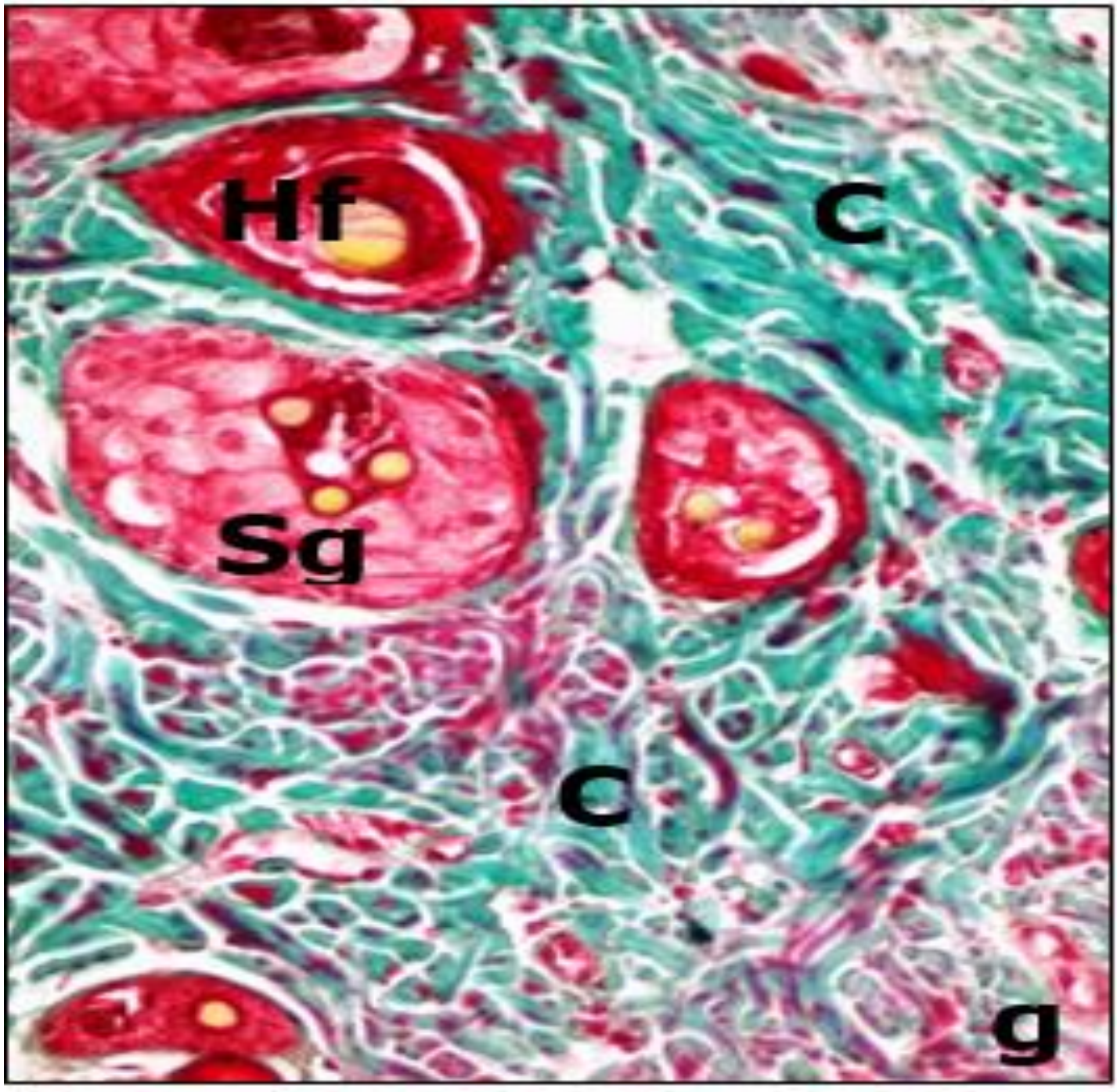
MSD Jan. 2021, Vol 8, No 1

MSD

Medical Science & Discovery



International Journal of Medical Science and Discovery
Open Access Scientific Journal
www.medscidiscovery.com,
Lycia Press London UK ISSN: 2148-6832

Medical Science and Discovery (**http://www.medscidiscovery.com**) is an international open access, peer-reviewed scientific research journal that provides rapid publication of articles in all disciplines of human health, clinical and basic medical science such as Biophysics, Biochemistry, Histology, Physiology, Genetics, Pathology, Toxicology, Anatomical Sciences, Pharmacology, Embryology, Internal and Surgical Medicine.

The policy of top priority of MSD is to put forward and highlight medical innovations and inspiring patents.

MSD offers an exceptionally fast publication schedule including prompt peer-review by the experts in the field and immediate publication upon acceptance. The editorial board aims at reviewing the submitted articles as fast as possible and promptly including them in the forthcoming issues.

This journal is published under ethical publishing policy of international scientific Bioethics and publication rules.

MSD supports the Open Access Initiative. Abstracts and full texts (HTML and PDF format) of all articles published by MSD are freely accessible to everyone immediately upon publication.

Indexed Databases: Chemical Abstracts (CAS), Index Copernicus, Open Air, ULRICHS Database, ProQuest, Advanced Science Index, Turkish Citation Index, Research Bible, Scholar Google, NLM Catalog

Medical Science and Discovery is an international open access, peer-reviewed scientific research journal.

ISSN: 2148-6832 (Print) E-ISSN: 2148-6832 (Online)

Category: Multi Disciplinary Health Science Journal

Abbreviated key title: Med. Sci. Discov.

Frequency: Monthly

Review System: Double Blind Peer Review

Circulation: Globally, Online, Printed

Article Processing Charge (APC): Free

Licensing: CC-BY-NC 4.0 International License Environmental

Editor-in-Chief: Assoc. Prof. Dr. Dr. Ahmad Rajabzadeh, Anatomical Department of lorestan, University of Medical Sciences, Tabriz, Iran

Established: 30.04.2014

Web address: www.medscidiscovery.com

E-mail : editor [at] medscidiscovery.com

Phone : +44 020 3289 9294

Design and preparation of PDFs, Language editing, Web site design, Graphical design Services of international Journal of Medical Science and Discovery has been contracted with Lycia Press LONDON, UK (as Publisher), by the MSD Board of Directors

Publisher: Lycia Press Inc.

