains, and a SEL1 domain at its C-terminal region (2).

Activation of eEF-2K

The activation of eEF-2K using a two-step allosteric activation mechanism is regulated by autophosphorylation in the presence of Ca^{2+} and CaM. In the first step of eEF-2K activation, high-affinity Ca^{2+}/CaM binding results in autophosphorylation at the Thr348 residue, giving rise to a conformational change. The binding of phosphorylated Thr348 to a phosphate-binding site in the kinase domain results in a second conformational change. Consequently, this change increases eEF-2K activity by increasing its affinity for eEF-2 and its intrinsic catalytic activity (19,20).
eEF-2K activity can be controlled by several other signals in addition to autophosphorylation reactions. It is affected by intracellular pH, hypoxia, stress, and nutrient restriction. AMP-activated protein kinase (AMPK) and glycogen synthase kinase 3 have been identified as positive regulators of eEF-2K activity (3,21,22) AMPK is the main sensor of cellular energy levels that is activated when ATP levels change (23). AMPK phosphorylates several substrates to maintain energy balance. The activation of AMPK induces the phosphorylation of eEF-2K and inhibits protein synthesis, potentially conserving energy (22). This probably includes the inhibition of the mammalian target of rapamycin complex 1 (mTORC1), a negative regulator of eEF-2K. AMPK may also contribute to its activation by directly phosphorylating eEF-2K (24).
Hypoxia causes the activation of eEF-2K. eEF-2K undergoes hydroxylation on the Proline-98 residue located between the CaM binding site and its catalytic domain. Proline hydroxylation is catalyzed by the oxygen-dependent enzyme proline

hydroxylase. Hydroxylation under normoxemic conditions partially impairs CaM binding to eEF-2K and significantly limits the CaM-induced activity of eEF-2K. At low oxygen levels, where proline hydroxylase function is impaired, this residue cannot be hydroxylated, and eEF-2K is more strongly activated by Ca^{2+}/CaM. This will slow down protein synthesis and save energy under conditions where oxidative metabolism cannot keep up with cellular energy demands and oxygen is insufficient (15,25).

Another factor affecting the activation of eEF-2K is pH. Low pH values (approximately pH 6.8-6.6) lead to eEF-2K activation and increased phosphorylation of eEF-2. Xie et al. argued that the histidine residues detected in the structure of eEF-2K play a key role in the formation of this effect. Due to their structural properties, histidines can be positively charged by taking protons at acidic pH values. Ultimately, the positive charge at the CaM binding site increases Ca^{2+} binding and positively regulates eEF-2K activation (26).

It has been reported that eEF-2K is negatively regulated by the mTORC1 signaling pathway via direct or indirect phosphorylation (21). The mTORC1 signaling pathway is activated by amino acids, hormones, or growth factors; it has been identified as a key cellular sensor of the nutrient or energy state of cells (27). There are several interactions described between mTORC1 and eEF-2K. By directly phosphorylating eEF-2K via Ser78/Ser396, mTORC1 can disrupt Ca^{2+}/CaM activity. In addition, an indirect phosphorylation reaction occurs when the p70S6 kinase activated by mTORC1 phosphorylates eEF-2K, resulting in the inhibition of eEF-2K activity. ERK signaling, which is an oncogenic signaling pathway, also causes the inactivation of the eEF-2K enzyme by phosphorylating it over serine residues (21,28).

In summary, eEF-2K supports cell survival by providing adaptation to hypoxia, nutrient, and energy deprivation, and by blocking translation. Even more remarkable, cancer cells use eEF-2K activity to adapt to a nutrient-restricted state (2,29).

Cancer and eEF-2K

Cancer cells are typically characterized by uncontrolled cell division and proliferation. In this state of uncontrolled growth and proliferation, protein synthesis is unregulated. The question of how the known role of eEF-2K in protein synthesis changes in cancer cells has drawn the interest of cancer researchers. Studies have reported that eEF-2K plays a critical role in the cell cycle, autophagy, and apoptosis. eEF-2K has been shown to be overexpressed in breast cancer, glioma, esophageal squamous cell carcinoma, and pancreatic cancer (7,12,30–32).

Breast cancer: In a study with MDA-MB-231 and MCF-7 breast cancer cell lines, the silencing of eEF-2K with the help of siRNA has been shown to significantly reduce cell proliferation and colony formation. However, *in vivo* targeting of eEF-2K expression by systemic liposomal siRNA in a mouse breast cancer model showed that it inhibited tumor growth, induced apoptosis, and increased sensitivity to the chemotherapy agent doxorubicin. It was concluded that the disruption of eEF-2K expression in breast cancer cells results in the downregulation of signaling pathways that affect the growth, survival, and resistance to chemotherapy agents (5). Similar results were also found evaluating the effects of silencing eEF-2K on autophagy, cell viability, tumorigenesis, and invasion in the breast cancer cell line. It has been shown that silencing eEF-2K significantly suppresses the tumorigenesis, invasion, and chemotherapy resistance of chemotherapy-resistant cancer cell lines while reducing the accumulation of LC3 protein, an autophagic marker, and suppressing autophagy (33).

Esophageal squamous cell carcinoma: Examining the biological function of eEF-2K in esophageal squamous cell carcinoma, Zhu et al. showed that eEF-2K expression is higher in esophageal squamous cell carcinoma tissues compared with non-tumor tissues. It was also found that the overexpression of eEF-2K had higher rates of migration and invasion and promoted tumor growth. Cells overexpressing eEF-2K had increased proliferation compared with the controls, while silencing eEF-2K decreased cell proliferation (30).

Pancreatic cancer: Pancreatic cancers with low survival rates constitute one of most aggressive types of cancer. Ashour et al. showed that eEF-2K is overexpressed in pancreatic cancer cells (PANC-1 and MIAPaCa-2). It has been shown that the inhibition of eEF-2K induces intrinsic and extrinsic factors in pancreatic cancer cells and contributes to the sensitivity of tumor cells to the chemotherapy agent doxorubicin (7).

Lung cancer: Bircan et al. reported that eEF-2K is overexpressed in lung cancer cell lines (A549, H1299, H322, H292, and H226) and is associated with shorter patient survival in lung cancer. The inhibition of eEF-2K expression by siRNA or a chemical inhibitor (Rottlerin) has been shown to significantly suppress lung cancer cell proliferation, colony formation, cellular survival, and tumorigenesis by inhibiting cyclin D1, Src, and MAPK/ERK signaling. In addition, the *in vivo* targeting of eEF-2K expression systemically by nanoliposomal siRNA in a lung cancer xenograft tumor model was found to inhibit tumor growth. Finally, it has been reported that the expression of eEF-2K promotes proliferation, invasion, and tumor growth of lung cancer (34).

Glioma: In human glioma cells, the inhibition of eEF-2K by siRNA or NH125 down-regulates the expression of the anti-apoptotic protein Bcl-xL. This inhibition reportedly increases TRAIL (tumor the necrosis factor-related apoptosis inducing ligand)-induced apoptosis (31). Since TRAIL has the ability to trigger rapid apoptosis in malignant cells, it is accepted as an anticancer agent candidate (35). It has been found that the inhibition of eEF-2K can sensitize glioma cells to TRAIL treatment, and this effect is mediated through the regulation of Bcl-xL. These results show that eEF-2K can regulate tumor cell apoptosis to protect tumor cells and increase drug resistance in cancer (31).

