ISSN: 2148-4724
E-ISSN: 2548-0030

TMSJ

TURKISH MEDICAL STUDENT JOURNAL

Volume: 7 Issue: 3 Oct 2020

https://tmsj.trakya.edu.tr/

TMSJ

TURKISH MEDICAL STUDENT JOURNAL

THE OFFICIAL JOURNAL OF
TRAKYA UNIVERSITY SCHOOL OF MEDICINE

Citation Abbreviation: Turkish Med Stud J

VOLUME 7 - ISSUE 3 - OCT 2020

Published three times a year

Free access to the Journal's web site: *https://tmsj.trakya.edu.tr*

Manuscript Submission*: tmsj@trakya.edu.tr*

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Printing at: Trakya Üniversitesi Basımevi
Edirne Teknik Bilimler M.Y.O Sarayiçi Yerleşkesi, 22020 Yeni İmaret, Edirne, Turkey
Phone: +90 (284) 224 02 83
Printing Date: October 2020
ISSN: 2148-4724 E-ISSN: 2548-0030

TMSJ
TURKISH MEDICAL STUDENT JOURNAL

Indexed In
Türk Medline

Publisher
Trakya University

TMSJ
TURKISH MEDICAL STUDENT JOURNAL

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TMSJ publishes original researches, interesting case reports and reviews regarding all fields of medicine. All of the published articles are open-access and reachable on our website. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications for stimulating students, doctors, researchers. Our mission is to feature quality publications that will contribute to the progress of medical sciences as well as encourage medical students to think critically and share their hypotheses and research results internationally.

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All original articles submitted to the TMSJ have to be approved by an ethical committee and include the name of ethics committee(s) or institutional review board(s), the number/ID of the approval(s). Additionally, informed consent documents obtained from patients involving case reports are required for the submission.

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The Journal publishes the following types of articles:

Original Research Articles: Original prospective or retrospective studies of basic or clinical investigations in areas relevant to medicine.

Content:
- Abstract (average 400 words; the structured abstract contain the following sections: aims, methods, results, conclusion)
- Introduction
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- Results
- Discussion
- Reference

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- Discussion
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- Submitted letters should include a note indicating the attribution to an article (with the number and date) and the name, affiliation and address of the author(s) at the end.
- The answer to the letter is given by the editor or the author(s) of the manuscript and is published in the journal.

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Content:
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Authors are encouraged to follow the following principles before submitting their material.

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ACKNOWLEDGEMENTS: Conflict of interest, financial support, grants, and all other editorial (statistical analysis, language editing) and/or technical assistance if present, must be presented at the end of the text.

REFERENCES: References should be numbered in the order they are cited. Only published data or manuscripts accepted for publication and recent data should be included. Inaccessible data sources and those not indexed in any database should be omitted. Titles of journals should be abbreviated in accordance with Index Medicus- NLM Style (Patrias K. Citing medicine: the NLM style guide for authors, editors, and publishers [Internet]. 2nd ed. Wendling DL, technical editor. Bethesda (MD): National Library of Medicine (US); 2007 - [updated 2011 Sep 15; cited Year Month Day] (http://www.nlm.nih.gov/citingmedicine). All authors should be listed if an article has three or less authors; first three authors are listed and the rest is represented by "ve ark." in Turkish articles and by "et al." in English articles. Reference format and punctuation should be as in the following examples.

Journal: Muller C, Buttner HJ, Peterson J et al. A randomized comparison of clopidogrel and aspirin versus ticlopidine and aspirin after placement of coronary artery stents. Circulation 2 Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. St Louis: Mosby; 1974.p.273-85. 000;101:590-3.

Book Section: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. St Louis: Mosby; 1974.p.273-85.

Books with Single Author: Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Editor(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

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Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

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EDITORIAL

Dear readers,

As TMSJ editorial board, we are proud to present the latest issue of this year. This issue consists of 9 articles including 3 original articles, 2 case reports, 3 reviews, and a letter to the editor.

Since the outbreak of COVID-19, a lot of things in our lives have changed and they are still changing. People around the world try to get over the pandemic period with as little damage as possible. Therefore, many of our daily practices has changed. The education system is one of them. At the beginning of the pandemic, most of the schools gave a break until figuring out what to do next. Afterwards, distance education was found to be the best solution, allowing the students to continue their education while avoiding the risks of getting infected. Medical schools also accepted these regulations and tried to adapt their education systems to distance learning. However, since practical lessons occupy a crucial place in medical education, students and academicians are having some problems during distance education. Çifcibaşı et al. tries to shed a light to this aspect with their original article by giving a place to the thoughts of medical students and academicians.

Nowadays, while the researches on COVID-19 are numerous, researchers also continue to their studies regarding other subjects such as cancer researches. The original article by Aksu et al. is one of them. Glioblastoma multiforme is a very aggressive brain tumor with poor diagnosis. Aksu et al. shared their findings on the treatment of glioblastoma multiforme with 5-Fluorouracil and Ruxolitinib.

For centuries, people have been struggling with chronic diseases. Chronic kidney disease is one of the most challenging ones. Since kidney transplant is the only curative method, it is not easy to live with this disease for patients. Therefore, hemodialysis is a commonly used renal replacement modality worldwide. Kıral et al. investigated the parameters that may predict the frequency of hemodialysis sessions weekly.

Ersoy et al. presented a case report regarding the role of ultrasonography as a diagnostic tool for juvenile polyps. Even though juvenile polyps are the most common intestinal polyps in children, ultrasound is not used primarily in the diagnosis. The case report by Ersoy et al. leans on the diagnostic effect of ultrasound imaging in juvenile polyps.

Cengiz et al. made a contribution to the October issue of TMSJ with a rare case about partial nephrectomy of a horseshoe kidney with renal cell carcinoma and cholecystectomy. I think our readers will find this case report interesting to read.

Cardiovascular diseases are one of the main causes of death and disability worldwide. Myocardial infarctions and angina are two of the main focus areas of the researches. Özkan et al. shared a review regarding the pathophysiology, clinical features and treatment of microvascular angina. Kurtoğlu et al. contributed with a review about myocardial infarction diagnosis and cardiac troponins.

As we clearly see, COVID-19 is changing our lives. The practice of medicine and the applications are also affected. Kadribey et al. shed a light to this matter with a review by providing a broader perspective.

Rasheed et al. made a contribution to an article published in our journal, 'Thoughts and awareness of medical students about COVID-19 pandemic', by a letter to the editor. I think, their comments enriched the discussion.

After this issue, my duty as Editor-in-chief in TMSJ comes to an end. Since June 2019, my editorial team and I published 5 issues consisting of 15 original articles, 8 case reports, 7 reviews and 3 letters to the editor. We tried to increase the quality of our journal and editorial policy day by day. In order to introduce our journal to as much as medical students possible, we attended many conferences and gathered a broad variety of articles. With the editors from different medical schools and different countries, our editorial board had put a great effort to every issue. I would like to thank each and every one of them. I am also thankful to our professors in the editorial advisory board for their support. Many thanks are also due to our authors for choosing our journal to submit their articles which they prepared with great efforts. On behalf of TMSJ editorial board, I also would like to thank our beloved readers.

As of this issue, I entrust my duty to Hilal Sena Çifcibaşı. I strongly believe that the editorial board is going to put great efforts to improve our journal in every aspect in her leadership.

Nur Gülce İŞKAN
Editor-in-Chief

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ORIGINAL ARTICLE

DISTANCE EDUCATION IN MEDICAL SCHOOLS: THE EXPERIENCE AND OPINIONS OF ACADEMICIANS AND STUDENTS

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ABSTRACT

Aims: This study aims to identify the thoughts and opinions of medical schools' students and academicians about the distance medical education and examination system that has been rapidly applied during the COVID-19 pandemic ***Methods:*** A questionnaire was prepared via Google Forms which consisted of a total of 3 sections. Informed consent was obtained in the first section and participants were directed to "student" or "academician" section. There were 28 questions for the students and 24 questions for the academicians. Categorical variables were demonstrated as numbers and percentages, whereas continuous variables were presented as minimum, maximum, and mean values. Chi-squared test was used to compare preclinic and clinic year students, and the academicians in preclinic, medical, and surgical fields. ***Results:*** A total of 321 participants completed the questionnaire. The mean participant ages were 21.4 years and 41.68 years for the students and the academicians, respectively. Only 30% of the students thought the distance education lessons were beneficial while it was 35.5% for the academicians. 25.8% of the academicians and 29.6% of the students were indecisive on the matter. When the examination process was taken into account 67.7% of the academicians and 56.9% of the students thought the online examinations were not reliable. ***Conclusion:*** It is predicted that the pandemic process will continue in the next academic years. Considering the current situation, distance education seems to be the best option to ensure that the learning process can continue while protecting the health of students and academicians. Although distance education is not sufficient by itself in medical education during and after the COVID-19 pandemic, it is a method that should be used in almost every field of medical education, especially in the preclinical phase. Both the distance education, and online examination process require improvements and they are needed to be supported with face to face lectures and practices.***Keywords:*** COVID-19, academician, medical student, distance learning, pandemic

INTRODUCTION

Coronavirus disease-19 (COVID-19) has made a definite entry into our lives since December 31, 2019 and continues to show its effects. With this entry viral diseases have been on the agenda of governments all across the world as well as the medical world (1).

Viruses, especially SARS-CoV-2, which increase and show up and affect the whole world, will obviously be in our lives for a while and we have to adapt our daily lives and most importantly education systems into the new normal (2).

Coronavirus disease-19 outbreak caused serious changes in the education field in Turkey, as well as all over the world. Upon the announcement of the first case in Turkey on March 10, 2020, all face-to-face education activities across the country were stopped as of March 16, and subsequently, online/distance education processes were initiated (3).

The primary purpose of this was to ensure the isolation of students and education staff, such as a very important part of the population, and to control the pandemic as much as possible (2).

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Received: 10.10.2020 Accepted: 25.10.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.01 Available at: tmsj.trakya.edu.tr

Cite this article as: Çifcibaşı HS, Kef B, Özyiğit İİ et al. Distance education in medical schools: the experience and opinions of academicians and students. Turkish Med Stud J 2020;7(3):115-29.

The necessity of making all these changes during the school period and without interrupting the education created difficulties in terms of both time constraints and feasibility. Online education is challenging, especially in education programs where practical applications are as important as theoretical applications such as medical faculties (4).

Considering that online education depends on the center, the instructor and of course the participation of the students, it is not possible to talk about a standard for now. Access to technological devices and internet, financial problems are among the issues that challenge students (5).

In addition, the fact that the practical training that medical students will receive takes place in the hospital makes the situation even more difficult as it requires the educators to pay attention to the safety of the students while providing care to the patients (5).

It is predicted that the pandemic process will continue in the next academic years (1). For this reason, determining the thoughts and opinions of medical faculty students and academicians about online education and the examination process is of great importance to increase the quality of education (5, 6). In this cross-sectional study, we aimed to identify the thoughts and opinions of medical students and academicians about the online education and examination system that has been rapidly passed through the COVID-19 pandemic process.

MATERIAL AND METHODS

This study was approved by the Scientific Research Ethics Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2020/343). This descriptive study was carried out between September and October 2020. Individuals other than medical students and medical school academicians were not included in the study. The study was conducted via a self-administrative online questionnaire in the Turkish language and delivered through scientific research communities of the medical schools. Information about the study was provided at the beginning of the questionnaire and the participants' consent was required to continue forward.

The questionnaire was prepared via Google Forms and consisted of a total of 3 sections. Informed consent was obtained in the first section and participants were directed to "student" or "academician" section.There were 28 questions for the students and 24 questions for the academicians (Table 1).

The age, gender, years in university, the name of the university, and chronic diseases of the participants were questioned in both groups for demographics. Categorical variables are demonstrated as numbers and percentages, whereas continuous variables are presented as the minimum, maximum, and mean values. The IBM SPSS version 23 was used for the statistical analysis of the data. Chi-squared test was used to compare preclinic and clinic year students, and the academicians in preclinic, medical, and surgical fields. A p-value <0.05 was set for statistical significance.

RESULTS

This questionnaire-based study was conducted among 290 medical students, and 31 academicians. Medical students are composed of 165 (56.9%) preclinical, and 125 (43.1%) clinical students from 15 different universities. The highest participation was from Trakya University with a rate of 69.1%. The mean of the students' age was 21.4 years (standard deviation [SD]: 2.267 years, range: 17-38 years). One hundred and eighty-two (62.8%) were female, and 108 (37.2%) were male. The dispersion of the students' grades is shown in Table 2. Thirty-one medical school academicians from ten different universities in Turkey participated in the online survey. The mean of the academicians' age was 41.68 years (SD: 7.512 years, range: 28-56 years). Eighteen (58.1%) were female, and 13 (41.9%) were male. The specialties, academic titles, and academic experiences (in years) of the academicians are shown in Table 3.

The majority of the students had no chronic disease (89.7%), however, 14 (4.8%) had asthma, 2 (0.7%) had diabetes, and 14 (4.8%) of them had some other chronic diseases. The number of academicians with no chronic disease was 23 (74.2%), and 1 (3.2%) of them had asthma, 1 (3.2%) of them had diabetes, and 6 (19.4%) of them had some other chronic diseases.

Of the students, two hundred and fifty-five (87.6%) used computers, 96 (33%) used smartphones, and 34 (11.7%) used tablets to follow their distance education lessons.

The leading problem students encountered was not having the proper study environment (46.7%) while this was not the leading problem for the academicians (12.9%). About 35.5% of the academicians had internet access problems, technological inefficacy, and systemic problems. One hundred and twenty (41.2%) students experienced internet access problems, 112 (38.5%) stu-

dents had systemic issues, and 95 (32.6%) had technological inefficacy. Eighty-nine (30.6%) students did not have proper time while only 4 (12.9%) academicians had no proper time during the distance education process.

The thoughts of medicals students' and academicians' on distance learning are shown in Tables 4 and 5, respectively. According to 40.3% of the students, distance education lessons are not beneficial and academicians are in co-decision with this answer. Also, 66.6% (40.7% do not agree at all, 25.9% do not agree) of the students think that distance education lessons are not useful as face-to-face lessons. Of the academicians, 45.2% who are in medical fields think that they are not as useful as face-to-face lessons. About 87.1% of the academicians think that distance education alone is insufficient in the field of medicine. By 43.1% do not agree at all, and 21.0% do not agree answers, medical students think that it is inconvenient to include interns in distance learning. Twenty-five (80.6%) academicians also think that interns should not be included in distance education. With the majority of 26 academicians, "practical lessons are not suitable" was the most common answer.

According to Table 6, 63 (38.2%) preclinical and 71 (56.8%) clinical students think that distance education was inadequate alone for the basic sciences, but most of them should be given as distance education, and this was statistically significant ($p<0.001$). Eighty-four (50.9%) preclinical students think that practical lessons in distance education were completely insufficient alone, and all of them should be in-class lessons, while 55 (44.0%) clinical students think that they are inadequate alone, and most of them should be face-to-face, and these results were statistically significant ($p=0.011$).

The overall responses for the user experience of distance learning applications are presented in Tables 7 and 8. Students predominately think that accessing the course was easy (n=182, 62.7%). One hundred and seventy-five (60.3%) students said that if they did not attend a live lecture, they can easily access and watch the recordings, and accessing the course contents is quick for the 196 (67.6%) students. Of the students, 125 (43.1%) occasionally encounter technical problems in virtual classroom applications, however, 182 (62.8%) of them find the distance education system not complicated. Twelve (38.7%) academicians know how to solve the problems they encountered and 12 (38.7%) were indecisive. For the question of whether the distance education lessons are interactive enough, 21 (49.8%) academicians think that they were not.

The medical students' and academicians' thoughts on online examinations are shown in Table 9. There was a statistically significant association between the academicians' answers on whether the online exams measure learning or not ($p=0.000239$). On the question of online exams' reliability, a statistically significant difference was found ($p=0.003$). None of the academicians chose the "do not agree at all" option for the question on whether they think that the students cheat in the online exam. Also, the answers to this question were statistically significant ($p=0.027$).

Table 1: The questionnaire used in the study.

Questions	Answers
For Medical Students and Academicians	
1) Do you have any chronic illnesses?	Asthma / COPD / Diabetes / Chronic renal failure / Other / None
2) I think distance education lessons are beneficial	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
3) I find distance education alone sufficient in the field of medicine	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
4) I think distance education lessons are as useful as face-to-face lessons	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
5) I find it convenient to include interns in distance education	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
6) What do you think about the competence of distance education for basic sciences?	Alone enough / Inadequate alone, but most of them should be distance education / Inadequate alone, and most of them should be in-class education / Completely insufficient alone, all of them should be in-class education
7) What do you think about the competence of distance education for clinical sciences?	Alone enough / Inadequate alone, but most of them should be distance education / Inadequate alone, and most of them should be in-class education / Completely insufficient alone, all of them should be in-class education
8) Apart from the pandemic, I think theoretical lessons can be given in the form of distance education	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
9) I would like distance education to be available also outside of working hours	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
10) Theoretical courses are suitable for distance education	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
11) Practical lessons are suitable for distance education	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
12) What kind of problems did you encounter during the distance education process?	Internet access / Technological inefficacy / Not having the proper study environment / Not having the proper time / Systemic issues / Other
13) I know how to solve the problems I encounter in the distance education system	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
14) I occasionally encounter technical problems in virtual classroom application	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
15) The distance education system seems very complicated to me	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
16) I think online exams measure learning	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
17) I think online exams are reliable	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
For Medical Students Only	
18) Which university do you study at?	
19) Which class are you currently enrolled?	Prep / 1 / 2 / 3 / 4 / 5 / 6
20) On which devices do you follow distance education lessons?	Computer / Smartphone / Tablet / Other
21) I regularly attend distance education lessons	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
22) I can easily access the course I want in the distance education system	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
23) I can easily access and watch the recordings of the live lectures I did not attend	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
24) The educational materials used in the lessons meet my needs	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
25) I can quickly access the course contents	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
26) I know how to use virtual classroom application	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
27) I can easily find the information I want in the distance education system	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
28) I think the information (announcement etc.) is sufficient	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
For Academicians Only	
29) What is your specialty?	Preclinical / Medical / Surgical
30) Which university do you teach at?	
31) Academic title	Guest teaching assistant / Teaching assistant / Associate professor / Professor
32) Academic experience (in years)	0-5 / 6-10 / 11-15 / 16-20 / 20+
33) I can use the virtual classroom application without support	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
34) I think the distance education lessons are interactive enough	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree
35) I think the students cheat in the online exams	Do not agree at all / Do not agree / Indecisive / Agree / Completely agree

Table 2: Medical students' demographics.

Grades	Number of Students [n (%)]
1	15 (5.2)
2	88 (30.3)
3	62 (21.4)
4	48 (16.6)
5	42 (14.5)
6	35 (12.1)
Total	290 (100.0)

Table 3: Demographics of academicians.

	Number of Academicians [n (%)]
Specialty	
Preclinical	2 (6.5)
Medical	24 (77.4)
Surgical	5 (16.1)
Academic title	
Guest teaching assistant	4 (12.9)
Teaching assistant	19 (61.3)
Associate professor	4 (12.9)
Professor	4 (12.9)
Academic experience	
0-5 years	18 (58.1)
6-10 years	3 (9.7)
11-15 years	4 (12.9)
16-20 years	3 (9.7)
20+ years	3 (9.7)
Total	31 (100.0)

Table 4: Medical Students' thoughts on distance learning.

	Do not agree at all n (%)	Do not agree n (%)	Indecisive n (%)	Agree n (%)	Completely agree n (%)	P-value *
I regularly attend distance education lessons						0.354
Preclinical Students	4 (1.4)	10 (3.4)	26 (9.0)	85 (29.3)	40 (13.8)	
Clinical Students	2 (0.7)	10 (3.4)	14 (4.8)	57 (19.7)	42 (14.5)	
Distance education lessons are beneficial						**0.041**
Preclinical Students	26 (9.0)	40 (13.8)	54 (18.6)	29 (10.0)	16 (5.5)	
Clinical Students	13 (4.5)	38 (13.1)	32 (11.0)	36 (12.4)	6 (2.1)	
Distance education alone is sufficient in the field of medicine						0.905
Preclinical Students	107 (36.9)	30 (10.3)	13 (4.5)	6 (2.1)	9 (3.1)	
Clinical Students	79 (27.2)	23 (7.9)	11 (3.8)	7 (2.4)	5 (1.7)	
Distance education lessons are useful as face-to-face lessons						**0.023**
Preclinical Students	62 (21.4)	54 (18.6)	19 (6.6)	19 (6.6)	11 (3.8)	
Clinical Students	56 (19.3)	21 (7.2)	19 (6.6)	23 (7.9)	6 (2.1)	
It is convenient to include interns in distance education						**0.017**
Preclinical Students	74 (25.5)	36 (12.4)	36 (12.4)	17 (5.9)	2 (0.7)	
Clinical Students	51 (17.6)	25 (8.6)	21 (7.2)	16 (5.5)	12 (4.1)	
The theoretical courses are suitable for distance education						0.133
Preclinical Students	18 (6.2)	31 (10.7)	39 (13.4)	48 (16.6)	29 (10.0)	
Clinical Students	6 (2.1)	17 (5.9)	27 (9.3)	49 (16.9)	26 (9.0)	
Practical lessons are suitable for distance education						0.362
Preclinical Students	111 (38.3)	33 (11.4)	13 (4.5)	4 (1.4)	4 (1.4)	
Clinical Students	73 (25.2)	31 (10.7)	17 (5.9)	2 (0.7)	2 (0.7)	
Apart from the pandemic, theoretical lessons can be given in the form of distance education						0.108
Preclinical Students	65 (22.4)	32 (11.0)	16 (5.5)	31 (10.7)	21 (7.2)	
Clinical Students	31 (10.7)	26 (9.0)	18 (6.2)	28 (9.7)	22 (7.6)	

**Statistically significant values are marked as bold.*

Table 5: The academicians' thoughts on distance learning.

	Do not agree at all n (%)	Do not agree n (%)	Indecisive n (%)	Agree n (%)	Completely agree n (%)	P-value *
Distance education lessons are beneficial						**0.005**
Preclinical academicians	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	1 (3.2)	9 (29.0)	8 (25.8)	6 (19.4)	0 (0.0)	
Surgical academicians	0 (0.0)	1 (3.2)	0 (0.0)	4 (12.9)	0 (0.0)	
Distance education alone is sufficient in the field of medicine						**0.007**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	14 (45.2)	8 (25.8)	2 (6.5)	0 (0.0)	0 (0.0)	
Surgical academicians	3 (9.7)	1 (3.2)	0 (0.0)	1 (3.2)	0 (0.0)	
It is convenient to include interns in distance education						**0.002**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	15 (48.4)	5 (16.1)	3 (9.7)	1 (3.2)	0 (0.0)	
Surgical academicians	0 (0.0)	4 (12.9)	1 (3.2)	0 (0.0)	0 (0.0)	
Distance education lessons are as useful as face-to-face lessons						**0.007**
Preclinical academicians	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	10 (32.3)	4 (12.9)	6 (19.4)	4 (12.9)	0 (0.0)	
Surgical academicians	0 (0.0)	1 (3.2)	3 (9.7)	1 (3.2)	0 (0.0)	
I would like distance education to be available also outside of working hours						0.180
Preclinical academicians	0 (0.0)	1 (3.2)	0 (0.0)	1 (3.2)	0 (0.0)	
Medical academicians	6 (19.4)	5 (16.1)	6 (19.4)	7 (22.6)	0 (0.0)	
Surgical academicians	4 (12.9)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	
The theoretical courses is suitable for distance education						0.382
Preclinical academicians	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	1 (3.2)	
Medical academicians	0 (0.0)	5 (16.1)	8 (25.8)	9 (29.0)	2 (6.5)	
Surgical academicians	0 (0.0)	1 (3.2)	3 (9.7)	1 (3.2)	0 (0.0)	
Practical lessons are suitable for distance education						**0.026**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	15 (48.4)	6 (19.4)	2 (6.5)	1 (3.2)	0 (0.0)	
Surgical academicians	3 (9.7)	1 (3.2)	0 (0.0)	1 (3.2)	0 (0.0)	
Apart from the pandemic, theoretical lessons can be given in the form of distance education						0.065
Preclinical academicians	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	1 (3.2)	
Medical academicians	11 (35.5)	3 (9.7)	4 (12.9)	5 (16.1)	1 (3.2)	
Surgical academicians	0 (0.0)	1 (3.2)	3 (9.7)	1 (3.2)	0 (0.0)	

**Statistically significant values are marked as bold.*

Table 6: Thoughts of clinical vs. pre-clinical students and academicians on distance education sufficiency.