Address: 3rd Floor 86 - 90 Paul Street, EC2A 4NE, London, UK

Web address: www.lycians.com

Phone : +44 020 3289 9294

E-mail : office [at] lycians.com

E-mail : info [at] lycians.com

Instruction for Authors

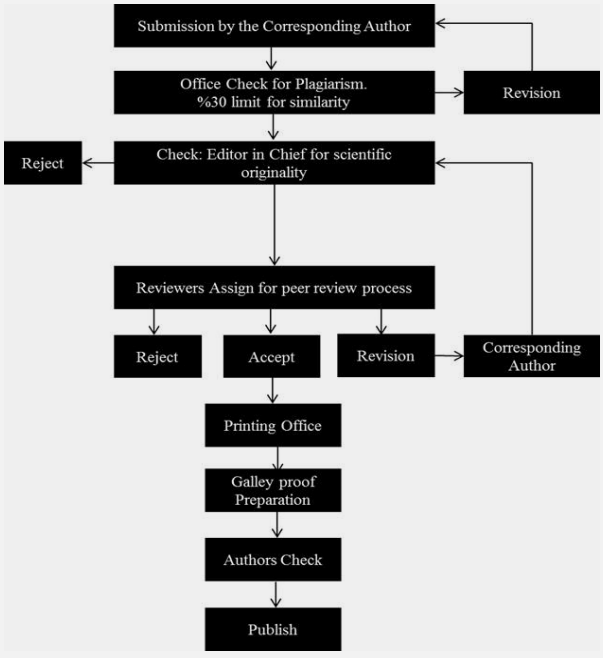
- **Important**
- MSD journal team, is committed to deterring plagiarism, including self-plagiarism. Your manuscripts will be screened for similarity detection with iThenticate, Similarity rate is expected under the %30 except for material and method section.
- For research studies using human or animal subjects, the trial's design, conduct and reporting of results must conform to Good Clinical Practice guidelines (such as the Good Clinical Practice in Food and Drug Administration (FDA)-Regulated Clinical Trials (USA) or the Medical Research Council Guidelines for Good Clinical Practice in Clinical Trials (UK)) and/or to the World Medical Association (WMA) Declaration of Helsinki
- Dear Authors, please upload just these three files to the manuscript submission system for article submissions.
- **1- Title Page Sample**
- **2- Manuscript Sample**
- **3- Copyright Transfer and Author Consent Form**
- Please select Keywords from the MESH source
- (https://www.nlm.nih.gov/mesh/MBrowser.html)
- Manuscripts should be prepared in accordance with the "Uniform Requirements for Manuscripts Submission to Biomedical Journals" proclaimed by the International Committee of Medical Journal Editors (www.icmje.org).
- MSD uses vancouver reference style, please prepare articles due to Vancouver reference style rules.
- Manuscript Preperation Rules
- 1.Cover letter
- a- A statement that the manuscript has been read and approved by all the authors.
- b- That the requirements for authorship have been met for all the authors, based on the criteria stated by ICMJE.
- c- Approval of all the authors regarding the order in which their names have appeared.
- d- That each author confirms the manuscript represents honest work.
- e- The name, address, and telephone number of the corresponding author who is responsible for communicating with other authors about revisions and final approval.
- f- The letter should give any additional information that may be helpful to the editor, such as the type or format of the article. If the manuscript has been submitted previously to another journal or in another language, it is helpful to include the previous editor's and reviewers' comments with the submitted manuscript, along with the authors' responses to those comments. Submitting previous evaluatory review of another journal accelerates the review process.
- g- For accepted manuscripts, the authors are requested to fill and sign the journal's cover letter to express their consent for its publication.
- h- To reproduce published material, to use illustrations or tables or report information about identifiable people, the author should submit a copy of the permission with the manuscript to the journal.
- 2.Top Ethic Committee Approval
- Inclusion of the approval letter from the relevant Ethics Committee or Institution's Review Board regarding the research protocol and the rights of the subjects (if applicable to the study)
- 3.Top Consent Form
- Attach a copy of the consent form to the letter, if applicable. Consent forms would be evaluated by the Ethics Committee and then signed by the participant.
- 4.Top RCT or NCT Registration
- Emailing the letter denoting registration of RCTs or NCTs in domestic or international databases (The trial's registration number needs to be mentioned, too).
- 5. Manuscripts submitted in English, must be type written, double-spaced, on good quality A4 paper, or paper of similar format. Authors are requested to reserve margins of at least 2.5cm all around the paper. Original drawings of photos, tables and figures should be furnished together with the manuscripts.
- 6. Manuscripts should be kept to a minimum length and should be subdivided into labeled sections (Title page, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgement, and References).
- 7. A title page is to be provided and should include the title of the article, authors' names with full first name (with degrees), authors' affiliation, suggested running title and corresponding author. The affiliation should comprise the department, institution (usually university or company), city and state (or nation). The suggested running title should be less than 50 characters (including spaces) and should comprise the article title or an abbreviated version thereof. For office purposes, the title page should include the name and complete mailing address, telephone and fax number, and email of the one author designated to review proofs.
- 8. An abstract no longer than 250 words for reviews and research articles is to be provided as the second page. Abstract should be structured as objective(s) (including purpose setting), materials and methods, results, and conclusion..

Instruction for Authors

- 9. A list of 3-8 keywords, chosen from the Medical Subject Headings(MeSH) listhttp://www.nlm.nih.gov/mesh/MBrowser.html, is to be provided directly below the abstract. Keywords should express the precise content of the manuscript, as they are used for indexing purposes. Provide abbreviations and nomenclature list in an alphabetical order and non-standard abbreviations contained in the manuscript (excluding references) with definitions after the keywords. Use abbreviations sparingly and only when necessary to save space, and to avoid repeating long chemical names or therapeutic regimes. In a figure or table, define the abbreviations used in a footnote.
- 10. Tables in limited numbers should be self- explanatory, clearly arranged, and supplemental to the text. The captions should be placed above.
- 11. Figures should be utilized only if they augment understandability of the text. The captions should be placed below. Drawings and graphs should be professionally prepared in deep black and submitted as glossy, black and white clean Photostats. Professionally designed computer generated graphs with a minimum of 300 DPI laser printer output is preferable. Color photographs are welcomed.
- 12. The same data should not be presented in tables, figures and text, simultaneously.
- 13. MSD uses Vancouver referencing Style. References in limited numbers and up-to-dated must be numbered consecutively in order of citation in the text (number in parentheses). Periodical titles should be abbreviated according to the PubMed Journals Database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=journals). Print surnames and initials of all authors when there are six or less. In the case of seven or more authors, the names of the first six authors followed by et al. should be listed.
- Please check all references with EndNote referencing System. Please check out and Download Vancouver Endnote Style.
- **Type of Articles**
- Type of articles are based on PubMed definitions. For more info please refer to: http://dtd.nlm.nih.gov/publishing/tag-library/3.0/n-w2d0.html
- **Editorial :**
- Editorial is Opinion piece, policy statement, or general commentary, typically written by staff of the publication (The similar value "article-commentary" is reserved for a commentary on a specific article or articles, which is written by an author with a contrasting position, not an editor or other publication staff.)
- **Letters to the Editor about a recent journal article :**
- Letters referring to a recent article in this journal must be received within three months of its publication. For example, a letter referring to an article published in the January issue must be submitted online no later than March 31st. Letters submitted after the allowed time will not be considered.
- The text, not including references, must not exceed 700 words. A maximum of three authors and 10 references are allowed. Neither tables nor figures are allowed.
- Letters to the Editor NOT referring to a recent journal article :
- Original research that is of interest but does not fulfill all the requirements needed for publication as a full-length manuscript can be submitted as a letter to the editor. The letter must have a title and a maximum of three authors.
- The text, not including references, tables, figures or legends must not exceed 700 words. No more than 10 references and either one table or one figure are allowed.
- Word Count Limit: Letters should contain 500 - 700 words, maximum number of references is 10, maximum Number of illustrations/Tables is 1.
- **Original Article:**
- The content of the paper must justify its length. For reports of original investigative work, traditional division into sections is required: Title, Keywords, Addresses and which author address for correspondence, Structured abstract, Background, Objectives, Materials/Patients and Methods, Results, Discussion, References and Acknowledgements, Legends for display items (Figures and Tables).
- Original Research articles should contain 2500 - 3500 words, maximum number of references is 35, maximum Number of illustrations/Tables is 5.
- **Review Article :**
- Review Articles should contain 3500 - 4000 words, maximum number of references is 50, maximum number of illustrations/Tables is 5. In a review article both abstract and text of the manuscript, include following items:
- 1) Context: Include 1 or 2 sentences describing the clinical question or issue and its importance in clinical practice or public heath.
- 2) Evidence Acquisition: Describe the data sources used, including the search strategies, years searched, and other sources of material, such as subsequent reference searches of retrieved articles. Explain the methods used for quality assessment and the inclusion of identified articles.
- 3) Results: Address the major findings of the review of the clinical issue or topic in an evidence-based, objective, and balanced fashion, emphasizing the highest-quality evidence available.
- 4) Conclusions: Clearly state the conclusions to answer the questions posed if applicable, basing the conclusions on available evidence, and emphasize how clinicians should apply current knowledge.