Nasopharyngeal Carcinoma: Zhao et al. investigated the combined antitumor effects of eEF-2K inhibitor NH125 and AKT inhibitor MK-2206 in nasopharyngeal carcinoma cell lines. eEF-2K-mediated autophagy induced by AKT inhibition played a protective role in nasopharyngeal carcinoma cells. The NH125-MK-2206 inhibitor combination showed a synergistic effect. This effect was found to further enhance the growth inhibitory effect of MK-2206 on nasopharyngeal carcinoma cells in *in vitro* and *in vivo* experiments. Based on these results, it was concluded that the inhibition of eEF-2K-mediated autophagy could increase the effects of chemotherapy agents (36).

Hepatocellular Carcinoma It has been found that eEF-2K is overexpressed, and its activity is high in hepatocellular carcinoma. Researchers have stated that this may be a prognostic marker (37). In another study, the suppression of eEF-2K expression in hepatocellular carcinoma cells led to decreased PI3K/Akt and STAT3 activity, which promoted cancer cell growth, survival, and migration. It has been posited that a high level of eEF-2K expression is associated with a high rate of metastatic and worsening liver cancer type, and eEF-2K promotes angiogenesis (38).

Colon cancer: It has been demonstrated that the atypical kinase eEF-2K does not have the same effect on all cancer cells. Cancer cell proliferation, growth, autophagy, and other related mechanisms were investigated by silencing and overexpressing the eEF-2K gene in HT-29 and HCT-116 colon cancer cell lines. Contrary to its previously described role, siRNA-mediated silencing of the eEF-2K gene was found to promote cancer cell growth and proliferation and result in increased autophagy mediated by the AMPK-ULK1 pathway (39).
It should be noted that although increased eEF-2K expression in many cancer types promotes tumor development by increasing proliferation, invasion, and metastatic activities of cancer cells, there are also studies that prove the opposite (39,40).

eEF-2K-mediated survival strategies of cancer cells

Rapidly growing cancer cells require protein synthesis at high rates for uncontrolled proliferation. However, cancer cells have a limited supply of nutrients (41). Therefore, it seems contradictory that proliferating cells overexpress eEF-2K, which negatively regulates protein synthesis. Several possible mechanisms have been proposed to explain how increased eEF-2K activation and reduced protein synthesis confer a survival advantage for cancer cells (Figure 2). First, considering that protein synthesis consumes a large proportion of cellular energy, eEF-2K may help cells conserve such resources, especially under nutrient-restricted conditions (22). Second, the activation of eEF-2K can inhibit the production of apoptotic and pro-apoptotic proteins that will induce apoptosis in the cell, which may promote the synthesis of proteins that are particularly important for cell survival. In addition, since the inhibition of eEF-2K sensitizes cells to chemotherapy, its overexpression may contribute to increased survival against chemotherapeutic agents (3,5). Another possible mechanism is that eEF-2K may mediate the induction of autophagy, which is one of the cell survival strategies, especially in nutrient deprivation (42). Apart from the above-mentioned mechanisms, there may also be unexplored interactions. The discovery of the eEF-2K molecule's effects on cell survival and proliferation has made it a potential target for cancer therapy. It should not be overlooked that the benefit of targeting eEF-2K in cancer treatment may vary depending on the type and stage of cancer.

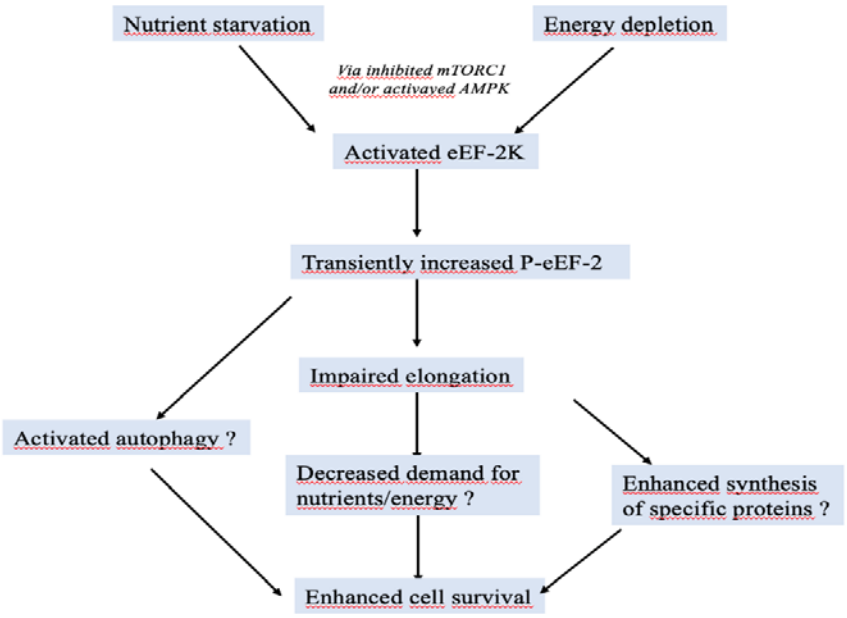


Figure 2: Regulation of eEF-2K activation through nutrient and energy balance (51)

eEF-2K inhibitors

miRNAs and siRNAs carried by nanoliposomes have a very important role in the targeted treatment of cancer for therapeutic purposes. Using these molecules, target genes can be silenced. Moreover, their related protein levels can be controlled by gene-level inhibition. In cancer research, the targeting of eEF-2K by siRNAs or miRNAs is frequently used in *in vivo* and *in vitro* studies (5,39). In addition, there are natural or synthetic compounds that are used to inhibit eEF-2K activity by targeting oncogenic pathways. (Figure 3) (3). Single or dual signal pathway targeted drugs and combination drugs (eEF2K inhibitor NH-125 and Akt inhibitor MK-2206 combination) are compounds used for this purpose (43). There are studies to inhibit eEF2K activity in cancer using re-purposed drugs (e.g. fluoxetine, pemetrexed) (44,45) The pharmacological agents exert their inhibitory effects through different mechanisms, such as cell proliferation inhibition, gene and protein expression inhibition, and ATP/CaM competitive inhibition. Rottlerin (7), NH125 (36,46), TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) and TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) (9), A-484954 (8), cefatrizine (47), β-phenylalanine derivative inhibitor 211 (48), DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile) (49), Thymoquinone (50) are among the eEF-2K inhibitors identified as a result of studies.

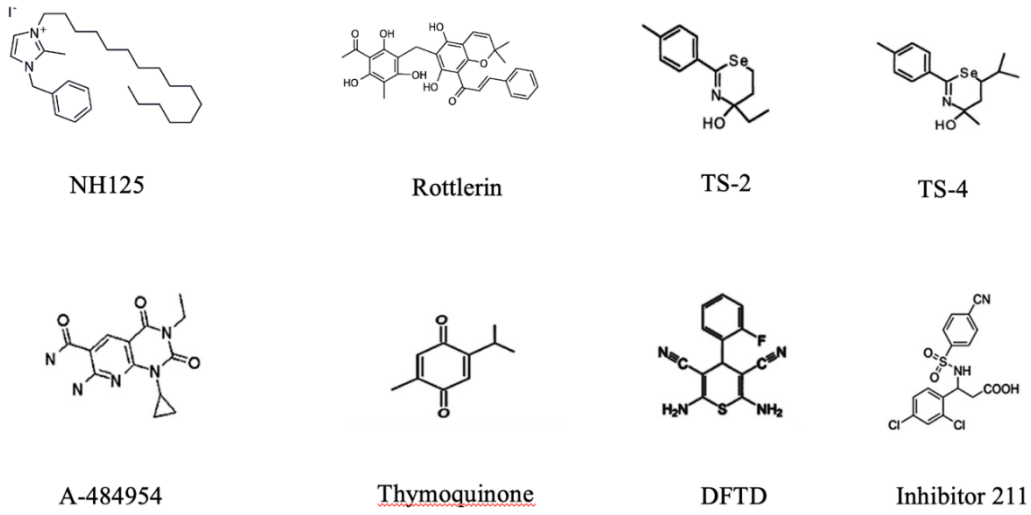


Figure 3: eEF-2K inhibitors. Rottlerin, NH125, TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), A-484954, β-phenylalanine derivative inhibitor 211, DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile), Thymoquinone are among the eEF-2K inhibitors.