	Medical Students			Academicians			
	Preclinical Students n= 165 (100%)	**Clinical Students n= 125 (100%)**	**P-value***	**Preclinical n=2 (100%)**	**Medical n=24 (100%)**	**Surgical n=5 (100%)**	**P-value**
The competence of distance education for basic sciences			**<0.001**				0.199
Alone enough	22 (13.3)	27 (21.6)		1 (50.0)	3 (12.5)	1 (20.0)	
Inadequate alone, but most of them should be distance education	63 (38.2)	71 (56.8)		0 (0.0)	12 (50.0)	0 (0.0)	
Inadequate alone, and most of them should be in-class education	58 (35.2)	26 (20.8)		0 (0.0)	7 (29.2)	4 (80.0)	
Completely insufficient alone, all them should be in-class education	22 (13.3)	1 (0.8)		1 (50.0)	2 (8.3)	0 (0.0)	
The competence of distance education for clinical sciences			**0.011**				0.078
Alone enough	2 (1.2)	4 (3.2)		1 (50.0)	0 (0.0)	1 (20.0)	
Inadequate alone, but most of them should be distance education	13 (7.9)	22 (17.6)		0 (0.0)	3 (12.5)	0 (0.0)	
Inadequate alone, and most of them should be in-class education	66 (40.0)	55 (44.0)		0 (0.0)	14 (58.3)	3 (60.0)	
Completely insufficient alone, all of them should be in-class education	84 (50.9)	44 (35.2)		1 (50.0)	7 (29.2)	1 (20.0)	

**Statistically significant values are marked as bold.*

Table 7: The students' user experience of distance learning applications.

	Do not agree at all n (%)	Do not agree n (%)	Indecisive n (%)	Agree n (%)	Completely agree n (%)	P-value *
Accessing the course is easy in the distance education system						**0.046**
Preclinical Students	12 (4.1)	27 (9.3)	32 (11.0)	71 (24.5)	23 (7.9)	
Clinical Students	2 (0.7)	12 (4.1)	23 (7.9)	70 (24.1)	18 (6.2)	
I would like distance education to be available also outside of working hours						0.540
Preclinical Students	42 (14.5)	43 (14.8)	33 (11.4)	32 (11.0)	15 (5.2)	
Clinical Students	32 (11.0)	29 (10.0)	32 (11.0)	26 (9.0)	6 (2.1)	
Accessing and watching the recordings of the live lectures I did not attend are easy						**0.006**
Preclinical Students	11 (3.8)	16 (5.5)	50 (17.2)	58 (20.0)	30 (10.3)	
Clinical Students	3 (1.0)	16 (5.5)	19 (6.6)	64 (22.1)	23 (7.9)	
The educational materials used in the lessons meet my needs						0.062
Preclinical Students	22 (7.6)	40 (13.8)	51 (17.6)	39 (13.4)	13 (4.5)	
Clinical Students	5 (1.7)	27 (9.3)	51 (17.6)	31 (10.7)	11 (3.8)	
I can quickly access the course contents						**0.027**
Preclinical Students	8 (2.8)	23 (7.9)	35 (12.1)	79 (27.2)	20 (6.9)	
Clinical Students	2 (0.7)	12 (4.1)	14 (4.8)	75 (25.9)	22 (7.6)	
I know how to solve the problems I encounter in the distance education system						0.135
Preclinical Students	19 (6.6)	39 (13.4)	54 (18.6)	41 (14.1)	12 (4.1)	
Clinical Students	7 (2.4)	21 (7.2)	52 (17.9)	38 (13.1)	7 (2.4)	
I know how to use virtual classroom application						0.151
Preclinical Students	24 (8.3)	35 (12.1)	37 (12.8)	55 (19.0)	14 (4.8)	
Clinical Students	11 (3.8)	23 (7.9)	22 (7.6)	50 (17.2)	19 (6.6)	
I occasionally encounter technical problems in virtual classroom application						**0.017**
Preclinical Students	10 (3.4)	17 (5.9)	69 (23.8)	53 (18.3)	16 (5.5)	
Clinical Students	13 (4.5)	19 (6.6)	37 (12.8)	52 (17.9)	4 (1.4)	
The distance education system seems very complicated to me						**0.021**
Preclinical Students	31 (10.7)	60 (20.7)	29 (10.0)	27 (9.3)	18 (6.2)	
Clinical Students	36 (12.4)	55 (19.0)	17 (5.9)	12 (4.1)	5 (1.7)	
Finding the information I want in the distance education system is easy						0.532
Preclinical Students	17 (5.9)	33 (11.4)	53 (18.3)	49 (16.9)	13 (4.5)	
Clinical Students	6 (2.1)	26 (9.0)	40 (13.8)	42 (14.5)	11 (3.8)	
The information (announcement etc.) is sufficient						0.298
Preclinical Students	31 (10.7)	38 (13.1)	28 (9.7)	50 (17.2)	18 (6.2)	
Clinical Students	22 (7.6)	35 (12.1)	27 (9.3)	35 (12.1)	6 (2.1)	

**Statistically significant values are marked as bold.*

Table 8: The academicians' user experience of distance learning applications.

	Do not agree at all n (%)	Do not agree n (%)	Indecisive n (%)	Agree n (%)	Completely agree n (%)	P-value *
I know how to solve the problems I encounter in the distance education system						**0.000206**
Preclinical academicians	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)	1 (3.2)	
Medical academicians	0 (0.0)	7 (22.6)	11 (35.5)	6 (19.4)	0 (0.0)	
Surgical academicians	0 (0.0)	0 (0.0)	0 (0.0)	5 (16.1)	0 (0.0)	
I know how to use virtual classroom application without support						0.181
Preclinical academicians	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	1 (3.2)	
Medical academicians	0 (0.0)	4 (12.9)	10 (32.3)	9 (29.0)	1 (3.2)	
Surgical academicians	0 (0.0)	0 (0.0)	3 (9.7)	2 (6.5)	0 (0.0)	
I occasionally encounter technical problems in virtual classroom application						0.681
Preclinical academicians	0 (0.0)	0 (0.0)	1 (3.2)	1 (3.2)	0 (0.0)	
Medical academicians	0 (0.0)	2 (6.5)	8 (25.8)	12 (38.7)	2 (6.5)	
Surgical academicians	0 (0.0)	1 (3.2)	0 (0.0)	4 (12.9)	0 (0.0)	
The distance education system seems very complicated to me						0.186
Preclinical academicians	1 (3.2)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	
Medical academicians	4 (12.9)	9 (29.0)	7 (22.6)	4 (12.9)	0 (0.0)	
Surgical academicians	0 (0.0)	5 (16.1)	0 (0.0)	0 (0.0)	0 (0.0)	
The distance education lessons are interactive enough						**0.007**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	6 (19.4)	10 (32.3)	6 (19.4)	2 (6.5)	0 (0.0)	
Surgical academicians	0 (0.0)	4 (12.9)	0 (0.0)	1 (3.2)	0 (0.0)	

**Statistically significant values are marked as bold.*

Table 9: Clinical vs. pre-clinical students and academicians on online examinations.

	Do not agree at all n (%)	Do not agree n (%)	Indecisive n (%)	Agree n (%)	Completely agree n (%)	P-value *
Medical Students						
Online exams measure learning						0.983
Preclinical Students	60 (20.7)	25 (8.6)	30 (10.3)	34 (11.7)	16 (5.5)	
Clinical Students	45 (15.5)	22 (7.6)	23 (7.9)	24 (8.3)	11 (3.8)	
Online exams are reliable						0.108
Preclinical Students	60 (20.7)	42 (14.5)	19 (6.6)	27 (9.3)	17 (5.9)	
Clinical Students	38 (13.1)	25 (8.6)	29 (10.0)	21 (7.2)	12 (4.1)	
Academicians						
Online exams measure learning						**0.000239**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	7 (22.6)	8 (25.8)	8 (25.8)	1 (3.2)	0 (0.0)	
Surgical academicians	0 (0.0)	2 (6.5)	0 (0.0)	3 (9.7)	0 (0.0)	
Online exams are reliable						**0.003**
Preclinical academicians	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	8 (25.8)	11 (35.5)	4 (12.9)	1 (3.2)	0 (0.0)	
Surgical academicians	0 (0.0)	1 (3.2)	3 (9.7)	1 (3.2)	0 (0.0)	
I think the students cheat in the online exams						**0.027**
Preclinical academicians	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (3.2)	
Medical academicians	0 (0.0)	2 (6.5)	3 (9.7)	16 (51.6)	3 (9.7)	
Surgical academicians	0 (0.0)	1 (3.2)	3 (9.7)	0 (0.0)	1 (3.2)	

**Statistically significant values are marked as bold.*

DISCUSSION

Since the COVID-19 outbreak, this pandemic has been a turning point for medical schools and medical students at many points (4). Universities have been closed all over the world. Many universities have turned face-to-face education methods into online education or a combination of online and traditional education (7). Online education suddenly became an academic principle. Experts predicted that it would take 5-10 years to recover from this pandemic (8). Therefore, it is important to identify the thoughts and opinions of medical students and academicians about the online education and examination system that is rapidly passed through the COVID-19 pandemic process. In this study, 290 medical students and 31 academicians from different medical schools in Turkey filled out an online survey, and results were evaluated descriptively.

Most of the students stated that they followed the online education lessons with computers. Computers played a major role in social distancing and rapid innovation, especially during the pandemic period (9). Many respondents (49% agree, 28.3% strongly agree) attended their online lessons. About 40% (26.9% do not agree, 13.4% do not agree at all) said online courses are useless, while 29.7% of students are indecisive about whether online courses are useful. Only about 9.3% of them (4.5% agree, 4.8% completely agree) found it sufficient, but 64.1% of the students stated that they did not find online education alone sufficient in the field of medicine. The fact that practical lessons occupy a large place and are very important in medical education may have been effective in this decision. For example, laboratory lessons are difficult or impossible to adapt to the virtual environment. However, according to Rajab et al. (8), 67% of Saudi Arabian medical students stated that the pandemic had a positive effect on online learning. Most of the students (40.7% do not agree at all, 25.9% do not agree) stated that online education classes are not as useful as face-to-face lessons. However, in the academic community, hybrid education has started to gain more acceptance than face-to-face education because it combines the best features of the two types of education (8).

Most of students can easily access the courses and course materials within the scope of distance education: 48.5% of the students stated that they can easily access the course they want in the distance education system, while 52.9% stated that they can access the course contents quickly. However, the majority of students with a rate of 35.1% declared that they were indecisive that the educational materials used in the courses were enough for their needs. This can be explained by the fact that each school follows different individual paths for distance education and the course materials may vary between schools and classes. According to the study done by Srinivasan (10), different platforms such as Zoom, PollEV can be used for e-learning. Besides in the study of Chick et al. (5), it was found that social media platforms like Facebook groups and video archives of the medical associations such as the American College of Surgeons can be used as educational materials for distance education as well.

Students could easily access and watch the recordings of the live classes that they did not attend on time, 60.1% of the students (18.2% strongly agree, 41.9% agree) stated. Despite this, almost half of the students (25.8% strongly disagree and 24.7% disagree) have a negative opinion on that they would like distance education to be available also outside of working hours. This quandary, that the students watch the recordings of lessons at their own time while not wanting distance education to be done outside of working hours, shows that the only function of face-to-face education is not only to attend live classes but at the same time, it provides a suitable environment for learning. Students who remained in isolation at home had difficulties in setting effective boundaries between home and school during COVID-19 (11).

Furthermore, 85.6% of students think that the practical lessons are not suitable for distance education, while 63.6% of which were strong on their opinion. Besides, 44.3% of the students believe that distance education is completely inadequate by itself, all the training should be face-to-face. Considering that most of the clinical sciences consist of practical courses, students especially attach importance to experience practical skills in clinics under the supervision of clinicians. Parallel to that, 64.3% of the students (43.3% strongly disagree, and 21% disagree) declared that "they find it incorrect to include interns in distance education" concerning the 6th-grade internship education, almost all of which are based on practical applications. In addition, 52.2% of the students expressed that the theoretical courses are suitable for distance education while 18.9% of which strongly agreed. However, 46% of the students believe that distance education is insufficient alone for the basic medical sciences, but most of the education in this field should be distant. Even though, the students answered "strongly disagree" with 33.3% and "disagree" with 19.9% to the question "I think that theoretical courses can be conducted in the form of

distance education even outside the pandemic process". This can be interpreted by considering that theoretical courses are taught with more conventional methods than practical courses and as a matter of fact that basic medical sciences are mostly composed of theoretical courses, basic medical sciences are more convenient to distance education. If the materials used in distance education can be made more diverse especially for practical courses and hybrid education models are used more widely, distance education may have positive results for students with different learning speeds and styles (12).

Applied courses are not required by students to be conducted online. Considering that the most important part of medical education is practicing on the patient, it is not surprising that this opinion is the majority. The research of Ruiz et al. (13) also supports this idea.

The biggest problem faced by the participants during their participation in online classes is seen as the inability to provide a suitable course environment. This affects the success of the students and also decreases their motivation to be interactive (14). Approximately 10% of the participants stated that they do not know how to use virtual applications and cannot solve if a problem occurs in the system. Compared to the literature, this outcome is not out of normal range (15).

The vast majority of students do not have difficulties with virtual classroom applications, Buckley et al. (16) stated that this is not a surprising outcome due to high access to the technology. Easy access to the information sought in distance education systems was avetaged by the students. It has been stated in many researches that the infrastructure and site designs are very effective at this point (14).

Although the majority of the students think that they are sufficient in informing, the differences in access to technology also affect the informational effectiveness (16). Although the majority of the students think that they are sufficient in informing, the differences in access to technology also affect the informational effectiveness (15).

Almost 1/3 of the participants thought that the exam was insufficient in measuring their knowledge level, but there was no difference in the scale of the exams taken in the classroom or online in the studies because nowadays most medical schools prefer test system in both cases (17).

When asked about the reliability of the exam, 33% of the participants stated that they did not find the exam reliable, but Buckley et al. (16) did not find a significant difference in reliability between online and paper exams in their study. However, the differences in exam systems and departments require a lot of new and large-scale studies to be done in this area.

A 2014 study, long before the pandemic, looked into the use of technology in education (18). One of the biggest technostress risk factors they found was age. They claimed the elder academicians experienced more stress using the online applications required for distance learning. Meanwhile the younger academicians supported the application of distance learning. The mean age of the participants in our study was 41.68 years ± 7.51, which is classified as young in the 2014 study (18). This could explain the relative ease in using the distance education applications in the academician group. However, contrary to that study, we found a majority of the academicians were not leaning toward distance education despite the ease of use. In addition, there was no difference between academic experience groups in terms of preferring distance education applications; disproving the idea of more experienced academicians being more traditional.

The majority of the academicians [n=23 (74.2%)] did not suffer from any chronical illnesses. In any case there was no significant difference between those who suffered from chronical illnesses and those who did not despite a greatly increased risk in terms of COVID-19 infection and prognosis (19, 20).

There is a split decision when it comes to distance lessons being beneficial. Eleven agreed, 12 disagreed, and 8 were indecisive. Academicians in the surgical field thought it was beneficial compared to other branches. The study conducted in Alfaisal University revealed that the 78.9% of the academicians thought the distance education was beneficial for the students (8). Interestingly this ratio was even higher than the students. Only 66.9% of the students thought distance education was beneficial (8). In our study 30% of the students thought online education was beneficial. The reasons behind the students preferring the more traditional face-to-face lessons should be evaluated. However, when the academicians were asked whether they find distance education alone sufficient in the field of medicine the decision was more in unity. Twenty-seven disagreed among which 18 strongly disagreed. The results show that the idea of medical education being a "master-apprentice relationship" is well accepted among the academicians.

The academicians did not want the interns to be a part of the distance education program. Some countries even proposed and practiced graduating the interns early to distribute the workload (21-23). We did not question whether the academicians would prefer the interns to be a part of the workforce in the medical field with early graduations. However, they would pre-

fer the interns to be a part of the face-to-face education where they work as practitioners under the supervision of the academicians. Academicians may have answered this question thinking that interns should work in hospitals to acquire the necessary practical skills in their professional life, since they are in the last year of their education. When compared with face to face lessons, 51.6% of the academicians said the distance education lessons were not as good. This is similar to the results of Alfaisal University study where only 12% of the participants thought online lessons alone could replace face-to-face lessons (8). The clinical branches disagree more compared to the preclinical branches. Considering the theoretical disposition of the preclinical branches, this result is expected.

The majority of the academicians disagreed when it came to distance education outside of working hours and both clinical and preclinical branches agreed on the topic. The pandemic caused healthcare workers to take extra shifts and work longer hours (24). Even the preclinical branches were called for duty in Turkey to help alleviate the heavy workload. Considering the already long working hours, it is understandable that the academicians do not want to extend distance education outside of work.

Academicians were more inclined towards distance education when the theoretical lessons were considered. Only 6 (19.4%) disagreed with the statement; "The theoretical courses are suitable for distance education" and none of the academicians completely disagreed. However, when it came to practical lessons 26 disagreed with 19 of them completely disagreeing. Yardley et al. (25) claims the transition from a freshman medical student to a qualified doctor was based more on experiential learning.

The majority of academicians thought distance education in the field of medicine was not enough by itself. While we compare the clinical and theoretical education, academicians predominately support face-to-face learning methodology for clinical education. This may be associated with reduced learning of clinical skills that model future physicians. This also explains why academicians find it inconvenient to include interns in this education model. However, some studies claim that effective distance clinical education is possible in favorable conditions. Marshall et al. (26) reported that including clinical students in tele-interactions with patients, using artificial intelligence, and receiving consultancy from trained clinicians can develop a positive distance learning environment. Usage of webinars, online atlases, simulations, and 3D models can be complementary to distance clinical and theoretical education to create a learner-centric manner (27).

On continuing theoretical lessons online after the pandemic, academicians mostly opposed the idea like the students. However, in our study, one of five academicians were hesitant who may adapt to change later by blended learning system as a complement to increase efficiency and effectiveness in their in-class lessons (13). For instance, a study conducted at the University of Dubai recommends continuing to offer a blended learning system after the pandemic (28). In another study, both students and faculty members agreed that online education is an effective way of learning, and teaching that includes resource, and time efficiency (29).

Most of the academicians are indecisive about whether they are capable of solving the problems they encountered or not. Following that option, a similar ratio was seen in the group that knew how to solve those problems. The results were similar between indecisive and agreed groups on knowing how to use the virtual classroom application without support. Despite this implication, more than 60% of the academicians encounter technical problems in virtual classroom applications. The problems academicians faced were slightly the same as the students, but in ratio, they had lesser difficulties. Assuming that effective internet connectivity is available to every student was wrong (30). The same case can be adapted to the academicians because relying on the idea that each of them has their uninterrupted connection is not accurate (31). They can experience systemic issues or computer related problems during lectures even though they use the university's facility.

More than half of the academicians predicated that virtual lessons were not interactive enough. The impact of asynchronous content delivery may result in a lack of communication (13). Instead, using real-time learning and instant transmission of questions can help to overcome this (13). Nevertheless, the virtual lessons and online examinations are part of the current learning system whether they are qualified or not to prevent more positive cases. However, when it comes to their measuring standards and reliability, online examinations may create doubt in academicians. Only around 16% of the academicians think online exams are measuring students' knowledge accurately. Contrary, Longhurst et al. (32) claim that preventing cheating, and improving examination conditions are possible to design traditional-like online examinations to measure knowledge accurately. On the question of reliability, not reliable (38.7%) followed by completely not reliable (29%) were the most selected options. Besides, 67.7%

of them think that students were cheating in the online exams. Schmidt et al. (33) had the same concern in their case study so they created unique exams for each student, but emphasized that even if the exam was not random, universities have their honor systems on not cheating.

Twenty-four medical, five surgical specialty academicians participated representing the clinical years. However, there were only two preclinical academicians who participated in the survey which is thought to be the greatest limitation of this study.

In conclusion, it is predicted that the pandemic process will continue in the next academic years. Considering the current situation, distance education seems to be the best option to ensure that the learning process can continue while protecting the health of students and academicians. Although distance education is not sufficient by itself in medical education during and after the COVID-19 pandemic, it is a method that should be used in almost every field of medical education, especially in the preclinical phase. Both the distance education, and online examination process require improvements and they are needed to be supported with face to face lectures and practices.

Ethics Committee Approval: This study was approved by the Scientific Research Ethical Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2020/343).
Informed Consent: Online informed consent was obtained from the participants of this study.
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: BK, HSÇ, İİÖ, SS, AG, SGS, SA. Design: İİÖ, BK, HSÇ, SS, AG, SGS, SA. Supervision: SA, SGS, BK, HSÇ, İİÖ, SS, AG. Resources: HSÇ, İİÖ, BK, SS, AG, SGS, SA. Materials: İİÖ, BK, HSÇ, SS, AG, SGS, SA. Data collection and/or processing: BK, HSÇ, İİÖ, SS, AG, SGS, SA. Analysis and/or Interpretation: İİÖ, HSÇ, BK, SS, AG, SGS, SA. Literature Search: İİÖ, HSÇ, BK, SS, AG, SGS, SA. Writing Manuscript: İİÖ, HSÇ, BK, SS, AG, SGS, SA. Critical Review: SA, SGS, BK, HSÇ, İİÖ, SS, AG.
Financial disclosure: The authors declared that this study received no financial support.
Editor in chief's Note: Five of the authors of this article, Hilal Sena Çifcibaşı, Berkay Kef, Irmak İrem Özyiğit, Sezin Sayın, and Aslı Göztepe are members of the editorial board of Turkish Medical Student Journal. However, they did not take place in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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ORIGINAL ARTICLE

THE EFFECT OF 5-FU AND RUXOLITINIB ON MITOCHONDRIAL APOPTOSIS IN GLIOBLASTOMA U87 CELL LINE

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ABSTRACT

Aims: The aim of this study is to carry out the effect of 5-Fluorouracil alone or combined with Ruxolitinib on both apoptosis and JAK/STAT pathway in U87 glioblastoma cells. ***Methods:*** We used U87 glioblastoma cell lines as the human brain cancer cells. We treated the cells with 5-Fluorouracil (3.125 μM-400 μM) alone and with a combination of Ruxolitinib (100 μM or 400 μM of Ruxolitinib with 3.125-25 μM 5-Fluorouracil), and performed the MTT test for calculating IC50 value. Molecular fluorescence staining was performed with Hoechst and acridine orange/ethidium bromide probes. The alteration in mitochondrial apoptosis and JAK/STAT pathways to drug treatment was analyzed by the qRT-PCR assay. ***Results:*** Decrease in cell viability was more prominent in U87 cells treated with a combination of 5-Fluorouracil and Ruxolitinib compared to those treated with 5-Fluorouracil alone. In gene expression analysis, apoptosis signals were observed in cells treated with 5-Fluorouracil alone and 5-Fluorouracil+Ruxolitinib treatment. ***Conclusion:*** Treatment with 5-Fluorouracil alone and 5-Fluorouracil+Ruxolitinib combination increased apoptosis in U87 glioblastoma cells. However, it is difficult to mention an evident difference between treatments. Therefore, further studies are needed. ***Keywords:*** 5-Fluorouracil, Ruxolitinib, glioblastoma, apoptosis, JAK/STAT pathway

INTRODUCTION

Glioblastoma multiforme (GBM), one of the aggressive, angiogenic and invasive brain tumors with poor prognosis (5-year survival rate is 4-5%), is the most common malignant primary brain tumor (1, 2). About 5% of patients with glioma are associated with hereditary syndromes, while other patients represent sporadic cases. Accumulation of genetic damage and abnormal growth factor signaling pathways leading to malignant transformation play an important role in the pathogenesis of glioma (3). In 2016, GBM was classified by World Health Organization as Isocitrate Dehydrogenase (IDH)-wild type, IDH-mutant, and otherwise specified. The type of epithelioid glioblastoma defined in 2016 is mostly found in IDH-wild type glioblastoma, giant cell glioblastoma, and gliosarcoma (2).