Instruction for Authors

- **Case Report**
- A case report is a case study, case report, or other description of a case that should contain 1500 - 2000 words with a structured abstract of 200 words maximum. Case reports should comprise sections of Introduction, Case Presentation, and Conclusions in Abstract and Introduction, Case Presentation, and Discussion in full text with not more than 2 tables or figures and up to 20 references.
- **Brief Report**
- Brief Reports should contain 1000 - 2000 words with a structured abstract of 200 words maximum. Short reports should comprise sections of Background, Objectives, Materials & Methods, Results and Discussion with not more than 2 tables or figures and up to 20 references.
- **Short Communication**
- Short Communication, follow the instructions for original articles, except that the total word number of the main text (excluding references, tables and figure legends) is limited to 2000 with no more than 2 figures and/or tables and no more than 15 references. An abstract, not exceeding 150 words, should be presented at the beginning of the article.
- **News**
- News should contain 1000 - 2000 words with a structured abstract of 200 words maximum. News should comprise sections of Background, Objectives, Materials & Methods, Results and Discussion with not more than 2 tables or figures and up to 20 references.
- **Publication Policies**
- Manuscripts, or the essence of their content, must be previously unpublished and should not be under simultaneous consideration by another Journal. The authors should also declare if any similar work has been submitted to or published by another Journal. By virtue of the submitted manuscript, the corresponding author acknowledges that all the co-authors have seen and approved the final version of the manuscript. The corresponding author should provide all co-authors with information regarding the manuscript, and obtain their approval before submitting any revisions. Manuscripts are only accepted for publication on the understanding that the authors will permit editorial amendments, though proofs will always be submitted to the corresponding author before being sent finally to press. Prior to the initial submission of a new manuscript, please carefully consider that all authors' names are included as no change to authors' details will be permitted after the acceptance. The decision to accept a contribution rests with the Editorial Board of the MSD.
- Manuscripts will be considered for publication in the form of original articles, Case report, short communications, Letter to editor and review articles. The work should be original or a thorough by an authoritative person in a pertinent field.
- **Peer review process**
- All submissions will be reviewed anonymously by at least two independent referees. All manuscripts will be acknowledged upon presenting to the Journal office, provided that all stated requirements are met. Authors are encouraged to suggest names of three expert reviewers, but selection remains a prerogative of the Editor. The whole review process depends on receiving referees comments and revising the manuscripts based on these comments to the author. On receipt of the revised article from the author, and after final approving by referees, the letter of acceptance is issued to the author. Authors have the right to communicate to the editor if they do not wish their manuscript to be reviewed by a particular reviewer because of potential conflicts of interest. No article is rejected unless negative comments are received from at least two reviewers. **MSD employs double blind reviewing process, where both the referee and author remain anonymous throughout the process**.



Instruction for Authors

- **Ethical Rules and Rights**
- **Conflicts of interest**
- Conflicts of interest arise when authors, reviewers, or editors have interests that are not fully apparent and that may influence their judgments on what is published. They have been described as those which, when revealed later, would make a reasonable reader feel misled or deceived. (The Committee on Publication Ethics (COPE) states in its Guidelines on Good Publication Practice 2003).
- Authors should disclose, at the time of submission, information on financial conflicts of interest or other interests that may influence the manuscript. Authors should declare sources of funding for the work undertaken.
- **Authors Responsibilities**
- 1. Authors must certify that their manuscript is their original work.
- 2. Authors must certify that the manuscript has not previously been published elsewhere, or even submitted and been in reviewed in another journal.
- 3. Authors must participate in the peer review process and follow the comments.
- 4. Authors are obliged to provide retractions or corrections of mistakes.
- 5. All Authors mentioned in the paper must have significantly contributed to the research. Level of their contribution also must be defined in the Authors Contributions section of the article.
- 6. Authors must state that all data in the paper are real and authentic.
- 7. Authors must notify the Editors of any conflicts of interest.
- 8. Authors must identify all sources used in the creation of their manuscript.
- 9. Authors must report any errors they discover in their published paper to the Editors.
- 10. Authors must not use irrelevant sources that may help other researches/journals.
- 11. Authors cannot withdraw their articles within the review process or after submission, or they must pay the penalty defined by the publisher.
- **Editorial Responsibilities**
- 1. Editors (Associate Editors or Editor in Chief) have complete responsibility and authority to reject/accept an article.
- 2. Editors are responsible for the contents and overall quality of the publication.
- 3. Editors should always consider the needs of the authors and the readers when attempting to improve the publication.
- 4. Editors should guarantee the quality of the papers and the integrity of the academic record.
- 5. Editors should publish errata pages or make corrections when needed.
- 6. Editors should have a clear picture of a researchs funding sources.
- 7. Editors should base their decisions solely one the papers importance, originality, clarity and relevance to publications scope.
- 8. Editors should not reverse their decisions nor overturn the ones of previous editors without serious reason.
- 9. Editors should preserve the anonymity of reviewers (in half blind peer review journals).
- 10. Editors should ensure that all research material they publish conforms to international accepted ethical guidelines.
- 11. Editors should only accept a paper when reasonably certain.
- 12. Editors should act if they suspect misconduct, whether a paper is published or unpublished, and make all reasonable attempts to persist in obtaining a resolution to the problem.
- 13. Editors should not reject papers based on suspicions; they should have proof of misconduct.
- 14. Editors should not allow any conflicts of interest between staff, authors, reviewers and board members.
- 15. Editors must not change their decision after submitting a decision (especially after reject or accept) unless they have a serious reason.
- **The Journal's Policy on Plagiarism**
- Any practice of plagiarism will not be tolerated by the journal regarding submitted manuscripts. Non-identifiable quoted segments of articles or close paraphrases from other author/s or even submitting the author's previously published work are known as the act of plagiarism by this journal unless proper use of quotations or paraphrasing with decent citation or referencing are in place. Heavy use of one or a couple of articles is discouraged, even if paraphrased fully. Advertent practice of plagiarism will abort reviewing process or later submission to this journal. All submitted articles will evaluate by iThenticate software belonged to cross check for stop any plagiarism and improve publication quality.