CONCLUSION

In this review, we summarize the main evidence describing the function of eEF-2K, the mechanisms regulating its activity, and its role in cancer cells. It is clear that eEF-2K protects cancer cells under stress conditions, such as nutrient and energy deprivation. In addition, it has been reported that eEF-2K plays a critical role in signaling pathways, such as the cell cycle, proliferation, autophagy, and apoptosis. Notably, it is overexpressed in many cancer types. These findings suggest that eEF-2K may be a new potential pharmacological target for cancer therapy. Moreover, the sensitization of cells to chemotherapy through the inhibition of eEF-2K may provide a new perspective on the combined treatment of chemotherapeutics. More studies are needed to evaluate the usefulness of eEF-2K as a therapeutic target in oncology.

Ethical Approval: *None*

Author Contributions: *Concept: S.T. Literature Review: S.T. Design : S.T. Data acquisition: S.T. Analysis and interpretation S.T. Writing manuscript: S.T. Critical revision of manuscript: S.T.*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Ryazanov AG, Pavur KS, Dorovkov M V. Alpha-kinases: A new class of protein kinases with a novel catalytic domain. Curr Biol. 1999;9(2):R43-5.
2. Fu LL, Xie T, Zhang SY, Liu B. Eukaryotic elongation factor-2 kinase (eEF2K): A potential therapeutic target in cancer. Apoptosis. 2014;19(10):1527–31.

3. Karakas D, Ozpolat B. Eukaryotic elongation factor-2 kinase (eEF2K) signaling in tumor and microenvironment as a novel molecular target. J Mol Med. 2020;98(6):775–87.
4. Parmer TG, Ward MD, Yurkow EJ, et al. Activity and regulation by growth factors of calmodulin-dependent protein kinase III (elongation factor 2-kinase) in human breast cancer. Br J Cancer. 1999;79(1):59–64.
5. Tekedereli I, Alpay SN, Tavares CDJ, et al. Targeted silencing of elongation factor 2 kinase suppresses growth and sensitizes tumors to doxorubicin in an orthotopic model of breast cancer. PLoS One. 2012;7(7).
6. Li S, Li Y, Bai Y. What is the impact of eukaryotic elongation factor 2 kinase on cancer: A systematic review. Eur J Pharmacol. 2019;857(June):172470.
7. Ashour AA, Abdel-Aziz AAH, Mansour AM, et al. Targeting elongation factor-2 kinase (eEF-2K) induces apoptosis in human pancreatic cancer cells. Apoptosis. 2014;19(1):241–58.
8. Chen Z, Gopalakrishnan SM, Bui MH, et al. 1-benzyl-3-cetyl-2-methylimidazolium iodide (NH125) induces phosphorylation of eukaryotic elongation factor-2 (eEF2): A cautionary note on the anticancer mechanism of an eEF2 kinase inhibitor. J Biol Chem. 2011;286(51):43951–8.
9. Cho SI, Koketsu M, Ishihara H, et al. Novel compounds, '1,3-selenazine derivatives' as specific inhibitors of eukaryotic elongation factor-2 kinase. Biochim Biophys Acta - Gen Subj. 2000;1475(3):207–15.
10. Beretta S, Gritti L, Verpelli C, et al. Eukaryotic Elongation Factor 2 Kinase a Pharmacological Target to Regulate Protein Translation Dysfunction in Neurological Diseases. Neuroscience. 2020;445:42–9.
11. Merrick WC. Eukaryotic protein synthesis: Still a mystery. J Biol Chem. 2010;285(28):21197–201.
12. Zhu H, Yang X, Liu J, et al. Eukaryotic elongation factor 2 kinase confers tolerance to stress conditions in cancer cells. Cell Stress Chaperones. 2015;20(2):217–20.
13. Kaul G, Pattan G, Rafeequi T. Eukaryotic elongation factor-2 (eEF2): Its regulation and peptide chain elongation. Cell Biochem Funct. 2011;29(3):227–34.
14. Ryazanov AG, Shestakova EA, Natapov PG. Phosphorylation of elongation factor 2 by EF-2 kinase affects rate of translation. Nature. 1988;334(6178):170–3.
15. Wang X, Xie J, Proud CG. Eukaryotic elongation factor 2 kinase (eEF2K) in cancer. Cancers (Basel). 2017;9(12):1–15.
16. Human eEF-2K. [cited 10 May 2022] Available from:https://www.proteinatlas.org/ENSG00000103319-EEF2K.
17. Pigott CR, Mikolajek H, Moore CE, et al. Insights into the regulation of eukaryotic elongation factor 2 kinase and the interplay between its domains. Biochem J. 2012;442(1):105–18.
18. Pavur KS, Petrov AN, Ryazanov AG. Mapping the functional domains of elongation factor-2 kinase. Biochemistry. 2000;39(40):12216–24.
19. Tavares CDJ, Obrien JP, Abramczyk O, et al. Calcium/calmodulin stimulates the autophosphorylation of elongation factor 2 kinase on Thr-348 and Ser-500 to regulate its activity and calcium dependence. Biochemistry. 2012;51(11):2232–45.
20. Tavares CDJ, Ferguson SB, Giles DH, et al. The molecular mechanism of eukaryotic elongation factor 2 kinase activation. J Biol Chem. 2014;289(34):23901–16.
21. Wang X, Regufe da Mota S, Liu R, et al. Eukaryotic Elongation Factor 2 Kinase Activity Is Controlled by Multiple Inputs from Oncogenic Signaling. Mol Cell Biol. 2014;34(22):4088–103.
22. Browne GJ, Finn SG, Proud CG. Stimulation of the AMP-activated Protein Kinase Leads to Activation of Eukaryotic Elongation Factor 2 Kinase and to Its Phosphorylation at a Novel Site, Serine 398. J Biol Chem. 2004;279(13):12220–31.
23. Hardie DG, Ross FA, Hawley SA. AMPK: A nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251–62.
24. Liu R, Proud CG. Eukaryotic elongation factor 2 kinase as a drug target in cancer, and in cardiovascular and neurodegenerative diseases. Acta Pharmacol Sin. 2016;37(3):285–94.
25. Moore CEJ, Mikolajek H, Regufe da Mota S, et al. Elongation Factor 2 Kinase Is Regulated by Proline Hydroxylation and Protects Cells during Hypoxia. Mol Cell Biol. 2015;35(10):1788–804.
26. Xie J, Mikolajek H, Pigott CR, et al. Molecular Mechanism for the Control of Eukaryotic Elongation Factor 2 Kinase by pH: Role in Cancer Cell Survival. Mol Cell Biol. 2015;35(10):1805–24.
27. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. T. 168, Cell. Cell Press; 2017. p. 960–76.
28. Wang X, Li W, Williams M, et al. Regulation of elongation factor 2 kinase by p90RSK1 and p70 S6 kinase. EMBO J. 2001;20(16):4370–4379.
29. Heise C, Gardoni F, Culotta L, et al. Elongation factor-2 phosphorylation in dendrites and the regulation of dendritic mRNA translation in neurons. Front Cell Neurosci. 2014;8:1–8.
30. Zhu H, Song H, Chen G, et al. eEF2K promotes progression and radioresistance of esophageal squamous cell carcinoma. Radiother Oncol. 2017;124(3):439–47.
31. Zhang Y, Cheng Y, Zhang L, et al. Inhibition of eEF-2 kinase sensitizes human glioma cells to TRAIL and down-regulates Bcl-xL expression. Biochem Biophys Res Commun. 2011;414(1):129–34.
32. Meric-Bernstam F, Chen H, Akcakanat A, et al. Aberrations in translational regulation are associated with poor prognosis in hormone receptor-positive breast cancer. Breast Cancer Res. 2012;14:R138.
33. Wang R-X, Xu X-E, Huang L, et al. eEF2 kinase mediated autophagy as a potential therapeutic target for paclitaxel-resistant triple-negative breast cancer. Ann Transl Med. 2019;7(23):783–783.
34. Bircan HA, Gurbuz N, Pataer A, et al. Elongation factor-2 kinase (eEF-2K) expression is associated with poor patient survival and promotes proliferation, invasion and tumor growth of lung cancer. Lung Cancer.