5-Fluorouracil (5-FU) has been in use since 1957. It has been used as an intravenous drug for various types of cancer including basal cell carcinoma, colorectal cancer, breast cancer, pancreatic cancer, gastric adenocarcinoma, head, and neck squamous cell carcinoma. Additionally, it is given locally for the treatment of corneal squamous cell carcinoma and actinic keratosis. 5-FU can be used at the operation site to prevent conjunctival scarring, and intraoperatively or postoperatively in glaucoma surgery (4). Uracil is required for nucleic acid synthesis, which is essential for tumor growth. While 5-FU is an analog of naturally occurring pyrimidine uracil and is metabolized by the same metabolic pathways as uracil, un-metabolized 5-FU cannot bind to the DNA structure, which is the main target site in the mammalian host and tumor cells. However, the cytotoxic effect of 5-FU is only against nucleotides after anabolism in actively proliferating cells (5). In the S phase of the cell cycle, it forms a complex with the enzyme thymidylate synthetase, blocks DNA synthesis and stops the cell cycle. It can also inhibit RNA synthesis by forming misconfigured ribonucleic acids (6).

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Received: 30.05.2020 Accepted: 15.08.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.02 Available at: tmsj.trakya.edu.tr

Cite this article as: Aksu G, Doğanlar O, Doğanlar ZB. The effect of 5-FU and ruxolitinib on mitochondrial apoptosis in glioblastoma U87 cell line. Turkish Med Stud J 2020;7(3):130-9.

Ruxolitinib (Ruxo), which is also known as INC424 or INCB18424, is a potent and selective inhibitor of Janus Kinase 1 (JAK1) and Janus Kinase 2 (JAK2). JAK1 and JAK2 are non-receptor tyrosine kinases, which are important components of pathways leading to the production and secretion of inflammatory cytokines and hematological growth factors. Inhibition of JAK1 and JAK2 may cause antiproliferative and antiapoptotic effects. Ruxolitinib causes Janus Kinase mutations. Its main effect is inhibition of cell division by preventing JAK to phosphorylate the signal transducer and activator of transcription (STAT) to induce apoptosis. Ruxo is the first agent used to treat myelofibrosis, which is a disease characterized by progressive bone marrow fibrosis, inadequate hematopoiesis, cytopenia, anemia, and splenomegaly and affecting the older age group (>60 years) (7). It has also been shown that it decreases the symptoms of polycythemia vera disease, by reducing the size of the spleen and circulating cytokine levels (8). Additionally, Ruxo inhibits glioblastoma invasion and angiogenesis (9).

5-Fluorouracil blocks DNA synthesis and stops the cell cycle. It can also inhibit RNA synthesis by forming misconfigured ribonucleic acids. Ruxo causes Janus kinase mutations. Inhibition of JAK1 and JAK2 may cause antiproliferative and antiapoptotic effects. Therefore, we aimed to carry out the effect of 5-FU alone or combined with Ruxolitinib on both apoptosis and JAK/STAT pathways in U87 glioblastoma cells.

MATERIAL AND METHODS

CELLS AND REAGENTS

U87 human brain cancer cell line was purchased from American Type Culture Collection (ATCC). It was cultured in Dulbecco's Modified Eagle Medium: F-12 (DMEM: F-12, ATCC 30-2006TM) supplemented with heat-inactivated 10% fetal bovine serum (Gibco Life Technologies, USA), 2 mM glutamine (Gibco-Life Technologies, USA), and 1% penicillin/streptomycin (Invitrogen, Life Technologies, USA). The U87 cell culture was maintained at 37 °C and 5% CO2 in the cell culture laboratory under sterile conditions.

5-Fluorouracil sodium hydroxide was obtained from the chemotherapy unit (Fluorouracil-GRYVR: 1000 mg/20 ml, Teva GmbH, Germany) and Ruxolitinib Phosphate was purchased from Santa Cruz Biotechnology (Chem Cruz, Sc-396768A, USA). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), ultrapure DNase/RNase-free water, NucBlue™ Live ReadyProbes™ Reagent, the PureLink® RNA Mini Kit, a High Capacity complementary DNA (cDNA) Reverse Transcription Kit, and SYBR® Select Master Mix were purchased from Thermo Fisher Scientific.

CELL PROLIFERATION ASSAY (MTT)

Cell viability and proliferation were determined by MTT assay. U87 cells were seeded in a 96-well plate and incubated for 12h in DMEM: F12 medium to ensure cell attachment. The cells were treated with 5-Fu alone and combined with Ruxo. For 5-Fu alone treatments, cells were treated with 400 µM to 3.125 µM with the 10-fold dilutions. For combined treatments, 100 µM or 400 µM of Ruxo and 25-3.125 µM of 5-FU were used. Treated cells were incubated for 24 h. At the end of this period, 20 µL MTT solution (5 mg/mL) was added to each well and the cells were incubated for 4 h at 37 °C. Then 180 µL dimethyl sulfoxide (DMSO) was added to the cells and the cells were analyzed at 492 nm with a microplate reader (Multiscan Go, Thermo Scientific, USA).

MOLECULAR FLUORESCENT STAINING

Cells were seeded into six-well plates with a density of 5×104 cells/well. After attachment of the cells, 5-FU and 5-FU+Ruxo treatments were performed. After the incubation period, cells were labeled with four drops of NucBlue® Live ReadyProbes® Reagent (40.6 µM Hoechst 33342, Thermo Scientific, USA), and ethidium bromide and acridine orange solution (10 µg/mL AO and 100 µg/mL EB) for 30 min at 37 °C. AO stains both live and dead cells, EB stains only dead cells that have lost their membrane integrity. NucBlue® Live Ready Probes were used for staining the nuclei. Then, cells were rinsed with phosphate-buffered saline, and a fresh medium was added. Images were acquired at ×40 magnification on a Zeiss Axio Vert. A1 fluorescence microscope with a DAPI filter for NucBlue®, and FITC/Texas Red filter for EB-AO.

ISOLATION OF TOTAL RNA, cDNA SYNTHESIS, AND QUANTITATIVE REAL-TIME PCR (qRT-PCR) ANALYSIS

Total RNA was isolated from three 25-cm²-tissue culture plates of each experimental group using the PureLink RNA Mini Kit (Life Technologies) according to the manufacturer's instructions. The extracted RNA concentrations were measured by the OPTIZEN NanoQ micro-volume photometer. The synthesis of the first strand of complementary DNA (cDNA) was performed

using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies). cDNA synthesis was performed using the thermal cycler Applied Biosystems ProFlex PCR System (step 1, 25 °C-10 min; step 2, 37 °C-120 min; step 3, 85 °C-5 min). The cDNA was stored at -20 °C for subsequent steps of the analysis. Expression levels of genes that involve in apoptosis pathway (tumor suppressor (P53), B-cell lymphoma 2 (BCL2), BCL2 associated X protein (BAX), cytochrome complex (Cyt-C), caspase3 and caspase 8), JAK1, JAK2, STAT3, Interleukin 6 (IL-6) and β-actin were analyzed by qRT-PCR using the SYBR Select Master Mix (Life Technologies) on Quant Studio 5 Real-Time PCR system. qRT-PCR conditions and Primer pairs are shown in Table 1. Relative fold changes were calculated by the 2(-Delta Delta C(T)) method and all data were normalized with control expression value that was set up as 1.

STATISTICAL ANALYSIS

The differences in the gene expressions were compared using the independent sample T-test and analysis of variance (ANOVA) with Tukey, HSD separation of means test using the IBM SPSS version 20 software at a significance level of $p \leq 0.05$.

Table 1: Primer sequences and qRT-PCR conditions.

Primer name			*Primer sequence*		*qRT-PCR conditions*
P53	F	5'	CACGAGCGCTGCTCAGATAGC	3'	
	R	5'	ACAGGCACAAACACGCACAAA	3'	
BCL2	F	5'	ATGTGTGTGTGGAGAGCGTCAA	3'	
	R	5'	ACAGTTCCACAAAGGCATCC	3'	
BAX	F	5'	TTCATCCAGGATCGAGCAGA	3'	
	R	5'	GCAAAGTAGAAGGCAACG	3'	
Cyt-C	F	5'	AGTGGCTAGAGTGGTCATTCATTTACA	3'	
	R	5'	TCATGATCTGAATTCTGGTGTATGAGA	3'	
CASP3	F	5'	GGTATTGAGACAGACAGTGG	3'	
	R	5'	CATGGGATCTGTTTCTTTGC	3'	
CASP8	F	5'	AGAGTCTGTGCCCAAATCAAC	3'	1 cycle of 2 min at 50 °C and 10 min at 95 °C followed by 42 cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min
	R	5'	GCTGCTTCTCTCTTTGCTGAA	3'	
JAK1	F	5'	ACAATTGGCATTCATTTTCCTG	3'	
	R	5'	CCTGGGCCCAAACTTCCTA	3'	
JAK2	F	5'	CAGTGGTCAAGAGGGAAACA	3'	
	R	5'	TGTCTGAGCGAACAGTTTCC	3'	
STAT3	F	5'	GGAGGAGTTGCAGCAAAAAG	3'	
	R	5'	TGTGTTTGTGCCCAGAATGT	3'	
IL-6	F	5'	ATGAACTCCTTCTCCACAAG	3'	
	R	5'	AGAGCCCTCAGGCTGGACTG	3'	
GAPDH	F	5'	TTGGTATCGTGGAAGGACTCA	3'	
	R	5'	TGTCATCATATTTGGCAGGTTT	3'	
β-ACTIN	F	5'	CCTCTGAACCCTAAGGCCAAC	3'	
	R	5'	TGCCACAGGATTCCATACCC	3'	

RESULTS

After incubation of U87 cells with different concentrations of 5-FU and 5-FU+Ruxo for 24 hours, cell viability was determined by MTT assay. As seen in Figure 1, the dose-dependent inhibition of cell viability was determined both 5-FU alone and 5-FU+Ruxo treatment. The viability of control cells was designated as 100%, and the others were expressed as percent compared to the control (Figure 1). The IC50 value was calculated as 118 μM for 5-FU alone and 50 μM 5-FU+100 nM Ruxo using Probit analysis.

On the other hand, the 5-FU and 5-FU+Ruxo treatment caused a 15.1 and 3.4-fold increase in BAX gene expression, the BAX/BCL2 ratio as indicative of apoptosis was determined as 9.68 and 1.46 at 5-FU and 5-FU+Ruxo, respectively. While P53 and caspase-3 gene expressions showed no significant changes at both 5-FU alone and combined with Ruxo, caspase 8 was significantly upregulated by 5-FU alone treatment (69.97-fold) (Figure 2).

For visualization of the apoptotic effect of 5-FU alone or in combination with Ruxo, we used both Hoechst 33342 and the EB-AO dual staining. As seen in Figure 3, cell morphologies were different between control, 5-FU, and 5-FU+Ruxo treatments. Additionally, cells treated with 5-FU caused more apoptosis and showed higher blue fluorescence due to fragmented nucleus and apoptotic body formation in Hoechst 33342 staining. Acridine Orange dye penetrates normal cells and early apoptotic cells (intact membrane integrity) and emits green fluorescence when bound to DNA. U87 cells treated with 5-FU and 5-FU+Ruxo have been observed to emit less green fluorescence when stained with AO and undergo more apoptosis compared to control. It was observed that viability is less especially in 5-FU+Ruxo treatment. EB enters cells with membrane damage, such as late apoptotic and dead cells, and emits orange-red fluorescence when bound to dense DNA fragments and apoptotic bodies. When U87 cells were treated with 5-FU and 5-FU+Ruxo, they were stained with EB, more intense apoptotic bodies were observed compared to the control group (Figure 3).

In this study, we investigated the effect of 5-Fu and 5-FU+Ruxo treatment on IL 6 /JAK1-2/ STAT3 axis of JAK/STAT pathway using gene expression levels in control and experimental groups of U87 cells. Our results showed that there was no significant change in IL6 and STAT3 gene expression levels in the control and administration groups. While JAK1 (16.2-fold) and JAK2 (3.3- fold) gene expressions were upregulated in 5-FU administration, the inclusion of the specific JAK inhibitor Ruxo in the treatment decreased the gene expression levels of both genes. However, these increases and decreases were statistically significant only for JAK2 (Figure 4).

DISCUSSION

In previous studies, 5-FU has been shown to be curative in the treatment of non-invasive basal cell carcinomas and has a positive activity in the treatment of gastrointestinal, ovarian, and breast cancers (5). Delen et al. (9) reported that Ruxo, used against myeloproliferative neoplasms in the clinic, has been shown to significantly inhibit tumor invasion in GBM cells. However, the effect of the combination of 5-FU and Ruxo on GBM cells has not been studied. In this study, we examined the apoptotic effects of 5-FU and 5-FU+Ruxo combination on U87 cells. According to the study, 5-FU and the combination of 5-FU+Ruxo decreased the proliferation of U87 cells. Bcl-2 gene family consists of antiapoptotic and proapoptotic members. While Bcl-2 suppressing apoptosis, another member of the family, BAX, speeds up cell death. BAX/Bcl-2 ratio plays an important role in cell apoptosis. In addition to the members of the Bcl-2 family, the p53 protein regulates cellular functions such as DNA synthesis, DNA repair, cell cycle arrest, and apoptosis (10). A significant increase in p53 expression was observed in colorectal adenocarcinoma cells treated with 5-FU in a study performed by Erdoğan et al. (11). Furthermore, it was observed that Bcl-2 expression decreased significantly in these cells and there was a significant increase in Bax gene expression (11). In our study, change in p53 expression was found statistically insignificant in U87 cells treated with 5-FU, and a combination of 5-FU+Ruxo. Bax expressions increased when 5-FU alone was used, while Bcl-2 expression increased in 5-FU+Ruxo combination therapy. On the other hand, the increased rate of BAX/Bcl-2 expression in U87 cells treated with 5-FU+Ruxo combination is lower than 5-FU alone, meaning that the apoptotic effect of only 5-FU is more evident.

When moderately and poorly differentiated prostate cancer samples were compared with healthy tissues, a decreased caspase 3 expression was observed (12). Caspase-8 expression loss was observed in high-grade small cell lung cancer, neuroendocrine lung cancer, and pediatric neuroblastoma (13, 14). In our study, a statistically significant increase was observed in caspase-8 expression in U87 cells treated with only 5-FU.

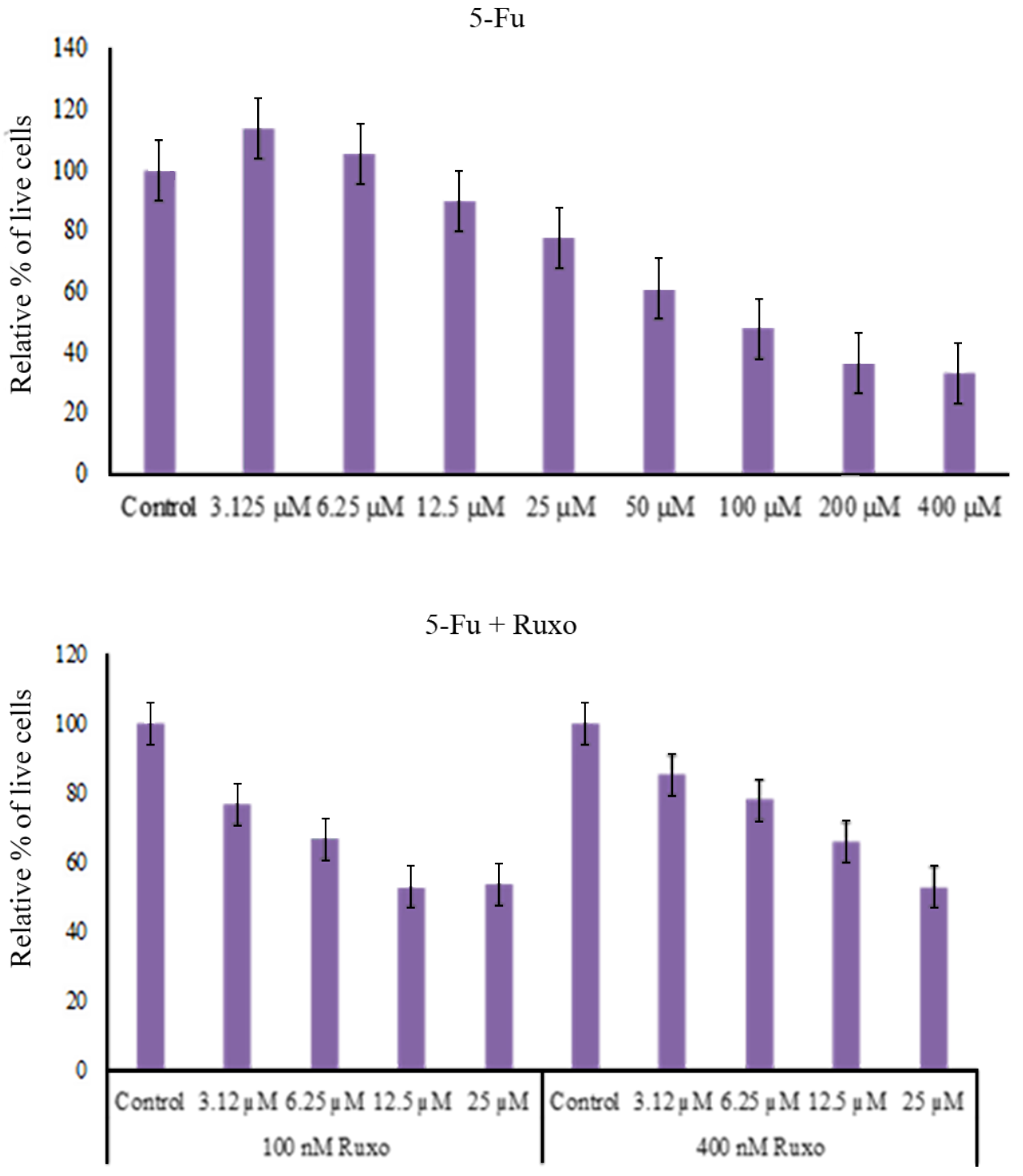

Figure 1: The effect of 5-FU and 5-FU+Ruxo treatment for 24 hours on cell viability in U87. (n=6. Data % viability mean ± standard error. 5-Fu:5-Fluorouracil Ruxo: Ruxolitinib.)