Instruction for Authors

- **The Journal's Policy on Plagiarism**
- Any practice of plagiarism will not be tolerated by the journal regarding submitted manuscripts. Non-identifiable quoted segments of articles or close paraphrases from other author/s or even submitting the author's previously published work are known as the act of plagiarism by this journal unless proper use of quotations or paraphrasing with decent citation or referencing are in place. Heavy use of one or a couple of articles is discouraged, even if paraphrased fully. Advertent practice of plagiarism will abort reviewing process or later submission to this journal. All submitted articles will evaluate by iThenticate software belonged to cross check for stop any plagiarism and improve publication quality.
- **Statement of Human and Animal Rights**
- All submitted articles involving human experiments should be performed only in accordance with the ethical standards provided by the responsible committee of the institution and in accordance with the Declaration of Helsinki (as revised in Edinburgh 2000), available at http://www.wma.net/en/30publications/ 10policies/b3/index.html. Papers describing animal experiments can be accepted for publication only if the experiment conforms the National Institute of Health Guide (National Institute of Health Publications No. 80-23, Revised 1978) for the care and use of Laboratory Animals for experimental procedure. Authors must provide a full description of their anesthetics and surgical procedures. All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming the informed consent was obtained from each subject or subject's guardian.
- Humans: When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.
- Animals: When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.
- All animal or human subjects should be used after approval of the experimental protocol by a local ethics committee.
- **Acknowledgements**
- Contributors: In acknowledgement section, name people for their contributions or their permission to reproduce their published material, to use their illustrations or provide information about them- try to fully name people who have helped from the conception of the idea to adoption of the hypothesis, to finalization of the study, etc., earnestly. Statement of financial support: Aside from the title page, state any financial or other relationships that might lead to a conflict of interest.
- **Copyright**
- After acceptance and publication; Medical Science and discovery allows to the author's to hold the copyright without any restriction. Please complete copyright form and send via email to editor. Download MSD Copyright Transfer and Author Consent Form
- **Creative Commons License**
- This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.(CC BY NC).
- **Copyright 2019:** The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. All Rights reserved by international journal of Medical Science and Discovery.
- **Disposal of material**
- Once published, all draft copies of the manuscript, correspondence and artwork will be held at least for 6 months before disposal. Authors and Readers may find original PDF file of article on backup servers such as LOCKSS (https://www.lockss.org/)
- **Digital Object Identifier DOI**
- Once a manuscript is accepted for publication it will be provided with a registered DOI number following the acceptance decision. Manuscripts accepted for publication by the MSD will be published as ahead of print articles prior to the printing date of their scheduled issue. Corresponding author will be provided with a PDF Proof by the publisher once the production process of an accepted manuscript is over.

Instruction for Authors

- **Article Processing Charge** is Fee
- MSD Article Submission Fee: Free
- MSD Fast Evaluation Process Fee: Free
- MSD Article Evaluation Fee: Free

- Please write your text in good English (American or British usage is accepted, but not a mixture of these). In case of insufficient writing on grammar and language, the authors may be directed to editing service of the journals publisher to eliminate possible grammatical or spelling errors (Lycia Press). Lycia Press proofreading service Fee for MSD is 40GBP /1000 words . for PDF design; service Fee for MSD is 40GBP /1000 words

- **MSD revenue sources and Sponsorships**
- All costs arising from the publications are covered by the Sponsor Companies. Sponsorship request evaluates by the MSD Journal Management Board, Lycia Press and the sponsor company logos will be included on the back page of printed magazine and in the sponsor section of journal website

- **References**
- Committee on Publication Ethics (COPE). (2011, March 7). Code of Conduct and Best-Practice Guidelines for Journal Editors. Retrieved from http://publicationethics.org/files/Code_of_conduct_for_journal_editors_Mar11.pdf
- World Association of Medical Editors (WAME). Principles of Transparency and Best Practice in Scholarly Publishing. http://www.wame.org/about/principles-of-transparency-and-best-practice

Medical Science and Discovery, January 2021, Vol.8, No.1

Contents

Research Articles

Ventricular hypertrophy and ischaemic changes in Children with Sickle Cell Anaemia and its relationship with the Haemoglobin Concentration in Steady State
Charles Ikegwuonu, Obumneme Ezeanosike, Chuka Manyike, Fortune Ujunwa, Cordis Ikegwuonu / 1-8

The Colistin-related nephrotoxicity and risk factors in the intensive care unit; a retrospective study
Colistin-related nephrotoxicity in the intensive care unit
Guler Doganay, Mustafa Ozgur Cirik, Ali Alagoz / 9-13

Autism Spectrum Disorder Referrals to a Rural Hospital in the Past Two Years – A Retrospective Evaluation
Mert Besenek / 14-18

The significance of Serum Vitamin D Levels on Changes in Hematological Parameters After Chemotherapy in Patients with Breast Cancer
Fatih İnci, Fatih Karataş / 19-23

Prognostic significance of ferritin, D-dimer, lymphocyte / monocyte ratio and some biochemical markers in patients with SARS-CoV-2
Ali Eser, Nang Hseng Kyio / 24-28

Is traditional herbal mix composition effective on wound healing?
Serap Gokce Eskin, Yucel Basimoglu Koca, Serdal Ogut /29-38

Effect of systemic immune-inflammation index on prognostic parameters and survival in patients with breast cancer under the age of 40 years
Hacer Demir, İsmail Beypınar / 39-44

The Reflections of Covid-19 pandemic: Turkey's results during the first month of the pandemic
Covid-19 results from Turkey
Suna Eraybar, Huseyin Aygun / 45-49

Medical Science and Discovery
ISSN: 2148-6832

Ventricular hypertrophy and ischaemic changes in Children with Sickle Cell Anaemia and its relationship with the Haemoglobin Concentration in Steady State

Ikegwuonu Charles[1]*, Ezeanosike Obumneme[1], Manyike Chuka[2], Ujunwa Fortune[1], Ikegwuonu Cordis[3].