2018;124(July):31–9.
35. Bellail A, Qi L, Mulligan P, et al. TRAIL Agonists on Clinical Trials for Cancer Therapy: The Promises and the Challenges. Rev Recent Clin Trials. 2009;4(1):34–41.
36. Zhao YY, Tian Y, Liu L, et al. Inhibiting eEF-2 kinase-mediated autophagy enhanced the cytocidal effect of AKT inhibitor on human nasopharyngeal carcinoma. Drug Des Devel Ther. 2018;12:2655–63.
37. Pott LL, Hagemann S, Reis H, et al. Eukaryotic elongation factor 2 is a prognostic marker and its kinase a potential therapeutic target in HCC. Oncotarget. 2017;8:11950–62.
38. Zhou Y, Li Y, Xu S, et al. Eukaryotic elongation factor 2 kinase promotes angiogenesis in hepatocellular carcinoma via PI3K/Akt and STAT3. Int J Cancer. 2020;146(5):1383–95.
39. Xie CM, Liu XY, Sham KWY, et al. Silencing of EEF2K (eukaryotic elongation factor-2 kinase) reveals AMPK-ULK1-dependent autophagy in colon cancer cells. Autophagy. 2014;10(9):1495–508.
40. De Gassart A, Demaria O, Panes R, et al. Pharmacological eEF 2K activation promotes cell death and inhibits cancer progression. EMBO Rep. 2016;17(10):1471–84.
41. Garcia-Bermudez J, Williams RT, Guarecuco R, et al. Targeting extracellular nutrient dependencies of cancer cells. Mol Metab. 2020;33:67–82.
42. Wu H, Zhu H, Liu DX, et al. Silencing of elongation factor-2 kinase potentiates the effect of 2-deoxy-D-glucose against human glioma cells through blunting of autophagy. Cancer Res. 2009;69(6):2453–60.
43. Zhu S, Liao M, Tan H, et al. Inhibiting Eukaryotic Elongation Factor 2 Kinase: An Update on Pharmacological Small-Molecule Compounds in Cancer. J Med Chem. 2021;64(13):8870–83.
44. Sun D, Zhu L, Zhao Y, et al. Fluoxetine induces autophagic cell death via eEF2K-AMPK-mTOR-ULK complex axis in triple negative breast cancer. Cell Prolif. 2018;51(2):1–10.
45. Ye WL, Zhang LX, Guan Y Di, et al. Virtual screening and experimental validation of eEF2K inhibitors by combining homology modeling, QSAR and molecular docking from FDA approved drugs. New J Chem. 2019;43(48):19097–106.
46. Devkota AK, Tavares CDJ, Warthaka M, et al. Investigating the kinetic mechanism of inhibition of elongation factor 2 kinase by NH125: Evidence of a common in vitro artifact. Biochemistry. 2012;51(10):2100–12.
47. Yao Z, Li J, Liu Z, et al. Integrative bioinformatics and proteomics-based discovery of an eEF2K inhibitor (cefatrizine) with ER stress modulation in breast cancer cells. Mol Biosyst. 2016;12(3):729–36.
48. Guo Y, Zhao Y, Wang G, et al. Design, synthesis and structure-activity relationship of a focused library of β-phenylalanine derivatives as novel eEF2K inhibitors with apoptosis-inducing mechanisms in breast cancer. Eur J Med Chem. 2018;143:402–18.
49. Devkota AK, Edupuganti R, Yan C, et al. Reversible covalent inhibition of eEF-2K by carbonitriles. ChemBioChem. 2014;15(16):2435–42.
50. Kabil N, Bayraktar R, Kahraman N, et al. Thymoquinone inhibits cell proliferation, migration, and invasion by regulating the elongation factor 2 kinase (eEF-2K) signaling axis in triple-negative breast cancer. Breast Cancer Res Treat. 2018;171(3):593–605.
51. Kenney JW, Moore CE, Wang X, et al. Eukaryotic elongation factor 2 kinase, an unusual enzyme with multiple roles. Adv Biol Regul. 2014;55:15–27.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Case Report

NAUSEA, VOMITING IN PREGNANCY AND ACUPUNCTURE

GEBELİKTE BULANTI, KUSMA VE AKUPUNKTUR

Ayşegül Elbir Şahin [1,2], Alperen Kuru[3], Rümeysa Karaçuha Sürücü[2]

[1]Samsun Education and Research Hospital, Traditional and Complementary Medicine Center
[2]Samsun Education and Research Hospital, Anesthesia and Reanimation Clinic
[3]Samsun Education and Research Hospital, Family Medicine Clinic, Samsun, TURKİYE

Corresponding author:
Dr. Aysegul ELBIR SAHIN, MD
Adress:
Samsun Eğitim ve Araştırma Hastanesi, Anestezi ve Reanimasyon Kliniği, Samsun/TURKİYE
email: aysegulelbir@yahoo.com
Received: 01.54.2022
Accepted: 07.06.2022
Cite as: Sahin.AE, Kuru. A, Surucu. RK, Vomiting and Acupuncture In Pregnancy ijcmbs 2022;2(2):150-152 doi.org/10.5281/zenodo.6800893

Abstract

Nausea and vomiting in pregnancy (morning sickness) is one of the most common symptoms. It is an important problem in terms of maternal and infant health. There is no generally accepted effective medical treatment. Acupuncture may be an advantageous option when evaluated with its efficacy and side-effect profile. In this article, acupuncture application in a patient, who was suffering from morning sickness that was not relieved with medical treatment, will be described.
Keywords: Acupuncture, Pregnancy, Nausea, Vomiting

ÖZ

Gebelikte bulantı ve kusma (GBK) en sık görülen rahatsızlıklardan biridir. Anne ve bebek sağlığı açısından önemli bir problemdir. Genel kabul gören etkili bir medikal tedavi bulunmamaktadır. Etkinlik ve yan etki profili ile değerlendirildiğinde akupunktur avantajlı bir seçenek olabilir. Bu yazıda GBK nedeniyle medikal tedaviden fayda görmeyen bir hastadaki akupunktur uygulaması sunulmaktadır.
Anahtar Kelimeler: Akupunktur, Gebelik, Bulantı, Kusma

Highlights

- Acupuncture in morning sickness is an effective alternative.
- Acupuncture is a safe option with low side-effect profile in morning sickness.

Introduction

Nausea and vomiting in pregnancy (morning sickness) is one of the most common symptoms during pregnancy (1). More than half of the pregnant women typically experience episodes of morning sickness that begin at 4th week and decrease after 16th week of pregnancy(2). Rarely, these complaints is observed to persist throughout the entire pregnancy and may turn into a severe form called “hyperemesis gravidarum” (1). In addition to increasing the cost of health care, causing loss of workforce and decreasing the quality of life of pregnant women, morning sickness is important factor causing deep anxiety in the family in terms of the course of pregnancy and fetal health (3).