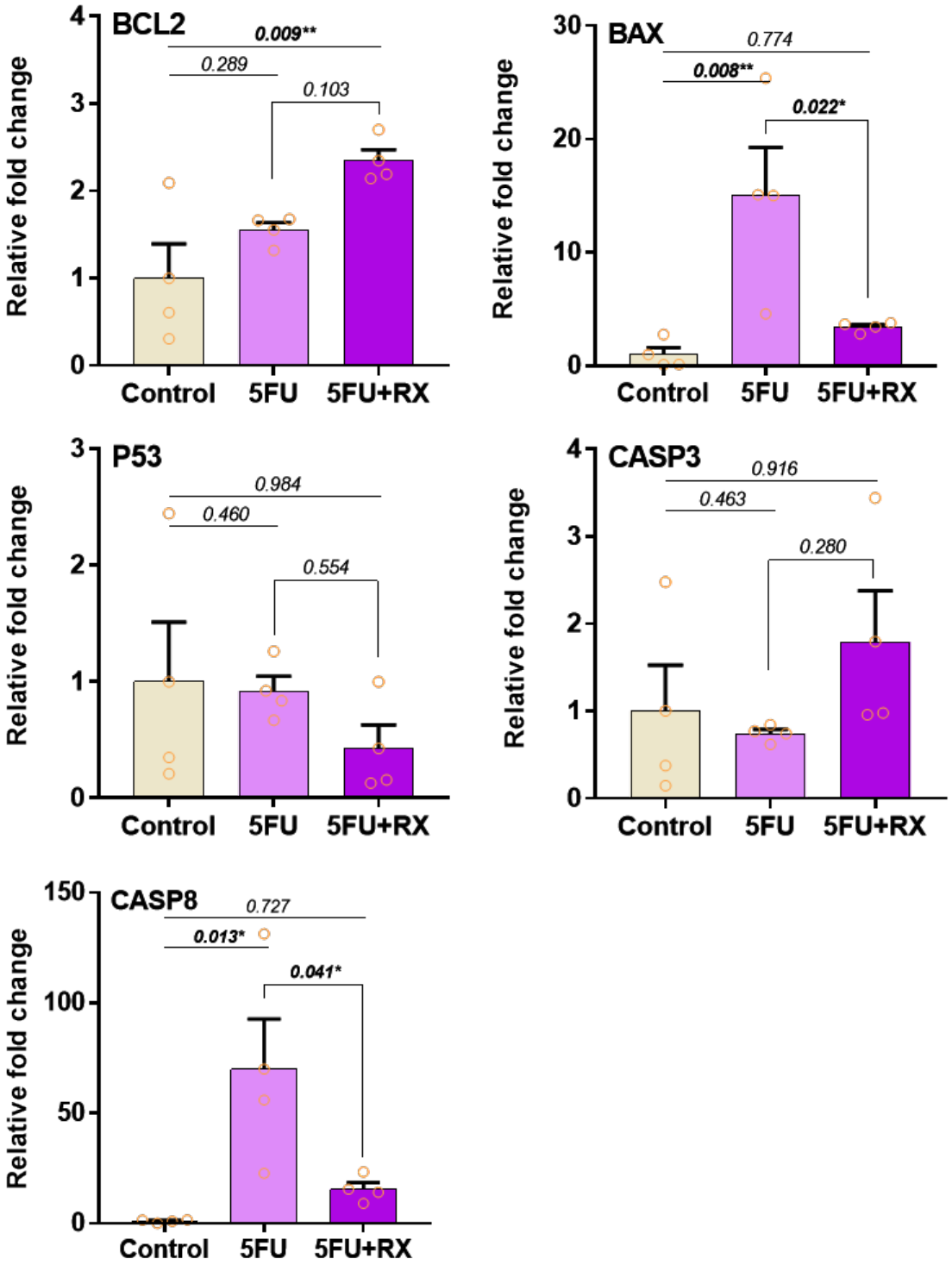

*Figure 2: Relative fold change qRT-PCR analysis of apoptosis pathway genes in IC50 doses of 5-FU and 5-FU+Ruxo exposed U87 cell line. (All data were normalized with β-actin expression and given as relative to control (control=1). Values expressed as mean±standard error (n=4). *Indicates significantly different values, one-way ANOVA, Tukey HSD test : $p \leq 0.05$, ** $p \leq 0.01$.)*

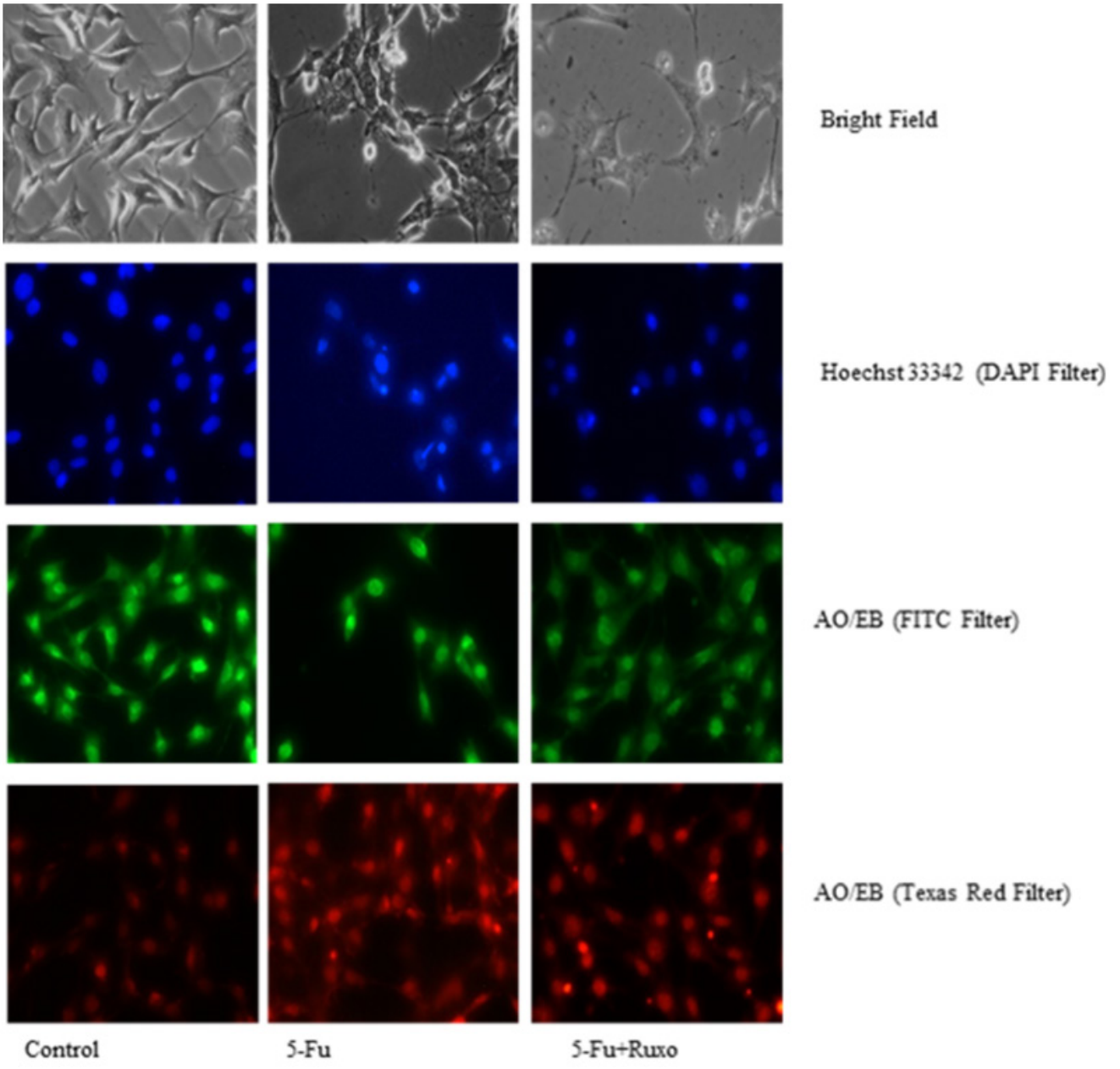

Figure 3: Bright field and Fluorescence microscopic images of U87 cells treated with 5-FU alone or in combination with Ruxo at IC50 dose after incubation for 24 hours of the U87 cell line.

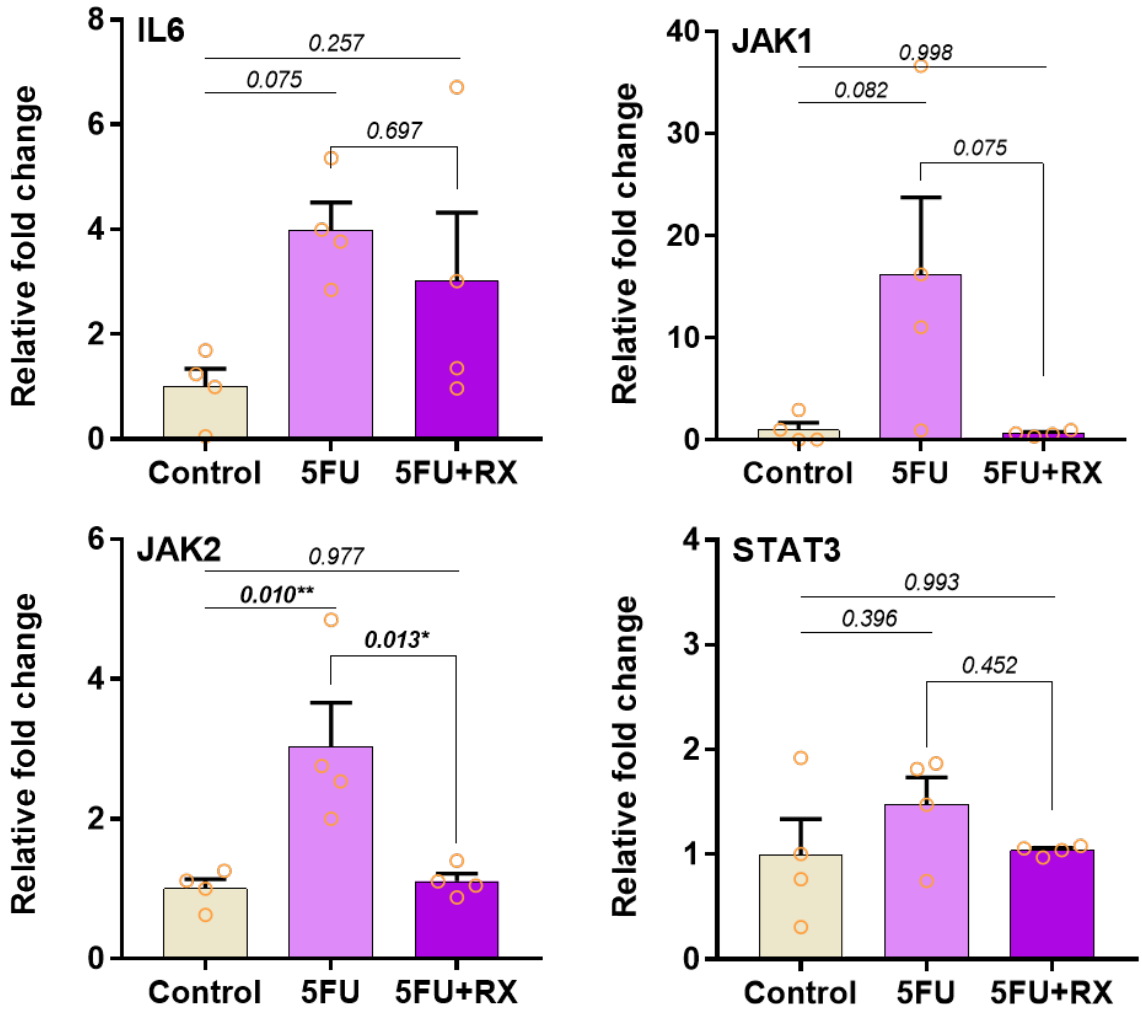

Figure 4: Relative fold change qRT-PCR analysis of IL-6, JAK1, JAK2, and STAT3 genes in IC50 doses of 5-FU and 5-FU+Ruxo exposed U87 cell line. (All data were normalized with β-actin expression and given as relative to control (control=1). Values expressed as mean±standard error (n=4). *Indicates significantly different values, one-way ANOVA, Tukey HSD test : p≤0.05, ** p≤0.01.)

Changes in caspase 3 and caspase 8 expressions were found statistically insignificant in U87 cells treated with a combination of 5-FU+Ruxo. However, the apoptotic effect of 5-FU alone is more pronounced than the apoptotic effect of the 5-FU+Ruxo combination. Acridine orange stains both living and dead cells, while ethidium bromide stains only membrane-lost cells. Therefore, while living cells are stained equally green, early apoptotic cells have bright green punctuation in the nucleus due to nuclear fragmentation. However, late apoptotic cells are stained orange with ethidium bromide (15). In our study, only 5-FU treated cells were stained bright green, while 5-FU+Ruxo treated cells were mostly stained bright orange. Both fluorescence staining and gene expression results showed that apoptosis induced in the glioblastoma cell line.

In our study, it was found that 5-FU+Ruxo administration suppressed JAK1 and JAK2 expression compared to 5-FU application (Figure 4). However, only the difference in JAK2 was statistically significant. The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is the principal signaling for immune response, growth factor or cytokine regulation, cell proliferation, differentiation, cell mi-

gration as well as autophagy and apoptosis (16, 17). JAK/STAT pathway also plays a critical role in the rapid transduction of signals from the cell surface to the nucleus (18, 19). Pro-inflammatory cytokine IL-6 signals through IL6-R-gp130/JAK1 and JAK2 complexes, resulting in the downstream phosphorylation of STAT3 and/or STAT5 (homo or heterodimers). These STAT complexes translocate nucleus and trigger a wide array of genes involved in processes ranging from cell survival or proliferation to inflammation. The phosphorylated sites on the IL6-receptor and JAKs serve as docking sites for the SH2-containing STAT3, and for SH2-containing proteins and adaptors that link the receptor to MAP kinase, PI3K/AKT/mTOR. Therefore, the IL6 / gp130 receptor complex is important in ensuring cell viability (20-22). In light of these findings, it is thought that the strong decrease in cell proliferation seen in MTT test and microscope images in ruxo treatment groups is due to the inhibition of cell proliferation due to JAK signal activated by 5-FU.

The limitations of our study are that we used only four replicates for control, and treatment groups and applied agents to a single cell line. However, we think that our data do reflect some potential clinical scenarios in GBM patients, especially patients who experienced exposure to both 5-FU and Ruxo. However, it is necessary to validate the results with studies using more repetition and types of cell lines as well as in vivo models.

In conclusion, this study is, to the best of our knowledge, the first to investigate the combined effect of 5-FU and Ruxo on the glioblastoma cell line. Additionally, our findings indicate that 5-FU alone or combined with Ruxo caused inhibition of cell proliferation of U87 glioblastoma cells via triggering mitochondrial apoptosis. While these initial results are important for the usability of 5-FU and Ruxo in glioblastoma, further detailed studies are needed.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: GA, OD, ZBD, Supervision: OD, ZBD, Resources: GA, OD, ZBD, Materials: GA, OD, ZBD, Data collection and/or processing: GA, OD, ZBD, Analysis and/or Interpretation: GA, OD, ZBD, Literature Search: GA, OD, ZBD, Writing Manuscript: GA, OD, ZBD, Critical Review: GA, OD, ZBD.
Financial disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

INVESTIGATION OF THE PARAMETERS THAT MAY PREDICT HEMODIALYSIS FREQUENCY

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ABSTRACT

Aims: This study investigates the relationship between the clinical parameters at the beginning of hemodialysis therapy and the changing in the frequency of weekly hemodialysis sessions in ongoing treatment. ***Methods:*** The study population was composed of all of the patients that were started chronic hemodialysis treatment between January 2015 and January 2020. The patients were classified as twice-weekly (2/7) and thrice-weekly (3/7) groups according to the hemodialysis schedule at the start of treatment. The 3/7 group is additionally subdivided according to the dialysis schedule switches to the thrice-weekly program. Basal demographics and biochemical parameters were obtained from medical records at the start of hemodialysis. ***Results:*** The total number of subjects in the study was 433 (141 in the twice-weekly program, 292 in the thrice-weekly program). Forty-six (32.6%) patients in twice-weekly hemodialysis program were shifted to a thrice-weekly program during the follow-up. The female/male ratio in the 2/7 program is different from the 3/7 program. Serum creatinine and C-reactive protein levels were higher in patients on the 3/7 program. Serum calcium and albumin levels were higher in patients on a 2/7 program. The statistically significant increments were found in calcium, hemoglobin, hematocrit, urea, creatinine, and potassium levels between the before and after switch results of the 3/7 group which switched from 2/7. ***Conclusion:*** Urea, creatinine, and potassium levels can guide the decision to compose a hemodialysis schedule. However, patients' future weekly treatment schedules cannot be predicted by biochemical parameters obtained at the start of hemodialysis. ***Keywords:*** Hemodialysis, dialysis frequency, chronic kidney disease, biochemical parameters

INTRODUCTION

Chronic kidney disease (CKD) is present in 9.1% of the world population, and approximately 10% of those patients have a glomerular filtration rate (GFR) less than 15 mL/min/m² (1). Hemodialysis (HD) is a well known and widely distributed renal replacement modality (RRT) modality worldwide. Despite the advances in hemodialysis techniques, patients on hemodialysis have higher mortality rates and lower quality of life than transplantation and peritoneal dialysis (2, 3). Hemodialysis modality has some disadvantages such as requiring the specified health care professionals, patients' dependency on a dialysis center three times a week with at least four hours in a day, and the increase in long-term cost that is a big burden to the health care system. Hemodialysis may be planned as a twice-weekly schedule considering to minimize all these disadvantages with patients who had the acceptable urinary output and biochemical evaluation.

Herein, we investigated the biochemical and clinical parameters that may affect the long term dialysis schedule in the patients who started the chronic dialysis program.

MATERIAL AND METHODS

PATIENT SELECTION

The study protocol was approved by the Trakya University Ethical Board (Protocol Code: TÜTF-BAEK 2020-345). Four hundred thirty-three patients that were started hemodialysis between January 2015 to January 2020 have been enrolled in this retrospective study. Inclusion criteria were being more than

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Received: 30.05.2020 Accepted: 15.06.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.03 Available at: tmsj.trakya.edu.tr

Cite this article as: Kıral G, Certel AT, Özyiğit İİ et al. Investigation of the parameters that may predict hemodialysis frequency. Turkish Med Stud J 2020;7(3):140-4.

18 years old and hemodialysis history for more than three months. Exclusion criteria were being younger than 18 years of age. Trakya University Hospital, as a tertiary medical facility, is one of the major hospitals in the Thrace region of Turkey and it serves high level nephrology care around 800,000 population. The patients were classified according to the dialysis schedule twice-weekly and thrice-weekly at the time of hemodialysis initiation. The twice-weekly group was classified into two subgroups that were ongoing with a twice-weekly schedule and switched to thrice-weekly schedule subgroups.

Age, gender, hemodialysis program, comorbidities, CKD ethiologies, urea, creatinine, uric acid, electrolyte levels, complete blood count, and C-reactive protein (CRP) values at the start of dialysis were recorded for statistical analysis.

STATISTICAL ANALYSIS

Categorical data are presented as frequencies and percentages. Continuous variables are expressed as mean ± standard deviation (SD) for normally distributed variables and as the median and interquartile range (IQR, 25th-75th percentile) for variables with skewed distribution. The Kolmogorov-Smirnov test was used to test for a normal distribution. The differences between groups were analyzed using the Student t-test and the Mann-Whitney U test as appropriate. A p-value of <0.05 was considered significant. Statistical analysis was performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 433 patients were included in this retrospective study. One hundred and forty-one (32.6%) patients were started twice-weekly HD schedule, and 292 (67.4%) patients were started thrice-weekly HD schedule at baseline. Forty-six (32.6%) patients in twice-weekly HD program were shifted to a thrice-weekly program during the follow-up. The mean age of all patients was 64.4 years, and 179 (41.3%) were female. The female to male ratio was higher in twice-weekly program group besides lower in the thrice-weekly program group (Table 1). Basal serum calcium and albumin levels were significantly higher in the twice-weekly group. Basal serum creatinine and CRP levels were significantly higher in the thrice-weekly group. Other basal demographic and biochemical parameters did not differ between the two treatment groups.

There was no demographic and biochemical difference observed between the patients who continued with the twice-weekly program and shifted the thrice-weekly program during the follow-up (Table 2).

The parameters at the start of the twice-weekly HD program and at the time of the switching the HD schedule to thrice-weekly were compared to elucidate the impact of biochemical parameters on the increase of HD frequency (Table 3). There were statistically significant differences in urea, creatinine, potassium, calcium, hemoglobin, and hematocrit values observed.

DISCUSSION

Chronic kidney disease is a worldwide health problem that significantly impacts patient morbidity and mortality (1). At the most severe CKD stage, called end-stage renal disease (ESRD), the patients need either dialysis or transplantation to survive. Hemodialysis is the most common renal replacement therapy applied in the whole world (4). Hemodialysis is generally received with a thrice-weekly schedule because of the limited supply of the dialysis centers, increasing demand for dialysis, and economic issues. Attachment to a dialysis center on certain days of the week, typical side effects like fatigue, nausea, vomiting, muscle cramps on dialysis days, a stricter diet, and increased mortality compared to other modalities are some disadvantages for HD treatment (5). In some HD patients, a twice-weekly HD program is sufficient for effective solute removal and volume control. In general, the ESRD patients who had preserved urine output, adequate solute clearance and water removal may have a chance to receive a twice-weekly schedule.

In this study, we investigated if any parameters, besides urine output, correlate the HD need at the beginning of the HD treatment in the future. There were more female patients in the twice-weekly group, but male patients predominate the whole study population. Data obtained from the Chronic Renal Insufficiency Cohort (CRIC)-Study showed a significantly lower risk for ESRD development in the female community (6). Our study showed that the females develop less end-stage renal disease and have better residual renal functions. The lower serum albumin and calcium levels were detected in the thrice-weekly group while better nutritional status and less-severe bone-mineral disorders in the twice-weekly group. Serum CRP was higher in the thrice-weekly group. This supports the previous

Table 1: Demographics and biochemical parameters at the start of hemodialysis treatment.

	Twice-weekly hemodialysis (n=141)	Thrice-weekly hemodialysis (n=292)	*P*-value*
Gender [n (%)]			**0.002**
Female	73 (52%)	106 (36%)	
Male	68 (48%)	186 (64%)	
Age (years)	66.04 ± 12.91	65.08 ± 13.62	0.516
Hypertension [n (%)]	95 (67%)	191 (65%)	0.330
Etiology of CKD			0.234
Diabetes Mellitus	7 (5%)	13 (4%)	
Hypertension	3 (2%)	11 (4%)	
Glomerulonephritis	13 (9%)	13 (4%)	
TIN	4 (3%)	7 (2%)	
PKD	5 (4%)	8 (2%)	
Amyloidosis	1 (1%)	7 (2%)	
Others/Unknown	108 (76%)	233 (82%)	
Urea (mg/dL)	113.13 ± 45.16	108.12 ± 46.33	0.207
Creatinine (mg/dL)	4.95 ± 1.69	5.78 ± 2.28	**0.001**
Uric Acid (mg/dL)	5.53 ± 1.77	5.73 ± 1.83	0.303
Na^+ (mEq/L)	136.83 ± 3.76	136.29 ± 3.96	0.356
K^+ (mEq/L)	4.40 ± 0.58	4.51 ± 0.66	0.101
Ca^{+2} (mEq/L)	8.48 ± 0.83	8.29 ± 0.86	**0.004**
PO_4^{-2} (mEq/L)	4.64 ± 1.17	4.86 ± 1.40	0.111
Albumin (g/dL)	3.09 ± 0.60	2.93 ± 0.68	**0.017**
CRP (mg/dL)	3.11 ± 0.39	4.48 ± 0.37	**0.004**
Hemoglobin (g/dL)	9.42 ± 1.26	9.35 ± 1.21	0.530
Hematocrit (%)	27.85 ± 4.05	27.80 ± 3.99	0.783

CKD: Chronic Kidney Disease, ***TIN:*** Tubulointerstitial Nephritis, ***PKD:*** Polycystic Kidney Disease, ***CRP:*** C-reactive protein
*Statistically significant values are marked as bold.

Table 2: Demographics and parameters of twice-weekly program patients.

	Ongoing twice-weekly (n=95)	Switched to thrice-weekly (n=46)	*P*-value
Gender [n (%)]			0.208
Female	44 (46%)	27 (58%)	
Male	51 (54%)	19 (42%)	
Age (years)	66.14 ± 13.48	65.54 ± 11.69	0.588
Urea (mg/dL)	116.47 ± 40.02	105.69 ± 40.874	0.264
Creatinine (mg/dL)	4.93 ± 1.74	4.99 ± 1.58	0.733
Uric Acid (mg/dL)	5.48 ± 1.76	5.63 ± 1.82	0.781
Na^+ (mEq/L)	136.80 ± 3.71	136.91 ± 3.93	0.893
K^+ (mEq/L)	4.40 ± 0.56	4.34 ± 0.59	0.410
Ca^{+2} (mEq/L)	8.51 ± 0.85	8.41 ± 0.80	0.165
PO_4^{-2} (mEq/L)	4.69 ± 1.27	4.54 ± 0.95	0.627
Albumin (g/dL)	3.09 ± 0.60	3.08 ± 0.60	0.954
CRP (mg/dL)	3.06 ± 0.48	3.28 ± 0.75	0.968
Hemoglobin (g/dL)	9.50 ± 1.17	9.20 ± 1.39	0.180
Hematocrit (%)	27.89 ± 3.81	27.68 ± 4.57	0.690

CRP: C-reactive protein

Table 3: Biochemical parameters at the beginning and at the switching of twice-weekly patients.

	Beginning with a twice-weekly plan	Switched to the thrice-weekly plan	*P*-value*
Urea (mg/dL)	102.16 ± 41.37	121.92 ± 43.87	**0.009**
Creatinine (mg/dL)	4.99 ± 1.58	6.22 ± 2.17	**<0.001**
Uric Acid (mg/dL)	5.59 ± 1.77	5.9 ± 1.54	0.517
Na^+ (mEq/L)	136.82 ± 4.10	137.72 ± 3.67	0.212
K^+ (mEq/L)	4.37 ± 0.63	4.76 ± 0.77	**0.008**
Ca^{+2} (mEq/L)	8.41 ± 0.82	8.84 ± 0.76	**0.004**
PO_4^{-2} (mEq/L)	4.49 ± 0.95	4.57 ± 1.48	0.744
Hemoglobin (g/dL)	9.20 ± 1.41	10.06 ± 1.76	**0.019**
Hematocrit (%)	27.66 ± 4.57	30.37 ± 5.27	**0.016**

*Statistically significant values are marked as bold.

studies about increased microinflammation negatively correlated with residual renal functions in the HD population (7).