1 Alex Ekwueme Federal University Teaching Hospital, Department of Paediatrics, Abakaliki, Ebonyi State, Nigeria
2 Ebonyi State University Teaching Hospital, Department of Paediatrics, Abakaliki, Ebonyi State, Nigeria
3 Alex Ekwueme Federal University Teaching Hospital, Department of Public Health, Abakaliki, Ebonyi State, Nigeria
*** Corresponding Author:** Ikegwuonu Charles **E-mail:** realici@gmail.com

ABSTRACT

Objective: The prevalence and burden of Sickle Cell Anaemia (SCA) in Nigeria are high and they contribute to childhood morbidity and mortality. Chronic anaemia and vaso-occlusion usually involve different organs. The involvement of the heart is a common complication of SCA, thus the need for early detection of cardiac abnormalities in children with SCA. To assess cardiac structure using ECG in children with SCA in steady-state and to determine the relationship between abnormal ECG findings and the participants' haemoglobin concentration.

Material and Method: It was a cross-sectional study done in one of the tertiary hospitals in southeast Nigeria. The study participants were 164 children with SCA in steady state within the ages of 2 -17 years. A 12-lead ECG was carried out on the participants and their haemoglobin concentrations determined. The relationship between the presence of cardiac abnormality and independent variables like haemoglobin concentration, age and gender were analyzed.

Result: The prevalence of cardiac abnormality was 59.1%. The commonest cardiac structure abnormality was Left ventricular hypertrophy (LVH) (39.6%). Abnormality in the P-R interval was seen in 14.6%, while ST segment abnormality was seen in 18.3%. The mean Hb of those with abnormal ECG was lower than that of those without abnormal ECG, across each age group. Severe anaemia was a significant ($p< 0.01$) predictor of LVH. However, there was no significant association between the degree of anaemia and the occurrence of ST-segment (p: 0.26) and PR interval abnormalities (p: 0.52).

Conclusion: Cardiac anomalies are common findings in SCA children.

Keywords: SCA, ECG, Ischaemia, LVH, Infarction

Received 22-10-2020

Accepted 29-12-2020

Published 09-01-2021

INTRODUCTION

Sickle cell anaemia (SCA) is an inherited condition that results from abnormalities of haemoglobin synthesis (1). In Africa, SCA is a common cause of morbidity and mortality (2) In Nigeria, SCA is found in about 3% of the population,1 and the country has the highest burden of SCA worldwide (3)

In SCA, there is sickling of the red blood cells with consequent occlusion of blood vessels and chronic haemolytic anaemia, leading to chronic hypoxia and ischeamia with or without infarction (4, 5). A common cause of morbidity and mortality is the involvement of the heart (6) as sudden death from cardiac causes has been demonstrated in patients with SCA, contributing to about 25.6% of mortality among those with SCA (7)

A study in Tanzania showed that among patients with SCA who died between 1979 to 2005, 9% of deaths were due to cardiovascular causes, including ischaemic and non-ischaemic heart diseases (8). The chronic anaemia and vaso-occlusion from sludged sickled cells results in chronic tissue hypoxia, which can lead to ischaemia and infarction of cardiac tissues (5, 9). These may involve the myocardium and the conducting system of the heart, resulting in abnormality in cardiac conduction and heart block (10).

The chronic anaemia and occlusion of the coronary artery by the sickled red blood cell causes progressive changes in cardiac structure; proliferation of myocytes, focal degeneration and fibrosis, thus, the chronicity of the effect of SCA on the heart (5). The heart has few collateral blood vessels that will oxygenate the cardiac tissues if the primary arterial conduits are narrowed or blocked (11).

Thus, it is vulnerable to ischaemia and infarction, causing a delay or abnormal electrical activities during the repolarization phase of the myocardial cells (11) This is seen as changes in ST segment and T wave on an electrocardiogram (ECG) (11). Aside the myocardium, ischaemia also affects the conducting systems, affecting the cardiac rhythm and may result in different degrees of heart block (12). This is seen as abnormalities in PR interval (12).

This could signal the effect of ischaemia at a number of sites (sino-atrial node, atrial myocardium and atrio-ventricular node) of the conduction system, since it measures the time depolarization spreads from the atria to the ventricles (12). Consequent upon the chronic anaemia in SCA, there is an increase in the left ventricular stroke volume with some increase in heart rate due to a compensatory attempt to increase the cardiac output (6).

The most common ECG abnormality seen in patients with SCA is left ventricular hypertrophy (LVH) (13, 14, 15) and results from this compensatory increase in the left ventricular stroke volume, resulting to a significant dilatation of the left ventricle over time (16).

This later adapts to the increased wall stress by developing eccentric hypertrophy over time (17). This ventricular hypertrophy is dependent on the degree of anemia (18). Cardiovascular morbidities, such as heart failure, have been associated with left ventricular hypertrophy (19). However, if detected early and with proper management, regression may be achieved, with consequent decrease in the attendant cardiac pathology (19).

MATERIALS AND METHODS

Study Area: The study was carried out in the Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State.

Study Population: The participants were children with SCA aged 2- 17 years in steady state who presented in the sickle cell clinic of AE-FUTHA.

Inclusion and Exclusion Criteria: Children with SCA within the ages of 2–17 years who were in steady state. The steady state was defined as absence of crisis or blood transfusion in the preceding four weeks before recruitment (19, 20). Those with history of co-morbidities like congenital heart disease, rheumatic heart disease or other chronic diseases were excluded from the study.

Ethical Consideration and Consent: Before the commencement of the study, an ethical approval (REC Approval No: 05/05/2017-9/3/2018) was obtained from the Research and Ethical committee of the AE-FUTHA, and an informed consent and assent obtained from the parents and their children who were 7 years of age and above, respectively. Results of the investigations were made available and explained to the participants and caregivers.

Study Design: This was a cross-sectional study

Sampling Method: The study participants (164 children with SCA) were consecutively recruited from the SCA out-patient clinic until the minimum sample was achieved. The patients' folders were coded in order to avoid double recruitment.

Data Collection: Socio-demographic data such as age and gender were obtained. Twelve lead ECG was done using a portable ASPEL AsCARD Mint 12 Lead and 3-channel (ASPEL Company, June 2016, Poland) Electrocardiograph.

The electrodes were positioned as recommended by American Heart Association (21) Any evidence of PR interval anomaly and ST segment anomaly were recorded and the QRS complex was analysed for any evidence of ventricular hypertrophy (22, 23, 24, 25)

Data Analysis: The data collected were entered and analysed using the Statistical Package for Social Science (SPSS) version 23 for Microsoft windows 8. Descriptive statistics such as frequency and percentages were used to describe characteristics of the subjects such as gender and number of participants within each age group.