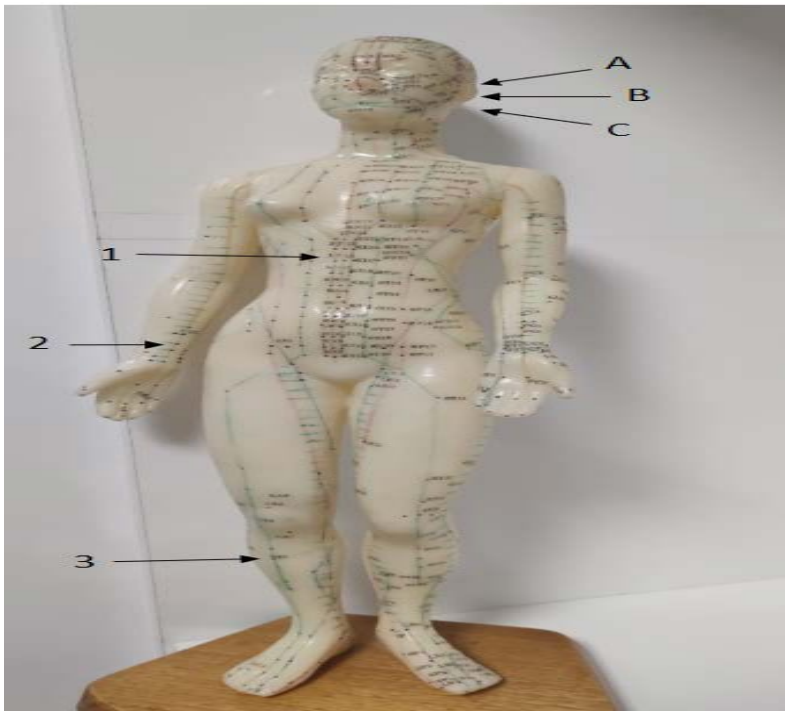
Early treatment aims to prevent serious complications, including hospitalization (3). The ethiopathogenesis of morning sickness is multifactorial, including genetic, endocrine and infectious factors. There is no effective treatment that can fully address the ethiopathogenesis. Dietary changes, symptomatic drugs prescribed with taking side effect profile for mother and fetus into consideration; and in severe cases, intravenous fluid support and intravenous nutrition are recommended in the treatment of morning sickness (1).

For patients who do not benefit from traditional medical methods, integrative methods that has no side effects can be considered. According to Traditional Chinese Medicine, the ear is a microsystem that represents our body. Auricular acupuncture, also known as auriculotherapy, can be done by holding seeds, balls and needles in place by a permanent band to specific points in the ear or by applying laser and electric current. It is a non-invasive, inexpensive, safe and easy form of treatment. Talking about body acupuncture, is a treatment applied by placing thin needles at defined points on the energy channels of human´s body. It has no known side effects or teratogenic complications (4,5). In this article, acupuncture application in a patient who applied to our Traditional and Complementary Medicine (TCAM) Center with the complaint of morning sickness is presented.

Case presentation

A 31-year-old, 14-week pregnant patient who had to use 2 tablets of doxylamine+pyridoxine and ondansetron daily in an attempt to relieve the complaints of severe nausea, sometimes vomiting, and aversion to odors, specifically of foods. These symptoms has started at the 6th week of pregnancy and reoccured every morning . Since the patient's complaints were not relieved, she left the medical treatment and applied to our TCAM Center for acupuncture treatment. First of all, the patient's detailed medical history was taken and physical examination was performed. According to the patient´s history, pregnancy follow-up visits were carried out regularly in the gynecology and obstetrics clinic of a tertiary health center. Complete blood count, liver and kidney function tests, electrolyte values and electrocardiography were evaluated within normal limits. The patient, who was found to have no medical issues to be treated, was informed about acupuncture and her informed consent was obtained. A single session of body acupuncture was performed on bilateral P6 (Neiguan), bilateral ST36 (Zusanli), REN12 (Zhong Wan) points for 20 minutes. 0.20x13mm disposable sterile steel needle was used. After body acupuncture, ear acupuncture was performed by placing sterile permanent seeds to Shen-men (bilateral), Antidepressant (bilateral), Stomach (left) points (Figure 1). Two days after the session, nausea decreased significantly, vomiting did not occur, but the symptom of aversion to odors persisted. When the patient felt nauseated, she managed to relieve her nausea by applying pressure to the bands placed on her ears. No side effects were reported. These advantageous effects continued until the end of pregnancy and the patient did not need any other treatment during pregnancy. In our case, a strong response to treatment was obtained by applying body and ear acupuncture simultaneously in a single session.

Figure 1*: Body and ear points used for acupuncture treatment in our case (A:* Shen-men**; B:** Stomach**; C:** Antidepressant**; 1**: Ren12**; 2:** P6**; 3:** ST36**)**



Discussion

In the current literature, there are conflicting studies on the effectiveness of acupuncture on morning sickness. While most studies reported complete efficacy, some reported partial or no effect. Similarly, the effectiveness of current medical treatments is controversial. The reasons for the conflicting results on efficacy are the insufficient level of evidence and number of studies (2,6-9). This suggests that there is a need for high-evidence studies in the treatment of morning sickness.

Pharmaceutical treatment of morning sickness include anticholinergics, antihistamines, dopamine antagonists, vitamins (B6 and B12), H3 antagonists, or combinations of these substances. Concern about probable teratogenicity of drugs results in hesitancy in prescribing and using drugs during the first trimester of pregnancy. These concerns about drugs in early pregnancy and the increase in the successful use of integrative therapies have made non-drug methods more popular in the treatment of morning sickness (7,9). In this respect, acupuncture seems to be an advantageous option without side effects.

The antiemetic effects of acupuncture are thought to be due to the inhibition of the chemoreceptor trigger zone and vomiting center as a result of increased pituitary secretion of beta-endorphins and adrenocorticotropic

hormone. Acupuncture also regulates gastric peristalsis and reduces gastric acid secretion by affecting the upper gastrointestinal system (5). The range of effects of acupuncture is wide and a strong response could be seen as in our case. In recent studies, it has been seen that body or auricular acupuncture is used alone in the treatment of morning sickness patients. In our case, the efficacy may have been increased and the need for additional sessions-treatment may have been avoided by using two methods consecutively. There are examples in the literature that the combined use of body and ear acupuncture can be more effective (10).

Studies have shown that P6 point stimulation is superior to several antiemetic drugs in preventing nausea and vomiting (11). In a study about the effects of body acupuncture on the P6 point, ondansetron and placebo on postoperative nausea and vomiting, it was shown that acupuncture and ondansetron were significantly more effective than placebo, equivalent to each other (12). In a systematic review of morning sickness treatments, ondansetron was found to be more effective in reducing nausea than doxylamine+pyridoxine (13). Our case decided to try acupuncture treatment because her complaints did not change despite the use of 2 tablets of Doxylamine+Pyridoxine and Ondansetron per day. After the session, the patient's complaint of vomiting completely disappeared, and the nausea decreased significantly. These effects which obtained in a single session continued until the end of pregnancy. These data suggest that acupuncture may be at least as effective as ondansetron.

Most of the acupuncture studies on nausea and vomiting focussed on the P6 point (9). In our case, it was aimed to balance the energy circulating in the stomach canal by adding ST36 and REN12 points to the P6 point, which are frequently used in stomach disorders. In order to prevent recurrences, permanent seeds were placed on the relevant acupuncture points in the ear and the possibility of needing retreatment with the acupressure method has been reduced. After the session, our patient managed to get over the nausea by applying pressure to the seeds in her ear when she felt nauseated again.