We could not observe any significant difference between thrice-weekly and twice-weekly groups comparing basal parameters. These findings support that the evaluation of residual renal function and urine output had a major role in the decision-making process of scheduled HD sessions.

While we compare the basal and shifting biochemical parameters, we observed a statistically significant increase in urea, creatinine, and potassium levels at the time of rescheduling. This can be explained by a decrease in residual kidney function in some twice-weekly patients with time. So not just in the start of HD treatment but also in the follow-up period, close monitoring of some biochemical parameters may make the doctors aware of the patients who need more frequent HD sessions.

In conclusion, hemodialysis schedule can be decided by urea, creatinine, and potassium levels. However, patients' future weekly treatment schedules cannot be foreseen by biochemical parameters obtained at the start of hemodialysis.

Ethics Committee Approval: This study was approved by the Scientific Research Ethical Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2020-345).
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: CG Design: CG Supervision: CG Resources: GK, ATC, İİÖ, BG, GA, CG Materials: CG Data collection and/or processing: GK, ATC, İİÖ, BG Analysis and/or Interpretation: GA, CG, GK, ATC, İİÖ, BG Literature Search: GA, CG, GK, ATC, İİÖ, BG Writing Manuscript: GA, CG Critical Review: GA, CG, GK, ATC, İİÖ, BG.
Financial disclosure: The authors declared that this study received no financial support.
Editor in chief's Note: Three of the authors of this article, Alperen Taha Certel, Irmak İrem Özyiğit, and Begüm Söyleyici are members of the editorial board of Turkish Medical Student Journal. However, they did not take place in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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TMSJ
TURKISH MEDICAL STUDENT JOURNAL
CASE REPORT

JUVENILE POLYP FROM SYMPTOM TO DIAGNOSIS AND THE ROLE OF ULTRASONOGRAPHY: A CASE REPORT

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ABSTRACT

Aims: Juvenile polyps are the most common intestinal polyps in children, but they are also found relatively frequently in adults. Although colonoscopy and pathologic examinations are essential for the final diagnosis of juvenile polyps, our aim is to show that ultrasound imaging can also reveal the juvenile polyps. ***Case Report:*** In a 21-year-old female patient with painless rectal bleeding and abdominal pain, blood tests showed no signs of anemia or infection, but the fecal occult blood test was found to be positive. Ultrasound imaging prior to colonoscopy revealed the juvenile polyp. The polyp was resected during colonoscopy via hot snare polypectomy and then it was pathologically diagnosed as a juvenile polyp. ***Conclusion:*** Ultrasonography is not a very common diagnostic tool for colonic polyps, but it may have the potential of revealing a colonic polyp such as a juvenile polyp. This can ease the process of diagnosis. ***Keywords:*** Juvenile polyp, ultrasonography, colonoscopy

INTRODUCTION

Juvenile polyps (JPs) are the most prevalent intestinal polyps in children and they are most frequently diagnosed in the first 10 years of life, but they also account for approximately 1% of all colonic polyps found in adults. JPs are more commonly seen in males and they are also more common on the left side of the colon (1).

The term "juvenile" refers to the histological characteristics of the polyp and not the age of onset (1). Regardless of age, the most common symptoms for JPs are blood in stool and abdominal pain. The gold standard of the confirmation of the diagnosis is a pathological examination of the polyp resected during colonoscopy, but ultrasonography may also help to reveal juvenile polyps (2). This case report underlines the effectiveness of ultrasound imaging during the diagnostic work-up of JPs since ultrasound is not a very common diagnostic tool for colonic polyps (3).

CASE REPORT

A 21-year-old female patient was admitted to the gastroenterology department of Acıbadem International Hospital due to blood in stool and abdominal pain. She had no fever, no loss of appetite, or weight loss and she was not taking any medications or herbs. Abdominal examination including digital rectal examination showed no pathological findings. Initially requested blood tests revealed no signs of anemia or infection (Table 1) but the fecal occult blood test was found to be positive and total abdominal ultrasonography pointed a hypoechoic nodule-like intraluminal polypoid lesion in sigmoid colon with 17x21 mm dimension with arterial blood flow on Doppler investigation (Figure 1). The colonoscopy that was performed afterwards showed a big polyp with a long stalk in the sigmoid colon which was resected via hot snare polypectomy (Figure 2). No other polyps were seen in the whole colon during the colonoscopy. Finally, pathological examination of the polyp was compatible with a JP with an edematous lamina propria with inflammatory cells and cystically dilated glands filled with mucus (Figure 3).

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Received: 25.07.2020 Accepted: 17.09.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.04 Available at: tmsj.trakya.edu.tr

Cite this article as: Ersoy B, Ersoy Ö, Akman H et al. Juvenile polyp from symptom to diagnosıs and the role of ultrasonography: a case report. Turkish Med Stud J 2020;7(3):145-8.

Table 1: Laboratory evaluation.

	Results with units	*Reference values with units*
Leukocytes	10.30 x10^3/uL	4.50-11.00 x10^3/uL
Hemoglobin	14.0 g/dL	12.5-16 g/dL
Platelets	347 x10^3/uL	150-400 x10^3/uL
Glucose (pre-prandial)	94 mg/dL	70-100 mg/dL
Alanine Aminotransferase (ALT)	28 IU/L	12-59 IU/L
Aspartate Aminotransferase (AST)	23 IU/L	10-37 IU/L
C-reactive protein (CRP)	0.50 mg/dL	< 0.50 mg/dL

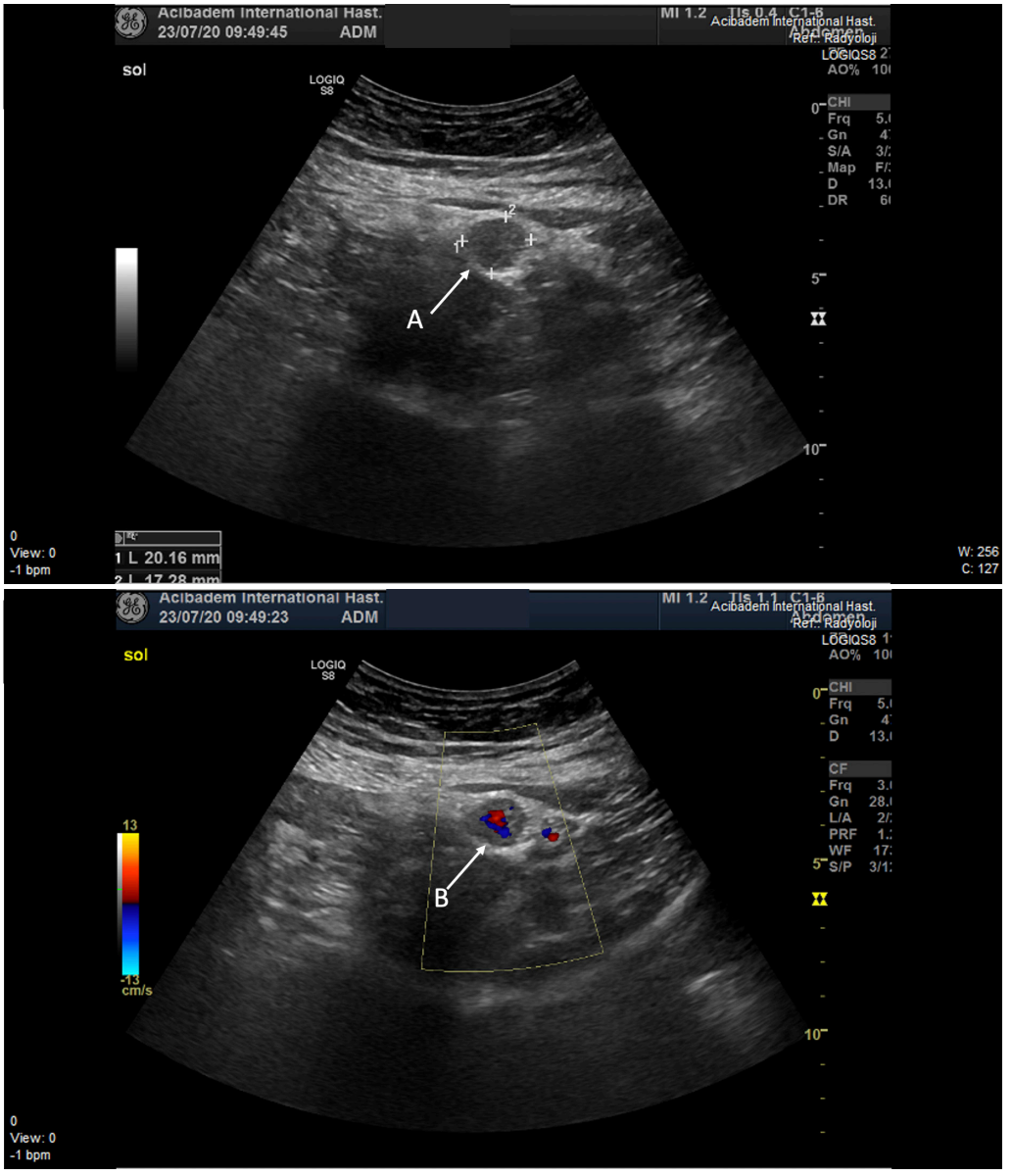

Figure 1: Ultrasound imaging. A: Abdominal ultrasound imaging indicating hypoechoic nodule. B: Vascularization of hypoechoic nodule on Doppler imaging.

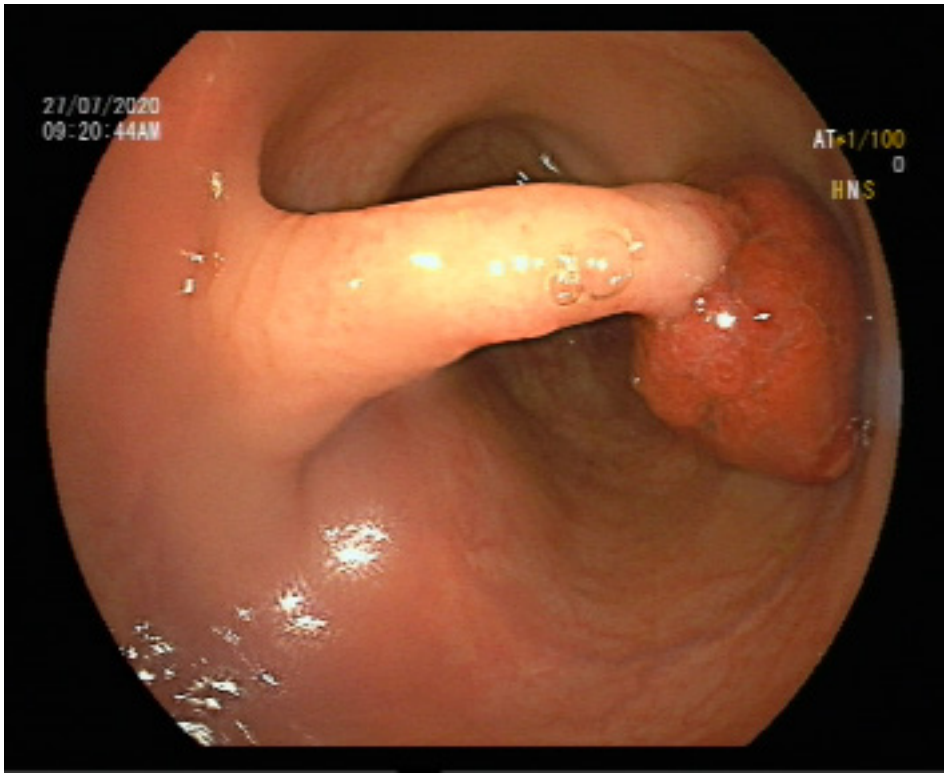

Figure 2: Endoscopic appearance of pedunculated polyp.

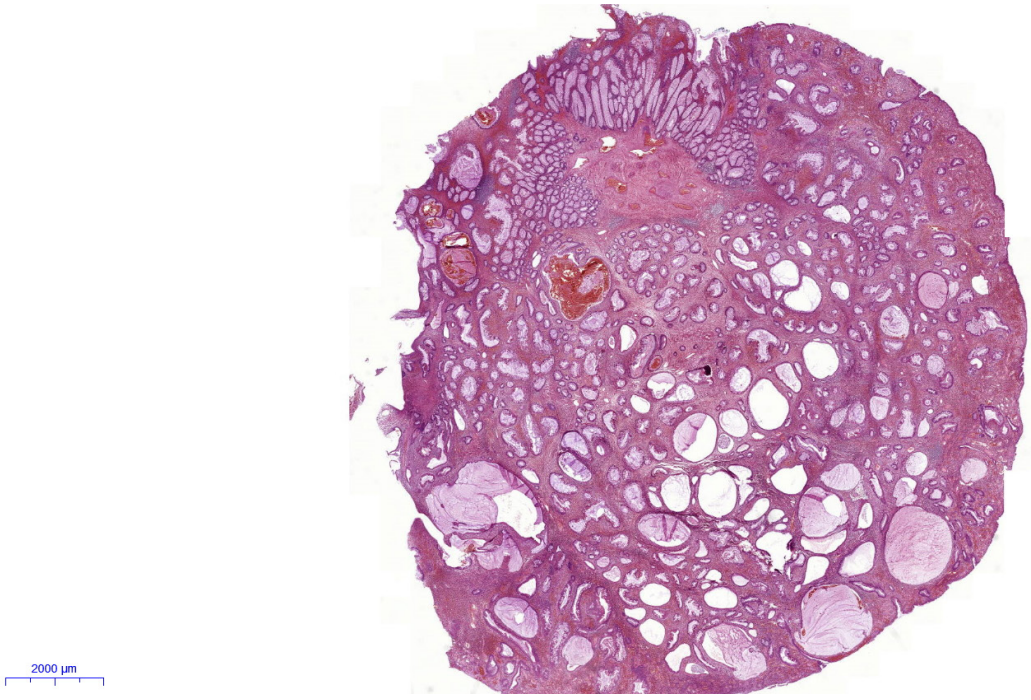

Figure 3: Pathological appearance of the polyp with cystically dilated glands with inflammation.

DISCUSSION

Juvenile polyps account for approximately 80% of polyps in children, but are more likely to be found and diagnosed on adults since colonoscopy is a more common way of diagnosis in adult age (1, 4). JPs are usually listed under hamartomas, which is one of the two major categories for gastrointestinal polyps (5). Patients of all ages with JPs typically complain of bloody stools and abdominal pain (2). Differential diagnosis of these symptoms commonly includes inflammatory bowel diseases and acute gastroenteritis in young patients. Therefore, blood and stool examinations together with ultrasound imaging are initial diagnostic investigations. In these young patients, a colonoscopy is rarely performed except when it is truly necessary (6). Our patient's blood tests showed no specific pathology whereas the fecal occult blood test was positive and ultrasound imaging revealed the polypoid lesion in the colonic lumen and colonoscopy was performed afterwards. According to Vitale V et al. (7), ultrasound findings could be able to differentiate a colonic polypoid lesion from intraluminal feces or inflammatory pseudopolyps which also look like nodules.

In this young case, besides blood tests and abdominal ultrasound imaging, diagnostic evaluations also included colonoscopy due to positive fecal occult blood test. However, in young patients with abdominal pain but with no rectal bleeding or any alarming symptoms and signs for colonic malignancies (weight loss, loss of appetite, anemia); a colonoscopy is not urgently indicated or planned. For these situations, ultrasound imaging can point a polypoid lesion and guide the referral physician to perform the colonoscopy (8, 9).

Since only one polyp was found during the colonoscopy, the final diagnosis of our patient was a solitary JP. Solitary juvenile polyps have minimal malignant potential (10). On the other hand, juvenile polyposis syndrome increases the colorectal cancer risk (11). Juvenile polyposis syndrome diagnosis is made if any of the following criteria is present; 5 or more juvenile polyps in the colorectum or juvenile polyps throughout the gastrointestinal tract or any number of juvenile polyps and a positive family history of juvenile polyposis (12). Thus, it is important to distinguish solitary JP from juvenile polyposis syndrome by performing total colonoscopy rather than proctosigmoidoscopy. Our patient was informed about this very low malignancy potential of her polyp and she was also informed that polypectomy was the definitive treatment and no further routine follow-up was needed.

As a conclusion, ultrasonography is not a very common diagnostic tool for colonic polyps, but it may have the potential of revealing a colonic polyp such as a juvenile polyp. This can ease the process of diagnosis.

Ethics Committee Approval: N/A
Informed Consent: Verbal informed consent was obtained from the patient through a phone call.
Conflict of Interest: The authors declared no conflict of interest.
Author Contributions: Concept: BE Design:BE Supervision: OE Resources:OE, HA, FB Materials:BE, OE, HA, FB Data collection and/or processing: BE,OE, HA, FB Analysis and/or Interpretation:BE Literature Search: BE Writing Manuscript:BE Critical Review:BE, OE.
Financial Disclosure: The authors declared that this study received no financial support.
Editor in chief's Note: One author of this article, Berkin Ersoy is a member of the editorial board of Turkish Medical Student Journal. However, he did not take place in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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TMSJ
TURKISH MEDICAL STUDENT JOURNAL
CASE REPORT

PARTIAL NEPHRECTOMY OF A HORSESHOE KIDNEY WITH RENAL-CELL CARCINOMA AND CHOLECYSTECTOMY: A CASE REPORT

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ABSTRACT

Aims: Horseshoe kidneys are the most common type of renal fusion anomaly and it may be associated with other anomalies and complications. Our case aims to present the partial nephrectomy of a horseshoe kidney with renal-cell carcinoma and cholecystectomy. ***Case Report:*** A sixty-year-old male patient was admitted to our outpatient clinic with a 3 cm suspicious mass in the horseshoe kidney that was detected during an attack of acute cholecystitis. Computed tomography revealed a heterogeneous hypodense lesion containing millimetric calcific foci of 35x31x33 mm in size at the ventral middle part of the right kidney. Partial nephrectomy was performed non-ischemically and then cholecystectomy was performed. Pathological examination revealed stage T1a clear cell renal carcinoma, WHO/ISUP Grade 2 with a negative surgical margin. During follow-up; urea, creatinine, and glomerular filtration rate were found to be normal. ***Conclusion:*** Horseshoe kidneys are fairly common among renal anomalies. Cholecystectomy following non-ischemic partial nephrectomy for a tumor in the horseshoe kidney is a rare case. ***Keywords:*** Horseshoe kidney, nephrectomy, cholecystectomy

INTRODUCTION

Horseshoe kidneys (HSK) are the most common renal fusion anomaly. It is seen in 0.25% of the population and is twice as common in men as in women (1). Horseshoe kidney is characterized by abnormalities in the kidney's position, rotation, and vascular supply. It has been found that functional renal masses fused with ureters on both sides of the vertebral column remain without crossing the renal hilum to the bladder (2). The position of the isthmus connecting the two renal masses may differ from case to case. It may be midline or asymmetrical, referred to as an asymmetrical horseshoe kidney. Seventy percent of the asymmetrical horseshoe kidneys are dominant, and 80% of the cases are composed of the renal parenchyma and the remainder of the fibrous band. Although fusion occurs in the lower pole in more than 90% of the cases, it may also occur in the upper pole in a small number of cases (2).

Horseshoe kidneys may also be present in 30% as an isolated condition, but there are different types of associated abnormalities (1). It mostly consists of lithiasis, ureteropelvic obstruction, and infections. Additionally, there is a higher risk of kidney lesions and an increased incidence of malignancy in trauma. It is important for physicians to have knowledge of embryology and anatomy in order to understand the complications affecting HSK.

Horseshoe kidneys in adults are mostly asymptomatic. It can be detected by intravenous pyelography, ultrasound, or computed tomography (CT) scanning. Scintigraphy can also be used to detect HSK (3). In normal embryonic development, kidneys come out of the pelvis in the 7th and 8th weeks. In the case of HSK, the inferior mesenteric artery blocks the ascent of the kidney. Therefore it gets stuck in the mid-abdominal area due to not migrating out of the pelvis as it does in normal embryonic development (3).

Horseshoe kidneys can be associated with other anomalies. Common anomalies for this instance are congenital gastrointestinal, central nervous system, skeletal and chromosomal abnormalities. Glenn (4) reported a 78.9% lower incidence of associated congenital anomalies in stillborn fetuses and infants, 28.5% in children, and 3.5% in adults.

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Received: 13.08.2020 Accepted: 18.09.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.06 Available at: tmsj.trakya.edu.tr

Cite this article as: Cengiz E, Şişman FO, Koçyiğit B et al. Partial nephrectomy of a horseshoe kidney with renal-cell carcinoma and cholecystectomy: a case report. Turkish Med Stud J 2020;7(3):149-53.

Benign and malignant tumors are also associated with HSK. Malignancy is mostly attributed to teratogenic factors. Having HSK can increase the risk of kidney cancer. For example, the incidence of horseshoe kidneys in the general population is 1 in 400, a child with HSK has twice the risk of developing Wilms' tumor than the general population (5).

Our case presents a patient who underwent non-ischemic partial nephrectomy followed by cholecystectomy for horseshoe kidney malignancy. We believe this case report will give physicians more insight into this event.

CASE REPORT

A sixty-year-old male patient was admitted to our outpatient clinic with a 3 cm suspicious mass in the horseshoe kidney that was detected incidentally during an attack of acute cholecystitis two months ago. According to the detailed history of the patient, he was using oral anticoagulants due to coronary by-pass. The patient's blood pressure, heart rate and body temperature were normal and he did not have hematuria. On the other hand, he had gastrointestinal symptoms and weight loss.On physical examination, there was tenderness in the right upper quadrant of the abdomen, the other quadrants were found to be normal. There was no bilateral costovertebral angle sensitivity.

The routine hemogram, urine analysis, and serum electrolytes were normal. A horseshoe kidney was detected in the urinary system ultrasound, and a heterogeneous lesion of approximately 3 cm in size was observed on the right. CT revealed a horseshoe kidney and a heterogeneous hypodense lesion containing millimetric calcific foci of 35x31x33 mm in size at the ventral middle part of the right (Figure 1, 2, 3). In addition, the gallbladder wall was inflamed, and calculus was found in it (Figure 3, 4).

The operation was performed with an anterior subcostal incision. The Gerota's fascia was opened and the mass was reached directly. The operation was continued non-ischemically, and the mass was excised in accordance with oncological procedures. Then, cholecystectomy was performed by the general surgery team, and the gallbladder was excised without any complication. The postoperative follow-up was uneventful.

Histopathological examination revealed stage T1a clear cell renal carcinoma, WHO/ISUP Grade 2, and negative surgical margin. The histopathology of the gallbladder was reported as chronic cholecystitis. During follow-up; urea, creatinine, and glomerular filtration rate (GFR) were found to be normal.