Continuous variables such as SV1, SV2, RV5 and RV6 amplitudes within each age and also, age distribution of each ECG anomaly were not normally distributed, using Shapiro-Wilks test for normality ($p > 0.05$) (26) hence, they were analysed using median and inter-quartile range.

Other continuous variables such as Hb concentration and PR interval duration were normally distributed, hence, they were analysed using mean and standard deviation. The prevalence of ECG abnormality was expressed in frequencies and percentages.

The Spearman's correlation test was used to evaluate for relationship between the Hb concentration and presence of cardiac anomaly. The strength of the relationship was said to be weak (rs= < 0 to > -0.3 or > 0 to < +0.3), moderate (rs= -0.3 to > -0.7 or +0.3 to < +0.7), strong (rs= -0.7 to -1 or +0.7 to +1) or no linear relationship (rs = 0), depending on the value of the correlation coefficient (rs) (27).

The chi-square test or fishers exact test was also used to assess for significant difference within each independent variable such as the gender and age of participants, with regards to each cardiac abnormality.

At confidence level of 95%, any difference observed was said to be significant if the p value was <0.05.

http://dx.doi.org/10.36472/msd.v8i1.458

RESULTS

A total of 164 children (96 males and 68 females) with SCA were recruited for this study. They were within the ages of 2-17 years, with a mean age of 9.7 years (SD, 4.56) and a male: female ratio of 1.4:1. This is illustrated in Table I

Anaemia was observed in the entire participants. Mild anaemia was seen in 11 persons (6.7%), 92 (56.1%) persons had moderate anaemia and 61 persons (37.2%) had severe anaemia. Severe anaemia was observed more in children between the ages of 9- 14 years (59%), moderate anaemia was observed more in children between the ages of 2–7 years (50%), while mild anaemia was observed more in children 15–17 years (54.5%). These are illustrated in Tables II and III

Table I: Gender and age distribution of children with SCA

	Characteristics	n (%)
Gender	Male	96(58.5)
	Female	68(41.5)
	Total	**164(100)**
Age (years)	2	9(5.5)
	3	7(4.3)
	4	12(7.3)
	5	10(6.1)
	6	6(3.7)
	7	18(11)
	8	8(4.9)
	9	8(4.9)
	10	10(6.1)
	11	12(7.3)
	12	12(7.3)
	13	12(7.3)
	14	8(4.9)
	15	14(8.5)
	16	8(4.9)
	17	10(6.1)
	TOTAL	**164(100)**

Table II: Mean haemoglobin of the different ages of children with SCA

Age (years)	Mean Haemoglobin, g/dl (SD)
2	7.69 (0.88)
3	6.63(1.10)
4	7.43(0.41)
5	7.56(1.42)
6	7.13(1.54)
7	7.38(1.11)
8	6.98(0.69)
9	6.53(1.11)
10	7.98(1.72)
11	7.55(1.64)
12	7.83(1.35)
13	7.20(1.61)
14	8.30(1.59)
15	8.25(1.20)
16	6.50(1.60)
17	7.42(1.30)

Abnormal ECG was detected in 97 (59.1%) of the children. It was observed more in males than in female participants, but the difference was not significant ($p = 0.40$, Z-score = -0.25).

Worthy of note was that no ECG abnormality was seen in children aged 2 years. The abnormal ECG results were observed more in older children, as the median age of children with abnormal ECG was 10 years, with an inter-quartile range of 9. This is shown in Table IV.

LVH was the most prevalent cardiac abnormality observed in the participants. This was seen in 65 (39.6%) participants, while none of the participants had right ventricular hypertrophy.

Abnormality in ST segment was seen in 30 (18.3%) participants; ST depression (80%) and ST elevation (20%). Abnormality in PR interval was seen in 24 (14.6%) participants; PR interval prolongation (20) and PR interval shortening (4).

The gender and age distribution of the ECG abnormalities are illustrated in Table VI. There was no significant relationship between gender and development of LVH and PR interval abnormality unlike ST segment abnormality seen more in females.

There was a significant relationship between the age and development of LVH, as 60% of children with LVH were above 10 years of age. Such was not seen with ST segment and PR interval abnormality, as these were fairly distributed among older and younger children.

The mean Hb of those with LVH was lower than the mean Hb of those without LVH, across all ages, difference being significant across almost all ages. This is shown in Table VII. There was a significant negative relationship between the Hb concentration and development of LVH ($rs = -0.71$, $p < 0.01$).

Though the mean Hb of those with ST anomaly in each age was lower than that of their counterpart age without ST anomaly across all ages, the difference was significant only in children that were 10 years and above. There was no significant relationship between the Hb concentration and development of ST anomaly ($rs = -0.14$, $p = 0.06$).

The mean Hb of those with abnormal PR interval was not significantly lower than that of those with normal PR interval across all ages. Also, there was no significant relationship between the Hb concentration and development of PR interval anomaly ($rs = -0.09$, $p = 0.24$).

There was a significant relationship between the degrees of anaemia and the development of LVH ($p < 0.01$), as 70.8% of children with LVH had severe anaemia. This was not seen between degree of anaemia and development of ST segment and PR interval anomalies. This is shown in Table VIII

http://dx.doi.org/10.36472/msd.v8i1.458

Table III: Frequency of anaemia among the different ages of children with SCA

Age (years)	Mild n (%)	Moderate n (%)	Severe n (%)
2	-	8 (4.88%)	1 (0.61%)
3	-	3 (1.83%)	4 (2.44%)
4	-	12(7.32%)	-
5	2 (1.22%)	5 (3.05%)	3 (1.83%)
6	-	4 (2.44%)	2 (1.22%)
7	-	14 (8.34%)	4 (2.44%)
8	-	4 (2.44%)	4 (2.44%)
9	-	2 (1.22%)	6 (3.70%)
10	1 (0.61%)	6 (3.70%)	3 (1.83%)
11	1 (0.61%)	5 (3.05%)	6 (3.70%)
12	-	6 (3.70%)	6 (3.70%)
13	-	3 (1.83%)	9 (5.49%)
14	1 (0.61%)	1 (0.61%)	6 (3.70%)
15	4 (2.44%)	7 (4.27%)	3 (1.83%)
16	-	6 (3.70%)	2 (1.22%)
17	2 (1.22%)	6 (3.70%)	2 (1.22%)
TOTAL	11(6.7%)	92 (56.1)	61 (37.2%)

Table IV: Abnormal electrocardiogram among different ages and genders of children with SCA

Age (years)	n	Gender	n	p-value	z-score
2	-	Male	56	0.4	-0.25
3	8	Female	41		
4	12				
5	6				
6	4				
7	8				
8	6				
9	4				
10	5				
11	6				
12	7				
13	6				
14	8				
15	6				
16	4				
17	7				
TOTAL	97		97		

Table V: Median amplitudes of SV1, SV2, RV5, RV6 waves and mean PR interval among different ages of children with sickle cell anaemia.