Conclusion

Morning sickness is an important problem in terms of maternal and infant health. Acupuncture may be an advantageous option for medical treatments in terms of efficiency and side-effect profile. Informing pregnant women about this issue in the early period may contribute to the quality of life of the patients, reduce health care costs and reduce the rates of hyperemesis gravidarum.

Acknowledgements: None

Ethical Approval: *N/A*

Author Contributions: *Author Contributions: Concept: Şahin AE, Kuru A; Literature Review: Sahin AE, Sürücü RK; Design: Kuru A,Sürücü RK; Data acquisition: Sahin AE, Kuru A; Analysis and interpretation: Şahin AE; Writing manuscript: Şahin AE, Sürücü RK; Critical revision of manuscript:Şahin AE, Kuru A*

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

Financial Disclosure: *Authors declared no financial support.*

References

1.Bustos M, Venkataramanan R, Caritis S. Nausea and vomiting of pregnancy - What's new? Auton Neurosci. 2017;202:62-72.

2. Festin M. Nausea and vomiting in early pregnancy. BMJ Clin Evid. [serial on the Internet] 2022 [cited 28 Apr 2022]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959188/pdf/2014-1405.pdf

3. Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin No. 189: Nausea And Vomiting Of Pregnancy. Obstet Gynecol. 2018;131(1):e15-e30.

4. Negarandeh R, Eghbali M, Janani L, et al. Auriculotherapy as a means of managing nausea and vomiting in pregnancy: A double-blind randomized controlled clinical trial. Complement Ther Clin Pract. 2020;40:101177.

5. Samuels N. Acupuncture for nausea: how does it work?. Harefuah. 2003;142(4):297-316.

6. Streitberger K, Ezzo J, Schneider A. Acupuncture for nausea and vomiting: an update of clinical and experimental studies. Auton Neurosci. 2006;129(1-2):107-17.

7. Matthews A, Haas DM, O'Mathúna DP, et al. Interventions for nausea and vomiting in early pregnancy. Cochrane Database Syst Rev. 2015;2015(9):CD007575.

8. Boelig RC, Barton SJ, Saccone G, et al. Interventions for treating hyperemesis gravidarum: a Cochrane systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2018;31(18):2492-505.

9. Heckroth M, Luckett RT, Moser C, et al. Nausea and Vomiting in 2021: A Comprehensive Update. J Clin Gastroenterol. 2021;55(4):279-99.

10. Elbir Şahin A, Göktaş Ç, Karaçuha Sürücü R, et al. Retinitis Pigmentosa and Acupuncture. Why Not! International Journal of Current Medical and Biological Sciences. 2022;2(1):78–81.

11. Ezzo J, Streitberger K, Schneider A. Cochrane systematic reviews examine P6 acupuncture-point stimulation for nausea and vomiting. J Altern Complement Med. 2006;12(5):489-95.

12. El-Deeb AM, Ahmady MS. Effect of acupuncture on nausea and/or vomiting during and after cesarean section in comparison with ondansetron. J Anesth. 2011;25(5):698-703.

13. O'Donnell A, McParlin C, Robson SC, et al. Treatments for hyperemesis gravidarum and nausea and vomiting in pregnancy: a systematic review and economic assessment. Health Technol Assess. 2016;20(74):1-268.

IJCMBS THE INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND BIOLOGICAL SCIENCES e-ISSN: 2791-8815

Case report

Pulmonary Hypertension Secondary to Dasatinib Use for the Treatment of Chronic Myeloid Leukemia

Kronik Miyeloid Lösemi Tedavisi için Dasatinib Kullanımına Sekonder Gelişen Pulmoner Hipertansiyon

Müge Tezer[1], Veysel Tosun[2*], Ünal Güntekin[3]

[1]Department of Cardiology, Cizre State Hospital, Şırnak, TURKİYE
[2]Department of Cardiology, Şanlıurfa Education and Research Hospital, Şanlıurfa, TURKİYE
[3]Department of Cardiology, Akdeniz University Medicine Faculty, Antalya, TURKİYE

***Corresponding author:**
Dr.Veysel Tosun, MD
Adress:
Department of Cardiology, Şanlıurfa Education and Research Hospital, Şanlıurfa, TURKİYE
email:
veyseltosun8810@gmail.com
Received: 05.07.2022
Accepted: 17.07.2022
Cite as: TEZER.M, TOSUN.V, GUNTEKİN.U Pulmonary Hypertension Secondary to Dasatinib Use for the Treatment of Chronic Myeloid Leukemia IJCMBS 2022;2(2):153-156
doi.org/10.5281/zenodo.6849397

Abstract

A 20-year-old male patient under the treatment of Dasatinib for chronic myeloid leukemia was admitted with symptoms and signs of right heart failure. As a result of the transthoracic echocardiographic evaluation of the patient, severe enlargement of the right heart chambers was detected and systolic pulmonary arterial pressure was measured up to 105 mmHg. As a result of the other tests performed for pulmonary hypertension, it was thought that it might be secondary to the tyrosine kinase inhibitor Dasatinib with the exclusion of the other causes of pulmonary hypertension. Echocardiographic and symptomatic improvement was observed in the follow-up after Dasatinib treatment was changed to another tyrosine kinase inhibitor, Nilotinib.
Key words: chronic myeloid leukemia, dasatinib, nilotinib, pulmonary hypertension, right heart failure

ÖZ

Kronik miyeloid lösemi nedeniyle Dasatinib kullanan 20 yaşındaki erkek hasta sağ kalp yetersizliği semptom ve bulguları ile başvurdu. Hastanın ekokardiyografik değerlendirilmesi sonucunda sağ kalp boşluklarında ileri derece genişleme tespit edildi ve sistolik pulmoner arteryel basıncının da 105 mmHg' ya kadar yükselmiş olduğu saptandı. Pulmoner hipertansiyon açısından yapılan tetkikleri sonucunda diğer nedenler ekarte edildikten sonra kullanmış olduğu Dasatinib etken maddeli tirozin kinaz inhibitörüne sekonder olabileceği düşünüldü. Dasatinib tedavisi başka bir tirozin kinaz inhibitörü olan Nilotinib ile değiştirildikten sonraki takiplerinde ekokardiyografik ve semptomatik iyileşme gözlendi.
Anahtar kelimeler: kronik miyeloid lösemi, dasatinib, nilotinib, pulmoner hipertansiyon, sağ kalp yetersizliği

Highlights

- Dasatinib should be kept in mind as a factor in Drug-related PAH
- It is reasonable to screen routinely for PAH by echocardiography before commencing dasatinib treatment

Introduction

Pulmonary arterial hypertension (PAH) is a chronic and progressive cardio-pulmonary disease with a mean pulmonary artery pressure (mPAB) above 25 mmHg which hemodynamically meets the definition of precapillary pulmonary hypertension (1). As a result of many etiological reasons, disruption of mediator balance acting on the pulmonary arterial wall in the direction of proliferation and vasoconstriction leads to clinic of PAH (2). Drug-related PAH is an example of this. In this case report, we aim to present a patient with a history of pulmonary hypertension and recurrent pleural effusion caused by the use of Dasatinib, a tyrosine kinase inhibitor used for treatment of chronic myeloid leukemia (CML).