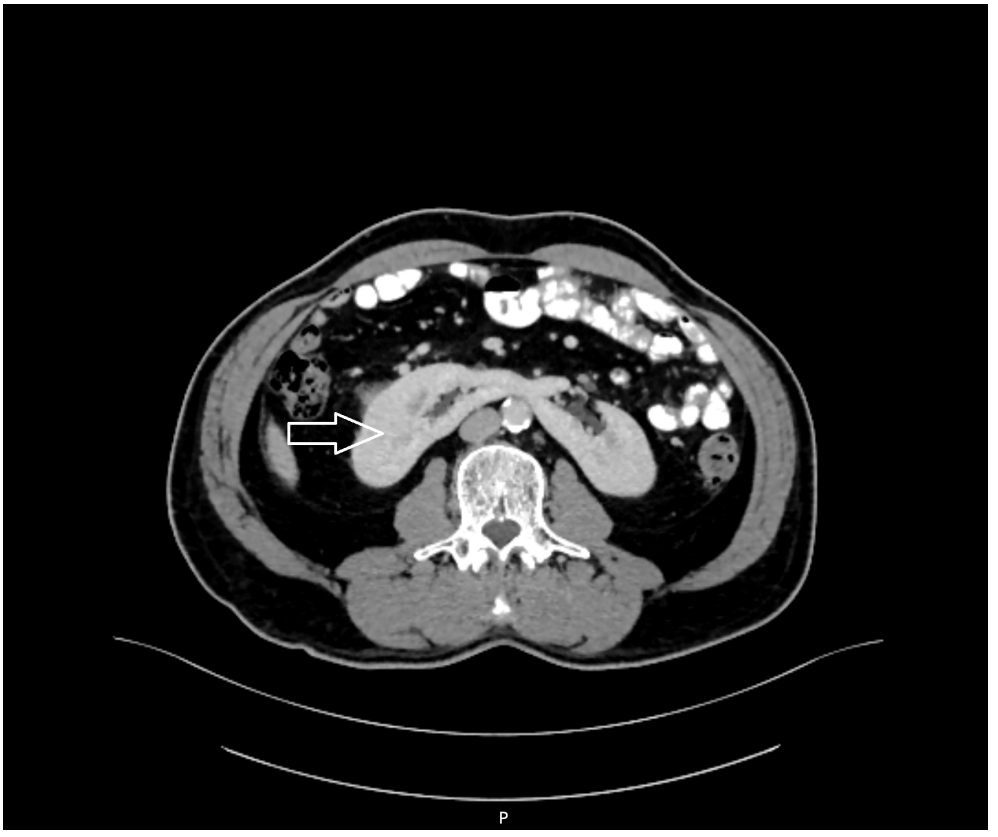

Figure 1: Transverse sectional view of the right horseshoe kidney on CT scan.

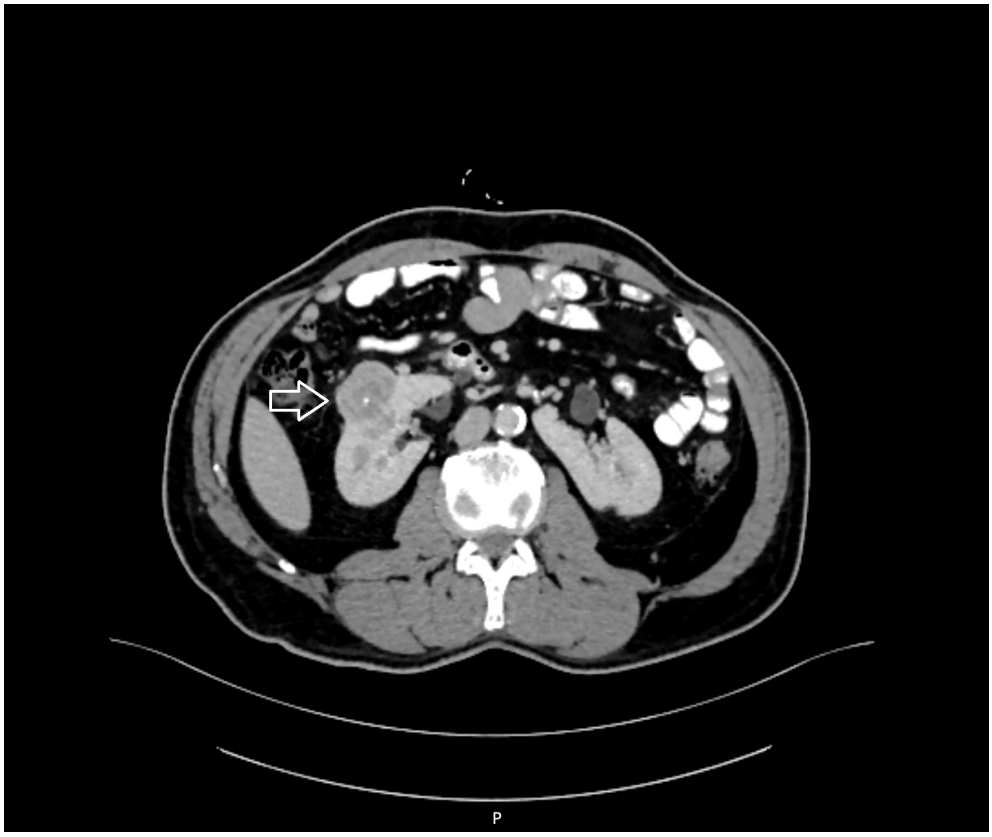

Figure 2: Transverse sectional view of the tumor in the right horseshoe kidney on CT scan.

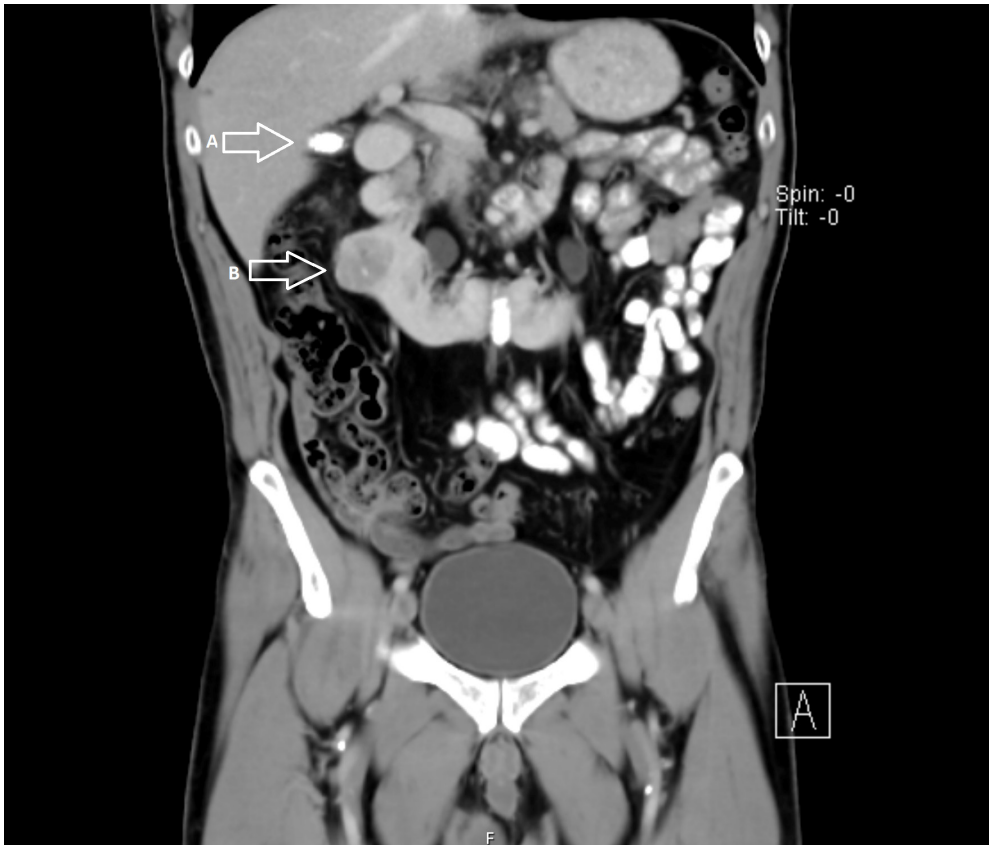

Figure 3: Coronal sectional view of the right horseshoe kidney and the gallbladder seen in the CT scan (A: calculus in the gallbladder and inflamed gallbladder wall B: tumor in the right horseshoe kidney).

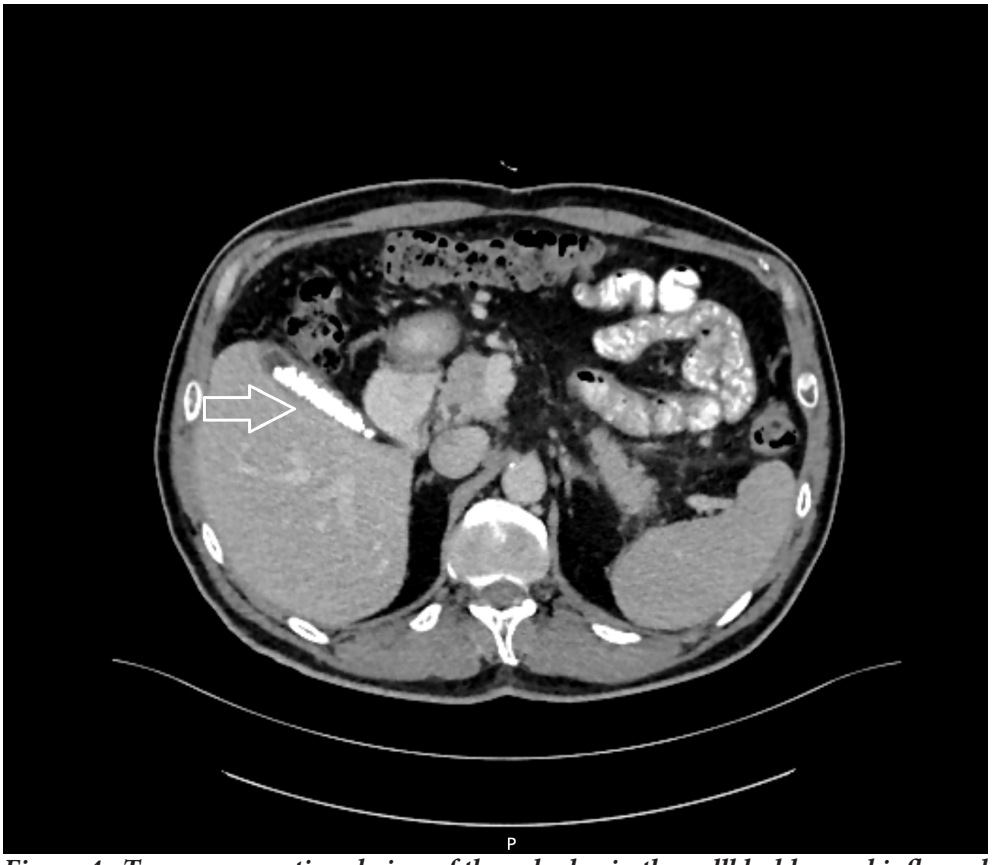

Figure 4: Transverse sectional view of the calculus in the gallbladder and inflamed gallbladder wall in the CT scan.

DISCUSSION

In our case, a sixty-year-old male patient was admitted to the hospital with an attack of acute cholecystitis, and a suspicious mass of 3 cm in the horseshoe kidney was found incidentally on CT. Bhandarkar et al. (6) stated that the probability of finding horseshoe kidneys by chance is approximately 50% of all cases.

Our case was diagnosed with horseshoe kidney incidentally which is similar to the case presented by Seker et al. (7). They presented a thirty-seven years old female patient incidentally diagnosed with HSK, similar to our case. Their patient had splenomegaly but after a CT scan, the patient had horseshoe kidney with a hypodense area 18 x 12.5 mm in size at the left kidney. The patient was operated by left-open partial nephrectomy zero ischemia with a modified Chevron incision without complications during and after the procedure (7).

Tkocz et al. (8) had a patient with HSK with a tumor. The tumor was removed through a median incision after organ detachment. Cholecystectomy was performed as in our case, due to the presence of gallbladder hydrocele.

The incidence of renal cell carcinoma in horseshoe kidneys is the same as in normal kidneys, and its development and course are not associated with the presence of the defect. According to the literature, transitional cell carcinoma is more common in horseshoe kidneys. This may be due to the presence of defects such as urinary obstruction, susceptibility to nephrolithiasis, and chronic infection (9, 10).

Ischemic segmental kidney necrosis due to insufficient collateral artery supply in HCC is a common complication. Ligation or division of an artery results in ischemic segmental renal necrosis (2). Justo-Janeiro et al. (11) had also operated on a HSK patient. Dissociation was by the ischemic line and total ischemic time was 125 minutes. However, in our case, the mass was excised non-ischemically. This is unusual for such a HSK surgery and there was no reduction in urea, creatinine, and GFR in postoperative follow-up. As a result, horseshoe kidneys are quite common among kidney anomalies. As mentioned earlier, the diagnosis of horseshoe kidneys is accidental in about 50% of all cases.

As a conclusion, horseshoe kidneys are fairly common among renal anomalies. Cholecystectomy following non-ischemic partial nephrectomy for a tumor in the horseshoe kidney is a rare case. We believe that this case report will provide physicians a different perspective and additional information on the literature.

Ethics Committee Approval: N/A
Informed Consent: Informed verbal consent was obtained from the patient for this study.
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: EC, FOŞ, BK, MGA, TA Design: EC, FOŞ, BK, MGA, TA Supervision: EC, FOŞ, BK, MGA, TA Materials: EC, FOŞ, BK, MGA, TA Data collection and/or processing: EC, FOŞ, BK, MGA, TA Analysis and/or Interpretation: EC, FOŞ, BK, MGA, TA Literature Search: AG, SA, TA Writing Manuscript: EC, FOŞ, BK, MGA, TA Critical Review: EC, FOŞ, BK, MGA, TA.
Financial disclosure: The authors declared that this study received no financial support.
Editor-in-chief's Note: Three of the authors of this article, Elif Cengiz, Fevzi Oktay Şişman, Beliz Koçyiğit are members of the editorial board of Turkish Medical Student Journal. However, they did not take place at any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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REVIEW

PATHOPHYSIOLOGY, CLINICAL FEATURES AND TREATMENT OF MICROVASCULAR ANGINA: A REVIEW

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ABSTRACT

Microvascular angina is a non-obstructive coronary syndrome which presents itself as anginal chest pain with different features compared to other causes of angina pectoris. Although, it occurs through a similar mechanism with the better-understood variant (prinzmetal) angina, unlike the former, microvascular angina affects the subendocardial thin, therefore less compliant vessels. It is essential for every medical practitioner to recognize a patient with microvascular angina, as the disease has some characteristic presentations, does not respond well to the classical treatments for angina pectoris, and eventually may result in serious complications later. ***Keywords:*** Microvascular angina, coronary syndrome, angina pectoris

INTRODUCTION

Angina pectoris, derived from the Latin verb "angere" (to strangle) and the noun "pectus" (chest), literally meaning strangling of the chest, is chest pain, clinically classified as either typical or atypical by whether special characteristics are present or not. This classification is based on three factors: presence of a substernal pain or discomfort that occurs after exercise or emotional stress, relief by rest or nitroglycerin (1). If all three criteria are present, the pain is classified as a "typical" angina pectoris according to the American College of Cardiology/American Heart Institute's 2002 Guideline Update on Exercise Testing (2). Being an important symptom, angina pectoris may be an indicator of many cardiac pathologies as well as imitated by other causes of chest pain such as those including the gastrointestinal tract, especially esophagus (3).

One of the causes of angina pectoris is microvascular angina (MVA). Acting with a similar mechanism to variant (prinzmetal) angina, MVA is an important condition, mostly considered benign, however responsible for 1.5 times increase in mortality of the affected patients. In addition, patients with MVA tend to have increased pain sensitivity due to the increased adrenergic and decreased parasympathetic activity; therefore more susceptible to the feeling of pain than other myocardial ischemia patients (4). For all these reasons, patients who show signs of MVA should be recognized by all the physicians and should not be mistaken as extracardiac causes of chest pain (3, 5).

PATHOPHYSIOLOGY

The most common cause of angina pectoris is the lack of oxygen supply through coronaries despite increased demand of the heart tissue. This results in ischemic changes in the area that is supplied by those specific coronary arteries. The main reason behind this lack of supply is the occlusion of the coronary vessels through atherosclerotic changes which is named as coronary artery disease (CAD) (6).

Coronary arteries, give branches reaching to the inner parts of the heart, which are in the proximity of lumens. First branch of these arteries is named, as subepicardial arteries, while they are in the close proximity of the epicardium. The latest branches, on the other hand, are named subendocardial arteries as they are closer to the subendocardium, just beneath the lumen of atria and ventricles (Figure 1).

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Received: 07.08.2020 Accepted: 10.09.2020 • DOI: 10.4274/tmsj.galenos.2020.07.02.06 Available at: tmsj.trakya.edu.tr

Cite this article as: Özkan HS, Kayıkçıoğlu M. Pathophysiology, clinical features and treatment of microvascular angina: a review. Turkish Med Stud J 2020;7(3):154-8.

The subendocardial layer of the heart is more vulnerable to ischemic changes than subendocardial vessels, which are more compliant as they are thinner. Hence as suggested by the foundational law of compliance, they are more likely to collapse after negative volume changes.

It's well understood that a flow redistribution with a decrease in the flow rate from subendocardium to subepicardium occurs with each myocardial contraction, and the more compliant vessels of the subendocardium induce a greater resistance to the flow (7).

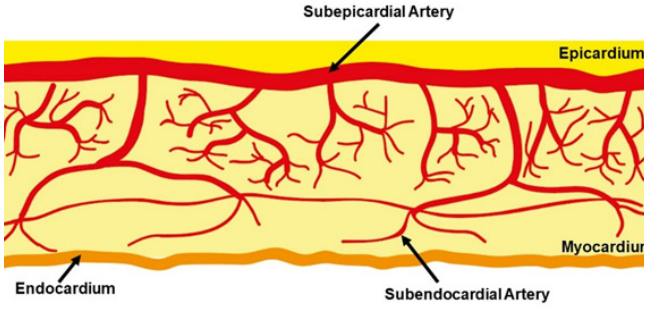

Figure 1: Branching of subepicardial vessels to the subendocardium.

On the other hand, there's another mechanism that can result in decreased perfusion by increasing the resistance against blood flow: abnormal constriction or decreased vasodilation of a coronary artery.

This mechanism is currently the main reason behind MVA (8). However, these erroneous vasodilative properties occur due to a phenomenon known as coronary microvascular dysfunction (CMD) and it is associated with different levels of both vasoactive (nitric oxide) and vasoconstrictive (endothelin-1) substances in different situations.

Coronary microvascular dysfunction can be further divided to 5 subtypes. Type 1, also known as primary CMD occurs in the absence of any previous myocardial disease or epicardial obstruction. Type 2 accompanies myocardial pathologies such as hypertrophic cardiomyopathy. Type 3 occurs in the case of an obstructed coronary artery and Type 4 is iatrogenic and secondary to myocardial revascularization. Finally, Type 5 follows cardiac transplantation (9).

Coronary microvascular dysfunction in MVA seems to appear alone in most cases, therefore can be named as CMD Type 1. Patients with other CMD types are diagnosed more rare than Type 1 (10).

Coronary microvascular dysfunction is a presentation of endothelial cell dysfunction that is associated with hypertension, diabetes mellitus, smoking, hypercholesterolemia and with elevated levels of homocysteine.

Homocysteine is known to inhibit vasodilation substances of endothelium such as nitric oxide. Methylenetetrahydrofolate reductase mutations, an enzyme responsible for break down of homocysteine, are also identified in MVA patients (11).

Among the other things, insulin-resistant or insulin-deficient states are also related to endothelial dysfunction, so can contribute to the pathophysiology of MVA (12).

The increased diastolic time is the backbone of another potential mechanism. Gender plays a significant role in this mechanism, older women whose arteries are stiffened tend to have left ventricular hypertrophy resulting in both decreased pressure and time of diastole to supply the coronary flow. It is already known that a decrease in diastolic duration from 33 to 27 s/min can have the same effect as an increase in coronary stenosis from 40% to 90% (13).

Histopathological features of MVA are mainly thickened small arteriolar walls due to medial layer hypertrophy, intimal proliferation and shorter small vessels (14).

CLINICAL FEATURES

Microvascular angina has a clinical presentation of anginal chest pain, normal or almost-normal epicardial coronary angiography without any spasms and myocardial ischemia in noninvasive stress tests.

Systemic hypertension with or without left ventricular hypertrophy, atypical CAD presentation with diabetes mellitus and as previously stated also from extracardiac causes of chest pain, especially those involving esophagus is a necessity for the specific diagnosis (15). Another common cause of coronary spasm is variant angina (prinzmetal angina). Variant angina patients are usually present with normal coronary angiography although the disease affects subepicardial vessels, and tend to have lower cholesterol and triglyceride levels compared to MVA patients. Additionally, MVA patients have a decreased endothelium-dependent vasodilation of brachial artery after application of a vasodilator compared to the variant angina patients, which suggests a systemic rather than local dysfunction of microvascular bed (2).

Chest pain in MVA is similar to obstructive CAD, mimicking the radiation to the medial part of left arm and substernal compressing pain. However, it occurs at rest in many patients unlike typical angina and can

last hours and does not respond to nitroglycerin as well as in the typical angina. It is not known to cause systolic ventricular dysfunction (can be determined by a decrease in ejection fraction) as common as it does in CAD. The phenomenon behind this is the layer-selective nature of CMD which only affects the thin layers of the myocardium, thin enough to be supplied also by thin vessels, and does not cause significant tissue damage as in CAD (10).

The electrocardiography (ECG) in MVA is similar to CAD, and shows significant changes in ST-segment such as depression, rarely an elevation during stress. An ambulatory 24-hour-ECG is also no different than chronic stable angina occurring in CAD with circadian episodes of ST-segment depression. ST-segment changes occur in a prolonged manner in MVA than CAD, and mostly accompanied by tachycardia in order to increase blood flow through narrowed arteries. The main characteristics of MVA can be summarized as atypical anginal features (long episodes of chest pain lasting sometimes hours and poor nitrate response), exercise-induced chest pain due to the ineffectiveness of physiological vasodilatory mechanisms and pain occurring even at rest, there is no significant systolic ventricular dysfunction present and rare myocardial ischemia can be seen afterward. MVA is more common in women with estrogen deficiency and women have a poorly defined ECG stress test response (15). Hence, a post-menauposal female patient, complaining of prolonged atypical angina pectoris or angina pectoris at rest, should be considered for MVA in the differential diagnosis.

Table 1 compares different causes of angina pectoris by pathophysiology, ECG changes and clinical features.

Table 1: Different characteristics of causes of angina pectoris.

Causes of Angina Pectoris	*Affected Vessels*	*ECG Changes*	*Clinical Features*
Microvascular Angina	Subendocardial vessels	Circadian episodes of ST-segment changes (elevation or depression)	Prolonged chest pain of angina characteristics which may occur both at rest or exertion, lasting hours to days and responds poorly to nitrates, accompanied by tachycardia
Variant (Prinzmetal) Angina	Subepicardial vessels	Transient ST-segment elevation (≥0.1 mV)	Chest pain of anginal characteristics, poor response to nitrates and occurs at rest
Coronary Artery Disease	Every vessel in coronary circulation, mostly affecting larger vessels	Permanent ST-segment changes, mostly ST-segment depression (in chest leads ≥0.2 mV, in extremity leads ≥0.1 mV)	Chest pain of anginal characteristics, good response to nitrates, occurs at rest or stress, often last minutes

DIAGNOSTICS

Although there are studies with non-invasive tests to assess indirect myocardial resistance to perfusion during stress, typically administering a systemic vasodilator such as adenosine, the perfusion assessment is not sensitive enough to determine if the problem occurs in the epicardial layer or subendocardial layer.