AGE (years)	SV1 mv (IQR)	SV2 mv (IQR)	RV5 mv (IQR)	RV6 mv (IQR)	PR interval ms
2	1.46(0.94)	2.02(1.09)	1.62(0.85)	1.19(0.36)	132.33(31.2)
3	2.43(1.37)	2.74(1.79)	3.41(3.26)	1.88(1.35)	161.43(17.5)
4	1.06(1.01)	1.55(1.02)	2.71(2.06)	1.65(0.29)	154.25(23.1)
5	1.74(1.06)	2.12(1.51)	3.13(3.01)	1.57(1.40)	141.20(23.2)
6	1.77(1.16)	2.23(2.19)	2.78(2.17)	1.83(1.02)	143.67(14.3)
7	2.05(1.42)	2.89(1.67)	4.01(1.44)	2.55(1.40)	161.89(34.2)
8	1.95(1.25)	3.13(2.16)	3.93(1.27)	2.33(1.31)	130.75(12.8)
9	2.81(0.92)	3.71(0.24)	3.85(1.20)	2.41(0.98)	139.00(10.8)
10	2.07(1.14)	2.79(2.07)	3.34(2.53)	2.07(1.47)	129.00(15.9)
11	2.71(1.18)	2.69(2.25)	3.50(1.68)	2.08(0.99)	152.50(27.6)
12	2.09(1.48)	2.01(1.45)	2.88(1.98)	1.76(0.84)	176.08(23.1)
13	2.18(1.40)	2.94(1.40)	3.42(2.01)	2.13(0.92)	162.50(32.4)
14	1.90(1.31)	2.41(1.91)	2.52(2.11)	2.36(1.20)	154.31(16.6)
15	1.83(1.10)	2.54(2.08)	3.02(2.65)	2.12(1.28)	166.28(30.1)
16	1.81(1.00)	1.55(1.07)	2.67(2.19)	1.97(0.61)	155.25(6.7)
17	1.85(0.73)	1.79(1.55)	2.64(0.75)	2.05(0.46)	179.10(37.7)

IQR- Inter-Quartile Range mv- millivolt ms- milliseconds

http://dx.doi.org/10.36472/msd.v8i1.458

Table VI. Gender and age distributions of the cardiac abnormalities

			LVH				ST segment anomaly				PR interval anomaly	
Gender	**Present**	**Absent**	χ^2	**P-value**	**Present**	**Absent**	χ^2	**P-value**	**Present**	**Absent**	χ^2	**P-value**
Male	35	61	0.98	0.32	12	84	5.2	**0.02**	13	83	0.22	0.64
Female	30	38			18	50			11	57		
Age (years)	**Present**	**Absent**	**F-exact**	**P-value**	**Present**	**Absent**	**F-exact**	**P-value**	**Present**	**Absent**	**F-exact**	**P-value**
2	0	9	27.1	**0.04**	0	9	19.5	0.07	0	9	33.2	0.11
3	1	6			4	3			3	4		
4	4	8			5	7			3	9		
5	3	7			2	8			1	9		
6	3	3			1	5			0	6		
7	6	12			1	17			5	13		
8	5	3			1	7			0	8		
9	4	4			2	6			0	8		
10	3	7			4	6			0	10		
11	6	6			2	10			1	11		
12	5	7			2	10			2	10		
13	6	6			2	10			2	10		
14	8	0			0	8			1	7		
15	6	8			0	14			1	13		
16	3	5			2	6			0	8		
17	2	8			2	8			5	5		
TOTAL	**65**	**99**			**30**	**134**			**24**	**140**		

Table VII: Mean haemoglobin of participants with and without ECG abnormalities

Age (years)	LVH present	LVH absent	p-value	ST-anomaly present	ST-anomaly absent	p- value	PR-anomaly present	PR-anomaly absent	p-value
2	-	7.69(0.88)	-	-	7.89(0.9)	-	-	7.69(0.9)	-
3	5.90(0.00)	7.60(0.01)	**<0.01**	6.47(1.2)	6.75(1.1)	0.66	7.00(0.8)	7.60(1.2)	0.26
4	7.40(0.41)	7.80(0.05)	**<0.01**	7.43(0.4)	7.45(0.4)	0.90	7.51(0.4)	7.80(0.2)	0.10
5	6.50(0.35)	8.27(1.43)	**<0.01**	7.20(0.1)	7.65(1.6)	0.40	6.90(0.0)	7.71(1.4)	0.15
6	5.20(0.03)	8.10(0.48)	**<0.01**	7.02(0.0)	7.70(1.7)	0.37	-	7.13(1.5)	-
7	7.33(1.37)	7.43(0.86)	0.57	7.50(0.0)	7.68(0.7)	0.44	7.46(1.6)	7.74(0.6)	0.65
8	6.83(0.76)	7.40(0.26)	**<0.01**	6.92(0.0)	6.98(0.7)	0.92	-	6.98(0.7)	-
9	5.90(0.01)	7.61(1.17)	**<0.01**	6.20(0.2)	6.63(1.3)	0.38	-	6.53(1.1)	-
10	6.57(0.64)	8.59(1.69)	**<0.01**	6.75(0.6)	8.80(1.7)	**<0.01**	-	7.98(1.7)	-
11	6.40(1.08)	8.70(1.26)	**<0.01**	5.30(0.2)	8.00(1.4)	**<0.01**	7.44(0.0)	8.00(1.0)	0.13
12	6.67(2.02)	8.22(1.26)	**<0.01**	6.80(0.1)	8.04(1.4)	**0.01**	7.66(1.4)	8.70(1.1)	0.06
13	6.14(0.94)	8.68(1.04)	**<0.01**	6.10(0.4)	7.42(1.7)	**0.02**	7.00(1.7)	8.20(1.3)	0.07
14	6.70(0.35)	8.83(1.47)	**<0.01**	-	8.30(1.6)	-	8.20(0.0)	8.31(1.7)	0.86
15	6.23(1.10)	8.49(1.71)	**<0.01**	-	8.25(1.2)	-	6.80(0.0)	7.48(1.8)	0.32
16	6.30(0.28)	8.50(1.56)	**<0.01**	5.30(0.1)	6.83(1.3)	**0.01**	-	6.45(1.6)	-
17	6.70(0.15)	8.03(1.13)	**<0.01**	6.50(0.0)	7.80(1.4)	**0.02**	6.34(1.1)	7.18(1.1)	0.11

Table VIII: Relationship between degree of anaemia and the ECG abnormalities.