Presentation of the case

A 20 year old male patient was admitted to the cardiology outpatient clinic due to shortness of breath for 15 days. His general condition was good during application and his vital signs were found to be normal. He had no tachypnea. On physical examination, cardiac sounds were rhythmic, S2 was rigid, and systolic murmur was in tricuspid focus. The respiratory sounds were decreased in bilateral basals. The peripheral system examination was normal. He was on follow-up with CML for seven years and had been using Dasatinib. When he had a similar dyspnea a year ago, pleural effusion was detected in the outer center and Dasatinib was suspended for a while considering that it was drug-related. After pleural effusion regressed and the clinic recovered, Dasatinib was started again and the patient was continued to be followed up by hematology clinik.

The electrocardiogram (ECG) of the patient had right axle deviation and was comatible with right ventricular hypertrophy **(Figure 1).**

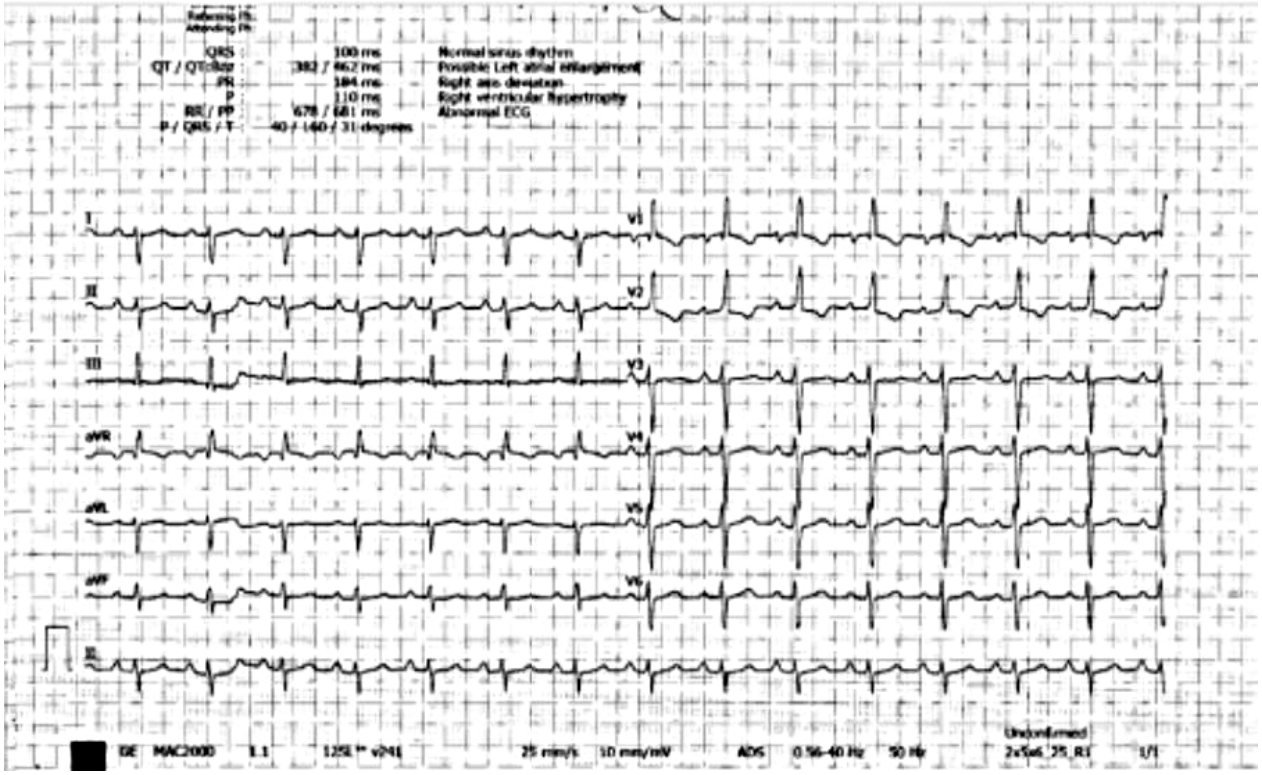


Figure 1: ECG; Right axle deviation and right ventricular hypertrophy

Transthoracic echocardiography showed normal left ventricle ejection fraction but wide right cavities, decreased right ventricular systolic functions, severe tricuspid regurgitation and the systolic PAB (sPAB) calculated on maximum TY velocity were 105 mmHg **(Figure 2).** The patient was hospitalized to the cardiology department due to decompensated heart failure. The volume of the patient was decreased with intravenous diuretic therapy and sPAP was still high on the control echocardiography. Other tests for the etiology of pulmonary hypertension were normal and the pulmonary hypertension of patient was thought to be related to Dasatinib which was then discontinued. Consulting with hematology for the treatment of CML, Dasatinib was changed with Nilotinib, which is another tyrosine kinase inhibitor. The patient was discharged and at 3 week follow-up, his complaints were significantly decreased. On echocardiography, the right cavities were wide, but the severity of tricuspid regurgitation decreased. sPAB was measured as 76 mmHg **(Figure 2).**

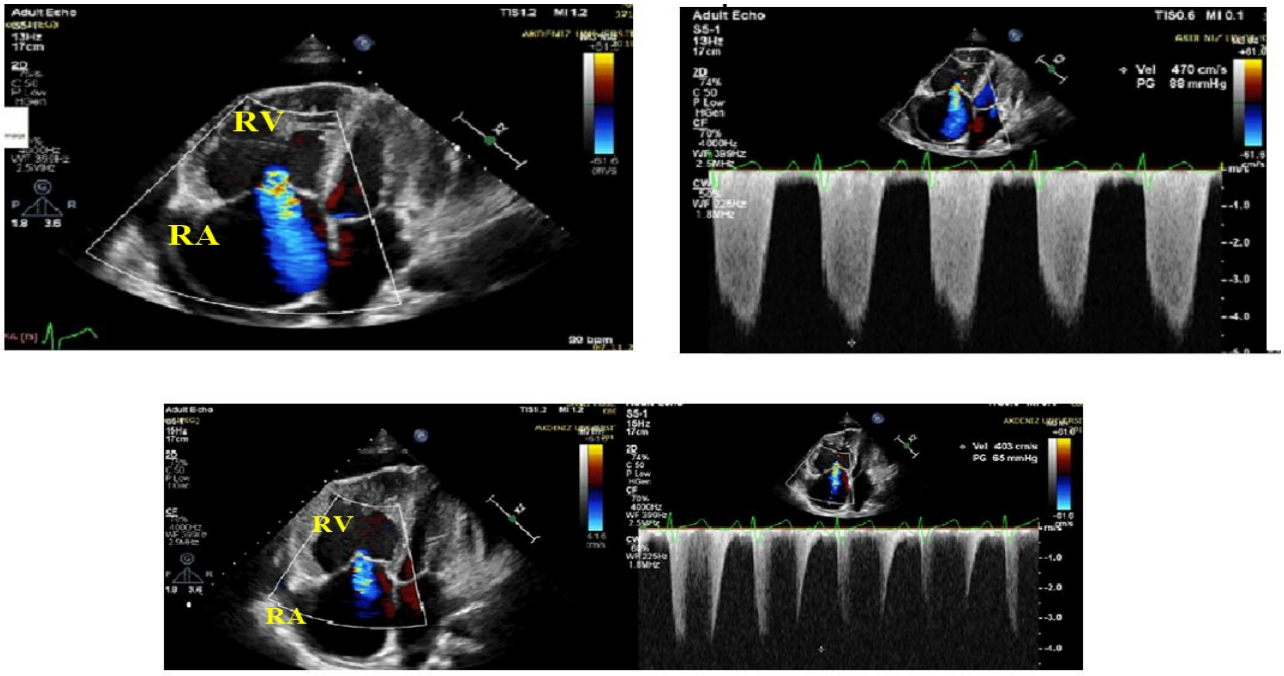


Figure 2: Comparative view with second echocardiography: moderate tricuspid regurgitation and PAB; calculated over maximum TY velocity; following drug discontinuation and clinical improvement.