Invasive coronary angiography can provide valuable information by guide wire-based assessment of the blood flow through coronaries at rest (at the time, where the vessels are vasodilated) and also by using pharmacological such as acetylcholine to mimic vasoconstriction.

The preferred vessel is the left descending coronary artery for this procedure because of its great impact on the perfusion of the myocardium. Multiple vessels can be interrogated but as the duration of the process gets longer the risk increases as well (16).

Nonetheless, in order to perform an invasive procedure such as coronary angiography the patient should exhibit the clinical presentations of angina. If the patient fits the MVA diagnosis criteria with atypical anginal features (long duration, poor response, occurring at rest) and presents with normal epicardial vessels in angiography but only with CMD alone and no other comorbidities, as in most of the cases, should be diagnosed as primary MVA accompanied by CMD Type 1. CMD can be detected by following angiographical parameters: impaired coronary flow reserve (≤2.0), coronary microvascular spasm (not accompanied by epicardial spasm after acetylcholine stimulation), abnormal coronary microvascular resistance indices (Index of Microcirculatory Resistance>25) and coronary slow flow phenomenon (TIMI frame count >25) (17).

In addition to the etiologies behind, CMD can also be classified as either structural CMD (SCMD) or functional CMD (FCMD). FCMD is characterized by low vascular tone both at rest and exercise, and can be recognized with increased nitric oxide synthetase (NOS) activity, normal forearm acetylcholine dilatation (FAD) during angiography and normal exercise blood pressure (EBP) and decreased myocardial coronary perfusion efficiency and slightly elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) levels. SCMD occurs due to high vascular tone both at rest and exercise, and can be recognized with increased NOS activity, however with reduced FAD and higher EBP levels and significantly increased levels of NT-proBNP and a decrease in myocardial coronary perfusion efficiency (18). Positron emission tomography with myocardial blood flow quantification can also be used to detect microvascular dysfunction with less invasive diagnostic tool, which needs further studies (19).

TREATMENT

Conventional anti-anginal drugs (nitroglycerine, isosorbide dinitrate, isosorbide mononitrate and erythrityl tetranitrate etc.) are proven to be poorly effective against MVA therefore a different approach should be applied to these patients.

As first-line treatment, beta-blockers are shown to be effective especially with patients of effort angina with increased heart rate (HR) on effort or patients with symptoms of increased adrenergic activity such as increased HR at rest. Ivabradine, a drug acting on If channels mostly located on the sinoatrial node, therefore used to induce bradycardia can be used but there is no evidence supporting its effectivity. To the patients with angina at rest, calcium channel blockers of non-dihydropyridine type, mostly preferred for their anti-arrhythmic properties in other settings, such as diltiazem and verapamil should be effective. Although, oral nitrates are proven to be not effective, nicorandil, a potassium channel activator shown to be effective in some studies, however another agent, ranolazine that reduces late sodium current is promising. In addition to all these, angiotensinogen converter enzyme inhibitors diminishing the oxidant feature of angiotensinogen II and decreasing microvascular dysfunction and alpha blockers, which interfere with adrenergic stimuli can be second-line treatment options. Also, statins are proven to be effective by improving the microvascular dysfunction (8, 20). In post-menopausal women, estrogens can also be a part of the treatment (10).

CONCLUSION

Microvascular angina as an insidious condition, mimicking many other similar etiologies, can easily be overseen, thus falsely diagnosed and managed by physicians. MVA patients are diagnosed mostly with extracardiac causes of chest pain or receive the classical antianginal drugs such as nitroglycerin. However, as the antianginals are poorly effective against MVA, the patient continues to suffer and due to the increased adrenergic stimulation the patient tends to feel the pain intensely. The increased mortality rate is another proof of the importance of this condition (4). Therefore, rec-

ognition of a patient with MVA by any physician is of utmost importance, just not to relieve the pain of the patient but also to avoid the possible follow-up comorbidities and mortality.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: HSO, MK Design: HSO, MK Supervision: HSO, MK Resources: HSO, MK Materials: HSO, MK Data collection and/or processing HSO, MK Analysis and/or Interpretation: HSO, MK Literature Search: HSO, MK Writing Manuscript: HSO, MK Critical Review: HSO, MK
Financial disclosure: The authors declared that this study received no financial support.

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TMSJ
TURKISH MEDICAL STUDENT JOURNAL
REVIEW

MYOCARDIAL INFARCTION DIAGNOSIS AND CARDIAC TROPONINS

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ABSTRACT

Acute myocardial infarction is a condition that develops due to the blockage of blood flow to the heart. Serial electrocardiography follow-up should be performed in patients with suspected acute myocardial infarction, serum cardiac troponin levels should be measured, and this measurement should be repeated at regular intervals. Cardiac troponins are the main markers for the diagnosis of acute myocardial infarction, as they are sensitive and specific biochemical markers of myocardial cell necrosis. Elevated levels of cardiac troponins indicate cardiac damage, but it does not explain the cause of the damage. Increases in cardiac troponin levels can be observed in many different disease states and do not necessarily indicate acute myocardial infarction. It is necessary to check cardiac troponin levels in a patient admitted to the hospital in order to exclude other diseases before establishing the diagnosis of myocardial infarction. The one-hour "rule-in" and "rule-out" algorithms are used in the tests performed on patients who come to the emergency department with non-ST elevation myocardial infarction. In addition, there are point-of-care cardiac troponin tests that can be used in emergency services and ambulances. However, while using point-of-care cardiac troponin tests for the diagnosis of myocardial infarction, it should be kept in mind that these tests are less sensitive and more costly than tests performed in central laboratories. ***Keywords:*** Myocardial infarction, acute coronary syndrome, troponin

INTRODUCTION

Cardiac troponins (cTn) are cardiospecific markers of ischemic myocardial damage that are used in the diagnosis of acute coronary syndrome (ACS) and the clinical diagnosis of myocardial injuries with different etiology and pathogenesis (1, 2).

As a result of the acceleration of biochemistry studies, the importance of cardiac troponins in the diagnosis of acute myocardial infarction (AMI) has been understood. Compared to other markers, it is tissue-specific and gives rapid results, which has led academic studies to focus on cardiac troponins (3).

CARDIAC TROPONINS

Cardiac troponins regulate the calcium-dependent interaction of actin and myosin and play a role in myocardial contraction. There are 3 subforms of cTn: cTnT, cTnI, and cTnC (4).

Structural Differences

When we examine the structural differences between cTns, there are 2 serine amino acids at the amino-terminal end of cTnI. These amino acids induce a cyclic adenosine monophosphate (cAMP) dependent phosphorylation. This situation reduces the affinity of cTnC to calcium. The reason for the low cardiac specificity of cTnC is its similar structure with its isoform which is found in skeletal muscle. However, the cardiac specificities of cTnT and cTnI are quite high. The reason for this is that they are encoded by genes different from the genes encoding their isoforms in skeletal muscle (4).

Functions

Cardiac troponins are sensitive and specific biochemical markers of myocardial cell necrosis. Even small damages in the myocardium can be detected by

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Received: 07.07.2020 Accepted: 17.08.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.07 Available at: tmsj.trakya.edu.tr

Cite this article as: Kurtoğlu B, Erkılıç B, Gürsel DY et al. Myocardial infarction diagnosis and cardiac troponins. Turkish Med Stud J 2020;7(3):159-70.

Table 1: Basic information on cardiac troponins. Table reprinted from reference (1).

	cTnI	cTnT
Source in the body	Myocardium	Myocardium
Molecular Weight (Dalton)	23.876	37.000
Biological half life	<120	120
Maximum concentration in the blood (hours)	12-30	12-75
Persistence in blood (days)	4-7	4-12
Kidney elimination	Happens	Does not happen
Increase after AMI (hours)	3-5	3-4
Applicability for ACS diagnosis	Similar	Similar
Efficiency in cardiovascular risk classification	Similar	Similar
Biphasic waveform during successful and early reperfusion	Less separator	Separator
Applicability for reperfusion assessment	Happens	Happens
Reinfarction diagnosis	Limited use due to long term persistence in the blood	Limited use due to long-term persistence in the blood
Increase in patients with stage 5 chronic kidney disease (National Kidney Foundation)	Less separator	Separator
Maximum limit of upper reference limit	300x	300x
AMI focal size estimation	Happens	Does not happen
Sampling timing for diagnosing AMI (CSCB, 2008)	6-9 hours upon arrival, 12-24 hours if negative	6-9 hours upon arrival, 12-24 hours if negative

cTnI: *Cardiac troponin I.* ***cTnT:*** *Cardiac troponin T* ***AMI:*** *Acute myocardial infarction* ***ACS:*** *Acute coronary syndrome.*

troponin measurements with high sensitivity (5). Cardiac troponins are used for the diagnosis of cardiovascular diseases due to myocardial necrosis and to evaluate the possibility of worsening prognosis (Table 1) (1).

For the early period, cTn sensitivity is higher than creatine kinase-MB (CK-MB). The reason for this is the presence of 13-15 times more cTn in the myocardium than the amount of CK-MB per gram (6, 7). Both the American College of Cardiology (ACC) and the European Society of Cardiology (ESC) recommend cTn as a biochemical marker for the diagnosis of AMI for these reasons (8, 9).

The specificity of cTn is high for the diagnosis of coronary ischemia. In addition, levels may increase in clinical conditions such as acute and chronic heart failure, cardiac contusion, cardioversion, pacing, ablation, endomyocardial biopsy, and hypertensive crisis (10). Furthermore, ACC and the American Heart Association determined that cTn can be used as a marker in the prognosis of unstable angina pectoris (11-13). Morbidity, mortality, and incidence in clinical pictures such as arrhythmia, ACS, pulmonary embolism, and stroke were examined and cTnT was observed to be a better marker in terms of showing the long-term mortality risk after an AMI (14).

It is necessary to exclude other diseases by examining cTns before reaching the final diagnosis of MI. A detailed history and physical examination of the patient are necessary to accurately identify the underlying cause, especially in small increases in serum cTn levels (15).

DIAGNOSTIC VALUE OF TROPONINS IN THE DIAGNOSIS OF ACS AND AMI

Acute myocardial infarction (AMI) is a condition that develops due to the blockage of blood flow to the heart (16). It is the coronary arteries that supply oxygen and nutrients to the heart. If the blood flow is interrupted in these arteries, the necessary oxygen, and nutrients cannot be provided, resulting in heart damage. If this situation develops suddenly, it is defined as AMI. However, when these arteries are partially obstructed, the amount of blood flow decreases, and a chest pain called angina occurs. This situation is an indicator of MI (17).

Clinically, ACS is a group of diseases that result in acute myocardial ischemia. ACS is a term that includes patients who have signs of acute myocardial ischemia, unstable angina pectoris, and MI with or without ST-segment elevation (18). The main objectives of the physician who encounters a patient with ACS are as follows: Keeping the left ventricular functions in place by minimizing myocardial cell death in the patient with MI and preventing the occurrence of heart failure to eliminate acute and life-threatening symptoms such as ventricular fibrillation/pulseless ventricular tachycardia, symptomatic bradycardia, unstable tachycardia (12).

In AMI; pressure, tightness, pain, or squeezing sensation radiating to the chest, arm, neck, jaw, or back; nausea, indigestion, heartburn or abdominal pain, shortness of breath, cold sweat, fatigue, and sudden dizziness are the signs and symptoms expected to be seen in the patient (16). While constant pain is being observed in 80% of the patients, the pain is observed in an accelerated manner in 20%. In another aspect, the incidence of atypical clinical pictures in ACS cases are also quite high. These atypical clinical scenarios are commonly seen in the young (25-40 years old), elderly (>75 years old), female patients, and in the patients with a history of diabetes (19). In patients with long-term diabetes history, the patient may not feel pain as a result of the effect of diabetes on the patient's nerves (17). In particular, pain during rest, epigastric pain, acute digestive disorders, stabbing chest pain, chest pains with some pleuritic features, or worsening dyspnea are atypical symptoms of unstable angina. Consequently, a reliable determination cannot be made in the diagnosis of AMI by assessing solely the character of the pain (19).

During the physical examination, most patients show signs of autonomic nervous system activation (sweating, pallor), hypotension, or conditions similar to a narrowed pulse pressure range. In addition, irregular heart rate, bradycardia or tachycardia, third heart sound, or basal rales may be encountered. The main purpose of physical examination is to eliminate non-cardiac conditions that may cause angina, cardiac diseases such as pericarditis, valvular diseases without ischemia, potential non-cardiac triggers, and pneumothorax in diagnosis; to examine left ventricular dysfunction and potential causes of hemodynamic instability (19).

In Patients With Suspected Acute Ischemic Myocardial Damage (19):

At rest, electrocardiography (ECG) should be taken and monitored continuously (The first ECG allows a correct diagnosis rate of only 50%. Therefore, serial ECG follow-up should be done especially during the patient's complaints.).

Cardiac troponin T (cTnT) or cardiac troponin I (cTnI) values should be measured at the first examination of the patient, and if normal values are observed, these measurements should be repeated at regular intervals.

Even though myocardial infarction (MI) can be diagnosed by symptoms, electrocardiographic abnormalities, elevated biomarkers of myocardial necrosis, and imaging such as echocardiography (ECHO); the patients without abnormal ECG, ECHO, and the patients who do not present with chest pain should be considered in terms of MI (20, 21).

Due to the similarity of symptoms and inconclusive ECG findings, several prominent international cardiac associations have made a universal definition of MI in 2012. According to this definition, "detection of a rise and/or fall of cardiac biomarker values, preferably cardiac troponin, with at least one value above the 99th percentile upper reference limit and at least one other diagnostic indicator such as wall motion abnormality that can be seen through imaging" is required for the diagnosis of MI (22).

Before the development of cardiac troponin sensors, only the electrocardiogram was used to diagnose acute myocardial infarction, and it was commonly misdiagnosed. Following the discovery of the significant correlation between the cardiac troponin levels and the onset of the AMI, cTnT and cTnI were used to diagnose AMI by detecting their levels in blood by using immunological assays with a recommended turnaround time of less than 1 hour by the National Academy of Clinical Biochemistry (23-25). Afterward, in Germany, faster techniques such as cTnT rapid strip tests were developed based on a sandwich enzyme-linked immunosorbent assay (ELISA), but this test was used for the patients who were already diagnosed with AMI (26).

In comparison between troponins, some studies in the literature stated that, among two cardiac enzymes, serum cTnT level has significantly higher sensitivity in the diagnosis of MI, whereas serum cTnI level has higher specificity (Table 2) (27-30).

Table 2: Comparison of cardiac troponins.

Study	Cardiac troponin	Test	Critical value (ng/mL)	Specificity (%)	Sensitivity (%)
Avcıküçük et al. (27)	cTnT	Roche	>0.03	70	92
	cTnI	Siemens Dimension	>0.017	90.9	80
Pagani et al. (28)	cTnT	Roche	>0.03	68.1	98
	cTnI	Beckman	>0.04	78.7	100
Elmalı et al. (29)	cTnT	Roche	>0.03	91	63
	cTnI	Beckman	>0.04	92	63
Ross et al. (30)	cTnT	-	-	-	-
	cTnI	Siemens Status II Analyzer	>0.6	81	94

cTnT: *Cardiac troponin T* ***cTnI:*** *Cardiac troponin I.*

Hemolyzed samples are one of the most important sources of preanalytical error. In the measurements made with hemolyzed sera, cTn values were found lower than they were supposed to be. Another source of error is biotin interaction. False high results of troponin values are seen in blood samples of patients who take too much biotin (31).

Various disease states besides the AMI can also cause serum troponin values to rise. Kalyon S (31) stated that other than AMI, non-coronary causes such as pulmonary embolism, myocarditis, pericarditis, sepsis, long-term exercise, heparin, and immunotherapy can result in elevated troponin levels in blood. In the study of Meyer et al. (32) an elevated level of cTnI was defined in 40% of patients with pulmonary embolism.

In the review article written by Lum et al. (33) reasons such as fibrin interaction, high level of alkaline phosphatase (ALP), and the interaction of immunocomplex formation were given to cause false troponin positivity. Bohner et al. (34) documented the first false troponin negativity with their study in 1996, they found that the reason for this was immunoglobulin G (IgG), which is an analyte-binding antibody and prevents cTnI recognition.

High Sensitivity Cardiac Troponins (hs-cTn)

Most of the clinics choose to use 5th generation hs-cTnT and hs-cTnI analysis rather than traditional cTn tests. These tests can demonstrate 10-100 times lower concentrations of troponin. For a patient who has come to the emergency room, the negative predictive hs-cTn value is >95% to eliminate the diagnosis of AMI. When the test is repeated after 3 hours, this rate can increase up to 100% (35).

Although these tests have high specificity, they cannot determine necrosis etiology alone. In addition, industrial standardization of hs-cTn tests has not been implemented yet. Therefore, there are difficulties in comparing them among medical systems (36).

Evaluation of Results

The one-hour "rule-in" and "rule-out" algorithms are used in patients who come to the emergency department with non-ST elevation MI (NSTEMI). As 0-1 hour refers to the time when the blood test was first performed; whether the test verifies the MI or not depends on the type of the troponin used in the test kit. There are many test kits that use this algorithm (37):

The high sensitivity cardiac troponin T-test is a high sensitivity immunological test that is used for the determination of cTnT in human serum and plasma. If the cTnT level in the blood sample at 0th hour is less than 12 ng/L and the difference between the samples between 0th-1st hours is <5 ng/L, MI is excluded. If the cTnT value of the blood sample at the 0th hour is ≥52 ng/L and the difference between the samples between the 0th-1st hours is ≥5 ng/L, then MI is considered.

The high sensitivity cardiac troponin I test is a highly sensitivity test used for the determination of the cTnI in serum. If the cTnI value in the blood sample at the 0th hour is <5 ng/L and the difference between the blood samples between 0th-1st hours is <2 ng/L, MI is excluded. If the value of the cTnI in the blood sample at 0th hour is ≥52 ng/L, and the difference between the value of samples between 0th and 1st hour is ≥6 ng/L, then the possibility of MI is considered.

For a patient who has chest pain for more than 3 hours; if the cTnT level for the hs-cTnT test is 5 ng/L, and the cTnI value for hs-cTnI test is <2 ng/L in the sample serum which is immediately taken, the possibility of NSTEMI is excluded.

SERUM CARDIAC TROPONIN ELEVATION OBSERVED IN OTHER DISEASES AND THEIR ROLE IN DIFFERENTIAL DIAGNOSIS

The most common reason that may increase the serum cTn levels is ischemic necrosis due to perfusion-restricted or blocked mural or occlusive thrombus in the coronary artery. However, high cTn concentration in the blood can be a sign of ischemic and/or non-ischemic myocardial damage. These damages may also be the result of the non-atherothrombotic events; such as mechanical cardiomyocyte disorders due to myocardial damage, the cumulative effect of potential cardiotoxic drugs, bacterial toxins, or a neurohumoral reaction (1).

Elevated cTnI levels indicate cardiac damage, but it is insufficient to explain the cause of the damage. Increases in cTn levels can be observed in many different disease states and do not necessarily indicate AMI (5). Other causes include cardiac muscle inflammation, various etiologies to cause circulatory failure (e.g. following polytrauma, multiple organ failure associated with sepsis, severe burn, etc.), systemic hypotension that can lead to myocardial ischemia, and renal failure which may result in increased levels of cTn (especially

cTnT) without any signs of infarction. Poisoning can also be counted among the causes. Carbon monoxide poisoning is quite common, and the decrease in the oxygen-carrying capacity of hemoglobin may cause myocardial hypoxia and increase in cTn (1).

Sometimes, prognostically, there may be elevated cTn concentrations owing to myocardial damage of iatrogenic origin following diagnostic or therapeutic procedures associated with coronary vessel catheterization and all cardiovascular procedures (1).

In the differential diagnosis, it may be difficult to interpret the serum cTn elevations in cases such as stroke, chronic obstructive pulmonary disease (COPD), pulmonary embolism, sepsis, exercise, acute perimyocarditis, Takotsubo cardiomyopathy (TC), acute heart failure, hypovolemia, renal failure, and tachycardia. The basic treatment strategy of these patients with increased cTn levels and various non-acute coronary syndromes is to eliminate the factors that cause an increase in cTn levels (5).

Chronic Renal Failure

Increases in cTn may be an indicator of poor prognosis in end-stage renal failure. It has been shown by several animal experiments that trauma and stress induce cTn in striated muscle (38). It is assumed that chronic striated muscle deformation and inflammation in dialysis patients induce cTn with a similar mechanism. Heart failure is a frequent comorbidity seen in renal failure in which elevated cTn levels occur without signs of ischemia or infarction (38).

In addition, decreased renal clearance level due to renal failure is another reason that may cause elevated cTn levels. In the presence of renal failure in a patient, the removal of cTn from the body is interrupted by the routine loss of myocytes. In these patients, increases in cTnT levels are observed more commonly than increases in cTnI levels due to differences in the elimination of cTnI and cTnT from the body (38).

Chronic Obstructive Pulmonary Disease (COPD)

Cardiovascular risk factors and cardiac comorbidity are conditions that are frequently observed in individuals with COPD. The more negative intrathoracic pressure results in increased left ventricular afterload. Increased pulmonary tension, hypoxia, and hypercapnia cause cardiac muscle deformations during acute exacerbation (38). Neukamm et al. (39) observed that stable COPD was independently associated with higher hs-cTnT levels in peripheral blood when randomly drawn individuals from the general population was compared with COPD patients in a stable state. In individuals with stable COPD, increased levels of hs-cTnT have been proven to be associated with immune activation and the degree of the disease. In addition, a positive correlation was found between neutrophil and serum cTn levels in COPD patients. On this account, it can be said that the excessive inflammatory response in COPD exacerbations causes damage to the heart muscle (40). It is demonstrated that increased cTnT level during COPD exacerbation is associated with increased mortality rates in the first year following discharge (39).

Strenuous Exercise

Both intense and light exercise can be misleading if the patient is suspected of MI due to high troponin levels (41). Physicians should be well informed about the exercise-induced cTn elevation and carefully evaluate the patient in terms of exercise history before diagnosing MI (42).

Studies have shown that myocardial demand, which increases in parallel with endurance exercise, can physiologically increase the turnover rate of cTns. Another possibility is the accumulation of stress-induced free radicals, which causes an inconsistent increase in myocyte membrane permeability and can cause troponin leakage from the cell cytosol (38).

However the mechanism works, there are many differences between the kinetics of asymptomatic cTn release after intensive exercise and the kinetics of the increase in serum cTn levels in ACS. Early peaking and rapid return to normal characteristics can be shown as examples (38). Exercise time, difficulty, and working distance are variables that affect the cTn increase, however, it has been shown that even short distance work/workout can cause serious cTn release in untrained people. Considering the available information, there is no prognostic effect of increased cTn level (38).

Takotsubo Cardiomyopathy

Takotsubo Cardiomyopathy (TC) is a disease that develops due to left ventricular dysfunction as a result of severe emotional or physical stress (43). Stress-induced TC shows similarities with ACS in ECG measurements and elevated cardiac enzyme levels. Therefore, the diagnosis of this syndrome depends on the exclusion of ACS (44). Cardiac troponin increases in

90% of patients who have TC. However, these increases are lower than the increase we observe in ST-segment elevation myocardial infarction (STEMI). The exact mechanism for the coordinated cTn increase in this syndrome is unknown. The possible mechanisms are demonstrated as; multi-vessel coronary artery spasm, ACS with reperfusion damage, impaired cardiac microvascular function, impaired myocardial fatty acid metabolism, and myocardial micro-infarction caused by endogenous catecholamines (15).