TAPSE and S' values showed right ventricular systolic functions elevated from 13 to 20 and from 9 to 14 respectively. sPAP decreased to 65 mmHg at the follow-up after 3 months, and decreased to 45 mmHg at the 6-month follow-up **(Figure 3).**

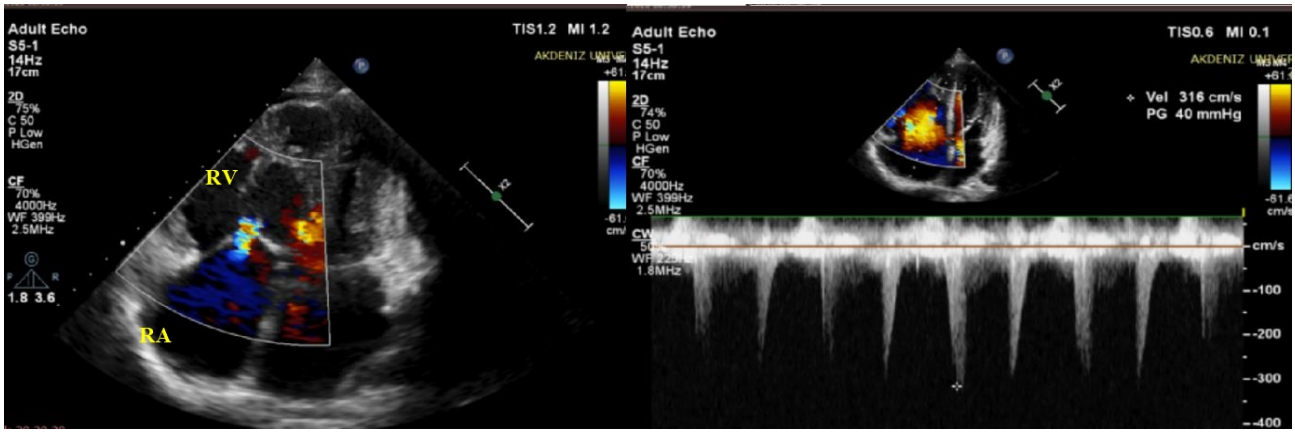


Figure 3: On echocardiography performed at the 3rd month following discharge, mild tricuspid regurgitation and measurement of PAB as 65 mmHg.

Discussion

Dasatinib is one of the second generation tyrosine kinase inhibitors which is preferred in early chronic phase treatment of CML in case of the first-line imatinib treatment failure, insufficient response, intolerance or resistance (3). Although the most common side effects are myelosuppression, nausea, vomiting, diarrhea and skin rash, it may also have serious side effects such as cardiac fluid retention, pleural and pericardial effusion, cardiac dysrhythmia, dysfunction, and pulmonary hypertension. The most common pulmonary toxic adverse effect is pleural effusion, but it usually creates a reversible clinical picture following drug discontinuation (4).

PAH is a chronic and progressive cardio-pulmonary disease that can result from many etiological reasons such as connective tissue diseases, HIV infection, portal hypertension, congenital heart diseases, schistosomiasis, hereditary causes like BMPR 2 mutation, drug-toxin-related and idiopatic. According to WHO's Updated Clinical Pulmonary Hypertension Classification, these patients are included in group 1 of pulmonary hypertension (5). Hemodynamically it meets the definition of precapillary pulmonary hypertension; mean pulmonary artery pressure (mPAB) above 25 mmHg and PVR is >3 Woods, PCWP and cardiac output are normal (6).

The underlying reason for the development of PAH is the disruption of the mediator balance on the pulmonary arterial wall in the direction of proliferation and vasoconstriction. The activity of serotonin, endothelin-1 and thromboxane increases. They lead to vasoconstrictive and proliferative effects. Since the activity of prostacyclin and nitric oxide decreases, vasodilator and antiproliferative effects decrease. Proliferation develops in intima and adventitia layers of vascular wall, hypertrophy and plexiform structures develop in smooth muscles. Consequently PVR and PAB increases due to in situ thrombosis (2). Because of the specific treatment for these underlying causes, Group 1 PAH patients have the best prognosis as reported in recent studies (7). Although the mechanism of drug-induced PAH remains unclear, it was thought that serotonin might play a role in the generation of this phenomenon (8).

A study in 138 dasatinib-treated patients found increased right ventricular systolic pressure in 18 of them from a median of 29 mmHg at baseline to 42 mmHg, with a return to baseline levels after the discontinuation of the drug (9). Then, in 2012, a study was published 9 case series reporting dasatinib-induced PAH from the French Registry Network. The median delay between initiation of dasatinib and PAH diagnosis was 34 month, suggesting that PAH may be a late complication of dasatinib. The positive mechanism of dasatinib-induced PAH may be hypoxic pulmonar vasoconstruction responses and endotelial cell disfunciton via increased production of reactive oxidants (10).

Conclusion

We reported a case of PAH and heart failure symptoms and signs with dasatinib use. The first-line treatment in drug-related pulmonary hypertension is discontinuation of the drug like our case. PAP may decrease and clinical improvement can be achieved. Spesific treatment methods, such as endothelin receptor blockers may be preferred to accelerate the process at this point. In our case, we stopped the dasatinib drug. The patient did not need specific PHT treatment in the follow-ups. However, it should be kept in mind that in some cases which causes Dasatinib pulmonary hypertension, the situation is irreversible (11). It is reasonable to screen

routinely for PAH by echocardiography before commencing dasatinib treatment, especially in patients with underlying cardiopulmonary disease.

Acknowledgements: None

Ethical Approval: *N/A*

Author Contributions: *Author Contributions: Concept: M.T ; Literature Review:M.T, V.T ; Design:M.T, V.T; Data acquisition:M.T ; Analysis and interpretation:M.T, V.T ; Writing manuscript:M.T, V.T ; Critical revision of manuscript: Ü.G*

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

Financial Disclosure: *Authors declared no financial support.*

References

1. Authors/Task Force Members, Galiè N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: The Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J 2009; 30:2493-537.
2. Travis WD, Colby TV, Koss MN, et al. Pulmonary hypertension and other vascular disorders in nonneoplastic disorders of the lower respiratory tract. AFIP 2002;767-92
3. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome–positive leukemias. N Engl J Med 2006; 354:2531-41.
4. Dingli D, Utz JP, Krowka MJ, et al. Unexplained pulmonary hypertension in chronic myeloproliferative disorders. Chest 2001; 120:801-8.
5. Simonneau G, Galie` N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. J Am Coll Cardiol 2004;43(Suppl 1):5-12.
6. Hoeper MM, Bogaard HJ, Condliffe R, et al. Definitions and diagnosis of pulmonary hypertension. J Am Coll Cardiol 2013;62(Suppl):42-50.
7. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med 1991; 115:343-9.
8. Eddahibi S, Adnot S. Anorexigen-induced pulmonary hypertension and the serotonin (5-HT) hypothesis: lessons for the future in pathogenesis. Respiratory research 2001; 3:1-4.
9. Quintás-Cardama A, Kantarjian H, O'brien, S, et al. Pleural effusion in patients with chronic myelogenous leukemia treated with dasatinib after imatinib failure. Journal of Clinical Oncology 2007; 25:3908-14.
10. Guignabert C, Phan C, Seferian A, et al. Dasatinib induces lung vascular toxicity and predisposes to pulmonary hypertension. J Clin Invest 2016;126: 3207-18.
11. Montani D, Bergot E, Gunther S, et al. Pulmonary hypertension in patients treated by dasatinib. Circulation 2012; 125:2128-37.