Sepsis and Septic Shock

Sepsis is seen as one of the most common causes of mortality in intensive care units. Organ dysfunction and multi-organ failure characterized by sepsis are encountered as well (45).

A decrease in ventricular performance is observed in approximately 50% of the cases with severe sepsis and septic shock (38). In addition, a significant relationship was found between sepsis, low cardiac function, and high stroke volume (46). Otherwise, 43-85% of patients who receive sepsis or systemic inflammatory response syndrome treatment in the intensive care units of hospitals have increased cTn values. Many studies prove that this increase in serum cTn levels is associated with mortality in sepsis cases (38). The mechanism of sepsis and its accompanying cTn increase is still not fully proven, but there are many theories about it. One of the accepted theories is based on the assumption of myocardial ischemia. According to this theory, increased oxygen demand in the myocardium is related to fever and tachycardia commonly observed in sepsis (38). Additionally, respiratory failure, hypotension, microcirculation dysfunction, and occasional systemic hypoxemia caused by anemia can result in a decreased amount of available oxygen to the myocardium. The myocardium releases cTn by being damaged due to mismatched supply and demand for oxygen (38). Apart from this theory, there are also theories explaining the increase in cTn level in sepsis with myocardial damage and myotoxic effect resulted from reactive oxygen radicals produced by endothelial cells, macrophages, and effective neutrophils triggered by inflammation (38).

OTHER BIOCHEMICAL PARAMETERS FOR DETERMINING AMI

Although cTns, which are indicatives of myocardial ischemia, are used as the main biochemical markers, the increase in cTns in some other diseases can be misleading in terms of AMI diagnosis (5, 38-46). However, it should not be forgotten that besides cTns, other plasma markers can also be used in the diagnosis of AMI (47).

Electrocardiogram (ECG) will be the first diagnostic procedure to be performed for the patient with suspected MI, however, ECG is not sufficient for diagnosis of MI since ECG changes may not be observed in all patients having MI. In addition to the ECG, cardiac markers should also be checked (47).

An ideal marker should have high tissue specificity, be intense in tissue and rise rapidly in blood after damage. The time when it is high in blood should be also long enough for measurement. There must be a direct proportion between the extent of the damage and the concentration of the marker in the blood. Its quantitative measurement can be made and it should not cost too much (48).

Creatine kinase (CK), CK-MB, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), myoglobin, and troponins are some markers that can be examined in plasma for MI (Table 3) (47-49).

Myoglobin is the first cardiac biomarker to appear in the blood after MI. It starts to rise in 2-4 hours, peaks in 12-24 hours, and declines within 7-10 days (50).

Troponins start to rise in blood subsequent to the myoglobin. It rises in 4-6 hours, peaks in 12-24 hours, and declines within 7-10 days. Cytosolic troponins are released relatively earlier compared to the troponins found in the contractile structure. The stage of the damage can be determined by assessing the levels of the different troponin variants (50).

Following the troponins, total CK rises in 4-6 hours, peaks in 24-48 hours, and declines within 2-4 days. Aspartate aminotransferase (AST) rises in 6-8 hours, peaks in 24-48 hours, and declines within 4-6 days.

Finally, LDH rises in 12-24 hours, peaks at 48-72 hours, and declines within 12-14 days (50).

Knowing these values tells us at what time to look at which indicator and shows when to do the next test. If the marker remains high in the blood for a long time, it is important to evaluate the risk of subsequent infarction. It is difficult to diagnose a subacute infarction by markers with a short half-life (48).

Although AST has a high sensitivity in infarction, it is not specific for cardiac tissue. In cases such as hepatic congestion secondary to heart failure, myocarditis, post-cardioversion, pericarditis, etc., AST levels may be elevated in the blood (49).

Lactate dehydrogenase is a cardiac enzyme with 5 isoenzymes. LDH-1 mainly exists in cardiac myocytes and erythrocytes (51). The level of LDH-2 in the blood

Table 3: MI markers and the chronological history (49).

Name of the marker	The years it started to be used	Limitations
AST	1950s	It has less specificity for myocardial tissue.
LDH	1950s	It has less specificity for myocardial tissue.
CK	1960s	Because it is also found in the skeletal muscle, it is found in high levels in the blood in other diseases.
CK-MB	Late 1970s	1. Low specificity for cardiac injury. 2. It cannot be used for late diagnosis due to its early release into the blood.
cTn	Late 1980s	Although it is specific to myocardial necrosis, it cannot differentiate ischemic myocardial injuries from non-ischemic myocardial injuries.

AST: *Aspartate aminotransferase* ***LDH:*** *Lactate dehydrogenase* ***CK:*** *Creatine kinase* ***CK-MB:*** *Creatine kinase muscle and brain* ***cTn:*** *Cardiac troponin.*

of a healthy individual is higher than the LDH-1 isoenzyme, however, this rate varies in favor of LDH-1 in MI. This is also referred to as the "flipped LDH pattern phenomenon". Nonetheless, since LDH-1 is also high in erythrocytes, this phenomenon may occur outside of MI due to an event that may cause hemolysis (e.g. many diseases such as hemolytic anemia, pancreatitis) (52).

Myoglobin is found in the skeletal muscle and cardiac tissue. The advantage of myoglobin is that it is released earlier than other markers, through its low molecular weight. In this way, early diagnosis can be provided in the presence of AMI (53). The biggest disadvantage is that it is not specific to the heart tissue due to its abundance in the skeletal-muscular system. A number of studies in the literature stated that it can only show 90% sensitivity in AMI (53, 54).

Creatine kinase (CK) has 3 isoenzymes: MB, BB and MM. Similar to myoglobin, CK is also found in many tissues other than cardiac muscle tissue, especially in skeletal muscle tissue. That's why high levels of CK does not necessarily indicate cardiac tissue necrosis (53). The success rate in the diagnosis of infarction can be increased by calculating the CK-MB/Total CK ratio (55). Cardiac troponins are more sensitive and specific in the diagnosis of MI than CK-MB, which was formerly known as a gold-sensitive cardiac marker (3). However, since troponin serum levels begin to fall over the next 4-10 days down to normal levels; CK-MB is still used in the diagnosis of reinfarction (56).

Troponins have been found more successful than CK-MB in determining extensive infarction (55). In addition, increased troponin levels may be informative in terms of the risk of adverse cardiac events (53).

As a result, cardiac troponins are markers with high specificity and high sensitivity. The measurement of troponin in the blood has a dual role: Abnormally high concentrations indicate AMI, whereas mildly elevated troponin levels with chest pain indicate 5 times higher risk for a cardiac event over the next 4-6 weeks (3).

In patients without ST elevation, the baseline cTnI levels correlate with 6-week mortality. For this reason, troponin levels are monitored periodically after cardiac operations (57).

Parameters such as high sensitivity C reactive protein (hs-CRP), cardiac myosin light chain (MLC), D-Dimer, heart-type fatty acid binding protein (h-FABP), ischemia modified albumin (IMA), myeloperoxidase (MPO), glycogen phosphorylase isoenzyme BB (GP-BB), and carbonic anhydrase isoenzyme III (CA III) are other cardiac markers that can be found in the literature (51).

THE IMPORTANCE OF POINT-OF-CARE TESTS IN AMI DIAGNOSIS

Central laboratories are used to assess cTn concentrations as a regular method, but these laboratories can provide information with a one-hour recommended turnaround time (58). The use of rapid diagnostic tools is essential in the emergency departments and in ambulances notably for the acute coronary syndrome. Neumann et al. have shown that prevention of ischemic events that occur while waiting for the delayed procedure can be achieved by early intervention (59).

However, point-of-care (POC) cardiac troponin tests do not perform as good as hs-cTn tests and are not considered as troponin tests with high sensitivity (37). Tests provided by central laboratories are more sensitive than point-of-care cardiac troponin tests. Nonetheless, central laboratories are not available 24 hours a day and laboratories cannot be used in various settings such as ambulances and emergency rooms where rapid decision-making is crucial. In such cases, POC cTn tests provide results in 10 to 20 minutes by a shorter turnaround time for cardiac biomarker detection (60).

However, a Japanese study has shown that in order to use POC cTnI level for an ACS diagnosis, sampling should be performed more than 3 hours after the onset of symptoms (21). Similarly, in a guideline published by ESC in 2015, it is stated that POC cTn test has a low diagnostic sensitivity as a first-line analysis since it takes time for cTn to reach a significant detectable level (37).

Furthermore, the price of the tests should also be taken into account. Bingisser et al. (60) and the Canadian Agency for Drugs and Technologies in Health (CADTH) state that POC testing is not cost-effective compared to central laboratory testing (61).

CONCLUSION

Acute myocardial infarction develops when blood flow to the heart is blocked (16). Electrocardiographic changes, particular cardiac biomarkers indicating myocardial ischemia and ECHO can be used in the diagnosis of AMI. However, there are also patients without abnormal ECG, ECHO, or chest pain-like findings (21, 22). It should be kept in mind that the first ECG measured allows only 50% accurate diagnosis in AMI; therefore, markers indicating myocardial necrosis in such cases are among the most helpful agents for physicians to make an accurate diagnosis (19).

Some markers examined in plasma for MI can be listed as CK, CK-MB, AST, LDH, myoglobin, and troponins (47). In the 1980s cTns which are more specific than other markers have been used (49). Cardiac troponins are more sensitive and specific indicators of myocardial damage than CK-MB and ECG (6, 7, 55). Especially I and T isoforms are more specific to cardiac tissue and are used in the diagnosis of MI (1, 4).

Instead of the cTn tests, hs-cTnT and hs-cTnI analyses have started to take place in clinical routine. They show levels of troponin at 10-100 times lower concentration (35). Although hs-cTn tests have high specificity, they are insufficient in determining the etiology of necrosis alone. In addition, hs-cTn tests have not been industrialized yet (36). In cardiac troponin analysis, 0-1 hour "rule-out" and "rule-in" algorithms are used for patients with NSTEMI who come to the emergency room (37). In this way, AMI can be eliminated in the early period, the diagnosis of patients with AMI can be made in a shorter time, and the treatment is initiated.

Point-of-care tests are used as well as hs-cTn tests in the diagnosis of AMI (37). Point-of-care tests are required in places such as emergency rooms and healthcare transporters where the central laboratories are unavailable and rapid interventions are critical (59). Nevertheless, the convenience and the limitations of POC tests should be taken into account in the diagnosis of MI.

To conclude, owing to their high sensitivity and specificity, cardiac troponin levels alert the physicians to the ongoing and recent myocardial infarction and facilitate the early diagnosis of MI (1, 3, 6, 7, 49). Thus, cardiac troponins are clinically crucial for the MI diagnosis.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: YA Supervision: BK, YA Resources: BK, BE, DYG, GA, OB, PA, SK, YA Materials: BK, BE, DYG, GA, OB, PA, SK, YA Data collection and/or processing: BK, BE, DYG, GA, OB, PA, SK, YA Analysis and/or Interpretation: BK, BE, DYG, GA, OB, PA, SK, YA Literature Search: BK, BE, DYG, GA, OB, PA, SK, YA Writing Manuscript: BK, BE, DYG, GA, OB, PA, SK, YA Critical Review: BK, YA
Financial disclosure: The authors declared that this study received no financial support.
Editor in chief's Note: One of the authors of this article, Berra Kurtoğlu is a member of the editorial board of Turkish Medical Student Journal. However, she did not take place in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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TMSJ
TURKISH MEDICAL STUDENT JOURNAL
REVIEW

THE FOURTH HORSEMAN OF 21ST CENTURY: COVID-19 PANDEMIC

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ABSTRACT

As the end of 2020 is approaching, its finest message to all of us has been how a pernicious virus like Sars-CoV-2 can change the pace of the world, by being an impetus to a global pandemic. COVID-19 was declared a pandemic by WHO on March 11, 2020. Since then, it has infected more than 40 million and killed at least 1.1 million people as this review was written (October 2020). However, the 2020's come not only with challenges, such as the pandemic itself, but also with opportunities for possible breakthroughs. Throughout human history, physicians examined their patients by touching them, arranged appropriate treatments by diagnosing them applying both usual and unusual methods, and tried their best to protect public health, in some cases even by testing the drugs and vaccines they discovered on themselves first. Bedside medicine to hospital medicine, followed by laboratory medicine, are the three crucial cornerstones accepted in the medical history. We now face a fourth potential cornerstone-to-be: could the telecommunication of physicians with their patients through the internet become the fourth essential cornerstone in the history of medicine? ***Keywords:*** Covid-19, Sars-Cov-2, pandemic, healthcare

INTRODUCTION

Deadly epidemics have changed and rewritten world history, not just today, but for centuries. The pale horsemen, which is the fourth of the white, red and black horsemen of the apocalypse, and its rider symbolizes the death and epidemic disease. The Fourth Horsemen representing the pandemics such as plague, syphilis, malaria, smallpox, and HIV/AIDS that have tyrannized the word until today, is in continuity with COVID-19. As history has painfully taught us, that our journey on the world stage is temporary whereas theirs is everlasting.

Scientifically described, pandemic is the spread of infectious diseases in a transcontinental area, a term first used in 1666 (1). With the transition to an agricultural society and domestication of animals, diseases spread from animals to humans (zoonotic) have increased in numbers and took their place in history in the form of pandemics. While plague, which killed about 34 million people, is the most well-known pandemic; the Spanish flu left a soul-shattering trace in recent history by killing at least 50 million people (2, 3). Some infectious diseases, such as Cholera, which is still ongoing in several third world countries, together with Ebola and HIV/AIDS have been increasing rapidly in recent years, incessantly causing permanent damage. On the other hand, with a grim history of causing 300 million deaths in the 20th century alone, it was announced at World Health Organization's (WHO) 33rd General Assembly on 8 May 1980 that smallpox was finally eradicated (4). Characterizing a disease as a pandemic is not only due to its widespread infectious nature; it should possess 3 characteristics reported by WHO: the disease-causing agent should be able to infect and cause danger to society, it should spread rapidly among people, and the affected should not have encountered the disease before (5). Interestingly, WHO drew attention to obesity, which has an increase in prevalence lately, by announcing it as the first non-infectious disease resulting in an epidemic (6).

Sustainability and reverence towards nature by reducing consumerism could be steps taken to prevent the epidemic of not only today but the future as well, which will lift a huge weight off of healthcare professional's shoulders, who play a critical role in the continuity of human health.

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Received: 05.10.2020 Accepted: 20.10.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.08 Available at: tmsj.trakya.edu.tr

Cite this article as: Kadrıbey İ, Akay FE. The fourth horseman of 21st century: covid-19 pandemic. Turkish Med Stud J 2020;7(3):171-4.

Sars-CoV-2 and COVID-19

The changing living conditions, climate, population, trade routes, and transportation all together are among the global factors which accelerate the spread of diseases in general (7, 8). A new member, Sars-CoV-2, took its place along with other outbreaks we had to face at the beginning of the 21st century like HIV&AIDS (1981-current), SARS (2003), Zika (2007), and MERS (2012) (9-12). Turning into a global danger, coronavirus disease-19 (COVID-19) was declared a pandemic by WHO on March 11, 2020. At the time of writing (October 2020), the official number of infected are approximately 40 million, with 1.1 million deaths. Claimed to first appear in a wild animal market in December 2019 in Wuhan, China, the novel member of the coronavirus family has an RNA genome. According to current scientific data, Sars-CoV-2 belongs to the family with the largest known RNA genome. It binds to ACE2 receptors on the cell surface to enter the cells, in which the virus reproduces itself continuously, turning the cell into a factory for further damage (13). It has mutated approximately 12,000 times to this date (14). Regardless of age, the most common symptoms of COVID-19, which can be fatal as well, are: high fever (37.8 °C and above), dry cough, shortness of breath, and weakness. Other symptoms like painful diarrhea, loss of sense of smell, and discoloration of the skin may be present as well. The incubation period of the virus is 2 to 14 days. It can be transmitted by droplets or be taken from surfaces contaminated with the respiratory materials of the patients through hands. The duration of the virus on surfaces varies according to the temperature and humidity of the environment. However, correct use of masks to cover the mouth and nose, frequent handwashing with soap and water, and a physical distance of at least 1.5 meters are the easiest and cheapest methods of disease prevention. The asymptomatic cases constitute the greatest cause of spread of this highly contagious disease. Although there is no known effective treatment and/or vaccine for the disease yet, the search continues with international collaborations (15, 16).

Outstanding messages have been given by COVID-19, the newest member of diseases that changed and reshaped the world; among with malaria, plague, smallpox, cholera, Spanish flu and HIV/AIDS. One of the notable messages was about the carbon emission, which decreased by 17% with the measures taken by April 2020, demonstrating that the human damage on the atmosphere can be undone if wanted (15). The climate change and the decrease of biodiversity do not only contribute to the spread of infectious diseases, but also lead to an increase of average temperatures, extinction of endengared species and melting of glacieres (16, 17). All these factors combined, this will presumably double the number of people facing food crises in the next 10 years and create a disproportionate gap in food distribution (18). To hinder the rapid increase of climate crisis, global measures must be taken and due to the pandemic, we now know that we are capable of such. Another urgent topic that needs to be addressed is the global ban on the illegal sales at wild animal markets, which caused the COVID-19 outbreak in the first place. Bushmeat consumption which is a signature mark for higher status and wealth became a part of luxurious living. Kept in mind that this behavior is acknowledged as a luxury today, it also hosts and accelerates the spread of disease-causing organisms transmitted from animals to humans (19-21). It has now become essential to impede wild animal trade, which is a ticking time bomb for all human health (22).

FUTURE OF HEALTHCARE

Throughout human history, physicians examined their patients by touching them, arranged appropriate treatments by using uncustomary methods to the common people, such as diagnosing them based on the color and the taste of their urine, and tried their best to protect public health, in some cases even by first testing the drugs and vaccines they discovered on themselves (23). There are three important cornerstones accepted in the history of medicine: bedside medicine, hospital medicine, followed by laboratory medicine (24, 25). With the given circumstances and opportunities, could medicine through the internet and telecommunication become the fourth important cornerstone? With the rapid development of the internet and technology, doctors, who have been treating their patients with their hands and observational skills for centuries, are now also trying to adapt and change the patient-physician relationship to the most efficient way, benefiting from the technological means of the modern-day. (26, 27). For instance, more and more doctors and patients are now benefiting from remote consultations, as people tend to prevent going to medical centers due their concern to prevent possible contact with the coronavirus (28). But only time will tell, how rooted this change will become.

At this point, it must also be stated that despite rapid changes in the patient-physician relationship, the crucial elements that will still remain as confidentiality, trust, transparency, and honesty (29). No matter how

technology shapes medical service, doctors should seek to continue working in line with their professional oaths and ethics. Without a doubt, technology will make a difference in medical services, but the crucial step is to use it in the correct way. A further point to highlight here is that not only doctors, but also the rest of the society should have equal access to sources and the knowledge on the internet. Impeding the increasing imbalance in income would make it easier for people to access their basic rights, such as the one mentioned.

Last but not least, another issue that must be stressed amidst the global pandemic is the decrease in the living and working conditions of healthcare workers. As doctors are fighting in the front row against COVID-19, their living and working conditions keep on getting worse. Measures must be taken to prevent and reverse the decrease. Grievously, after the death of Dr. Li Venliang, first person to introduce the novel coronavirus to the world, the overall picture created by the healthcare workers passing away because of coronavirus is also increasing (27). The fight against the pandemic is not only with the doctors, but also with the state and the public, i.e. with the whole section of society.

CONCLUSION

Coronavirus disease-19 is not and will not be the last pandemic we will have to face. It reminded us that pandemics are a common problem, not only for the underdeveloped countries but for the entire human race. In order to make a contribution to control the pandemic, we should stay away from crowded environments, wash hands with soap regularly, obey the physical distance rules in the community and wear masks to cover the mouth and nose.

It should be emphasized that nature is not taking revenge, does not take revenge and will not take revenge. The coronavirus is a normal result of human greed and demand, changing the natural balance according to their own desires. As 1965 Nobel Laureate in Medicine/ Physiology Dr. André Lwoff said: "Virus is virus. Is the human race ready for The Four Horseman of the next Apocalypse?"

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: IK, FEA Supervision: IK, FEA Resources: IK, FEA Materials IK, FEA Data collection and/or processing: IK, FEA Analysis and/or Interpretation: IK, FEA Literature Search: IK, FEA Writing Manuscript: IK, FEA Critical Review: IK, FEA
Financial disclosure: The authors declared that this study received no financial support.
Editor in chief's Note: One of the author of this article, Fatih Erkan Akay is the member of the editorial board of Turkish Medical Student Journal. However, he did not take place in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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LETTER TO THE EDITOR

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Dear Editor,

We read with interest the article titled "Thoughts and awareness of medical students about COVID-19 pandemic" by Cifcibasi et al. (1) in your esteemed journal. The study sheds light on the awareness of the COVID-19 pandemic of medical students. This being a topic of interest for us, we would like to add a few points which we feel would enrich the discussion.

A study from Uganda, that have looked into the sources of information used by medical students to access information about COVID-19, found an expected massive reliance on online sources (2). This underscores the immense potential and the need to disseminate information to the public via online sources during times of crisis, such as the present pandemic. Unfortunately, another study surmised that official sites such as the Centers for Disease Control and Prevention website or medical search engines like PubMed, which should reflect reliable sources of information, were less commonly used by medical students to obtain information when compared to social media and news media, which may be potential conduits for false news or incomplete information (3). This is alarming and raises the need to train medical students to obtain evidence from reliable sources and to critically analyze the information available online.

Another issue that medical students seem to face in terms of the pandemic is the social stigma associated with the diagnosis of COVID-19. In a study among medical students of Jordan, when asked whether they would want the matter to be private if a family member was in contact with the virus, a third of the students believed that the information should not be shared (4). This problem of social stigma is even more acute in certain parts of our country, where violence against healthcare workers has been recorded in responses to suspicions of raising the risk of the spread of the virus in communities. It is of vital importance that the power of social media and other public information sources to be harnessed to raise awareness in communities to support their healthcare workers so that they may carry out their work without fear of harm.

Finally, we would like to focus on the global response of medical students in all countries who came forward ready to volunteer their services for the sake of their fellow man. A study from Uganda found that over 80% of medical students are willing to participate in the frontline response to COVID-19 if called upon (2). This enthusiasm among medical students to put their lives on the line in the face of this pandemic is heroic and makes it even more incumbent upon medical authorities to incorporate comprehensive training for these medical students, to make the best use of their abilities, while taking adequate protective measures.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: DTP. Design: DTP. Literature Search: AR. Writing Manuscript: AR. Critical Review: ACP.
Financial disclosure: The authors declared that this study received no financial support.

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Received: 03.10.2020 Accepted: 20.10.2020 • DOI: 10.4274/tmsj.galenos.2020.07.03.09 Available at: tmsj.trakya.edu.tr

Cite this article as: Rasheed A, Pulimoottil DT, Philip AC. Letter to the editor. Turkish Med Stud J 2020;7(3):175.

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4. *I have been given a copy of the Participant Information Sheet and Consent Form to keep.*

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