			LVH				ST segment anomaly				PR interval anomaly	
Degree of anaemia	**Present**	**Absent**	χ^2	**P-value**	**Present**	**Absent**	χ^2	**P-value**	**Present**	**Absent**	χ^2	**P-value**
Mild	1	10	52.41	**<0.01**	2	9	2.69	0.26	1	10	1.32	0.52
Moderate	18	74			13	79			16	76		
Severe	46	15			15	46			7	54		

DISCUSSION

All the children were found to be anaemic. The high prevalence of severe anaemia at 9-14 years may be due to the combined effect of the chronic anaemia of SCA and the effect of puberty. This is because, it has been observed that puberty induces a state of anaemia, resulting from a relative deficiency of iron needed for red blood cell production in both male and female, due to its consumption during the exaggerated growth of lean body mass and menstruation; for the females (28). Similar observation was made in a study by Hall et al, (29) where they observed that children within the ages of 12 – 14 years were 1.35 times more likely to be anaemic than children aged 7 – 11 years.

The cardiac abnormality detected in children with SCA portrays that the presence of SCA is a risk factor for the development of cardiac abnormality and has been attributed to the chronic anaemia and vaso-occlusion, with consequent hypoxia and ischaemia of cardiac tissues.16,18 Similar20 and different (13, 21, 30, 31) prevalence have been reported by different authors. The difference in prevalence among several studies may be due to some factors such as the ECG parameters used to define abnormal ECG, differences in diagnostic criteria or cut-off value used, the steady state status of the recruited children with SCA, the age range of the participants and differences in sample size.

http://dx.doi.org/10.36472/msd.v8i1.458

The lower prevalence observed in the 2 studies by Bode-Thomas et al (31, 32) may be due to the ECG parameters used to define abnormal ECG, as their focus was only on presence of arrhythmia (32) and myocardial ischaemic changes (31) The high prevalence observed by Adegoke et al (30) and Odike et al (13) may be due to difference in the diagnostic criteria used; Sokolow and Lyon criteria (33) and centile chart by Davignon et al34 respectively.

Also, the steady state status of children with SCA was not an inclusion criterion in the study by Odike et al, (13) as children that were not in steady state may also have been studied. There was no significant difference in the prevalence of abnormal ECG between males and females in this study, though the prevalence was higher in the former than in the later.

This is similar to previous reports that there is little or no difference in ECG between males and females among children with SCA (20, 30) and also in non-SCA children (30, 35). Abnormal ECG was not seen in any 2-year-old child, rather in older children, with a median age of 10 years. This shows that it may take some years for the cardiac complications of SCA to develop in a child, reflecting the chronicity of the condition. This is similar to that observed in other studies (14, 20)

This finding may also be explained by the observation that 59% of children with SCA who had severe anaemia were older children (9- 14 years), showing that the degree of anaemia may also determine the occurrence of cardiac anomaly, as a result of the cardiac response to the chronic anaemia (16)

LVH was the commonest abnormality detected. It has also been shown to be the commonest ECG abnormality in children with SCA.15 It is as a result of the chronic anaemia with consequent decrease in cardiac output, which then leads to a compensatory cardiac dilatation in an attempt to increase the cardiac output (17, 18)

Overtime, the ventricle adapts to the increased wall stress by developing eccentric hypertrophy (17) LVH has also been observed to be the commonest abnormality by other authors (13, 14, 30, 36) The lower prevalence reported by Naburi et al20 and Ali et al14 may be due to difference in diagnostic criteria, which was not stated. Also, they included infants, whom at that age, may not have developed the compensatory hypertrophy due to the presence of foetal Hb.1,37 LVH was seen more in males than in females, though not significant.

This is similar to that reported in a previous study (20). Regarding the R wave and S wave amplitudes, the median amplitudes were higher in older children with SCA, but without pattern, unlike the RS progression with age that occurs in non-SCA children (35)

There was a significant relationship between the age and development of LVH, as LVH occurred more in older children; 60% of children with SCA were $\geqslant$ 10 years. This may suggest that it may take some years before the heart develops compensatory hypertrophy, consequent to the chronic anaemic. Other studies (14, 20) have reported similar finding.

This finding may also be explained by the fact that severe anaemia was more in older children, as worsening severity of anaemia has been shown to correlate positively with LVH in SCA (14, 18) 70.8% of those with LVH also had severe anaemia, mean Hb of those with LVH was lower than that of those without LVH across all ages and a negative correlation between Hb concentration and development of LVH. This has been corroborated by other studies (13, 14, 20, 38)

The PR interval anomaly observed may be attributed to the effect of ischaemia and infarction on the conducting tissues of the heart (12). It has also been reported to result from increased vagal tone, which is a compensatory mechanism for the hypoxia resulting from the chronic anaemia of SCA (20). This is similar to that observed by Adegoke et al,30 with a smaller prevalence observed in other studies (13, 31, 36).

The similarity with Adegoke et al (30) may be due to similar centile chart used to analyse PR interval. Other studies (13, 31) observed that PR anomaly was commoner in non-SCA with acute anaemia than in children with SCA. The lower prevalence may be attributed to a better adjustment of the conducting system of the heart to chronic anaemia of SCA than to anaemia of acute onset non-SCA children in their studies. In addition, their finding is similar to the finding of a higher mean PR interval in non-SCA children than in SCA children by Odia et al (36). Other studies have also observed PR interval anomaly among children with SCA (14, 20)

In contrast to the present study, Anjan et al (38) did not observe PR interval anomaly among children with SCA. A smaller sample size may explain this and also, the Hb concentration of the participants may be higher, because they also recruited children who were on chronic transfusion. Males had PR interval anomaly than females, though the difference was not significant. This is similar to other studies (13, 20)

There was no significant difference in the prevalence of PR interval anomaly among the ages of the participants, as it was fairly distributed among younger and older children. Thus, it can be inferred that PR anomaly starts early in childhood. A similar finding was observed by Naburi et al. (20) There was no significant relationship between the Hb concentration and PR interval anomaly, though there was a relatively lower Hb concentration among children with SCA who had PR interval anomaly than those without the anomaly in all ages. Also, there was no significant difference among the degrees of anaemia, with regards to development of PR interval anomaly.

Unlike in LVH where majority of children had severe anaemia, majority of children with PR interval anomaly had moderate anaemia. This observation may be inferred to mean that the conducting system of the heart is affected by lesser degree of anaemia, unlike the compensatory hypertrophy that occurs with worsening severity of anaemia. This is similar to the finding by Naburi et al, (20) as they observed that all the children with SCA who had prolonged PR interval had Hb concentration <8g/dl. This is in contrast to the finding by Bode-Thomas et al, (32) where it was observed that the mean Hb of children with SCA who had arrhythmia (including PR anomaly) was higher than that of those without arrhythmia, thus suggesting the possible role of other risk factors in the

occurrence of PR interval anomaly, aside from anaemia and ischaemia.

The ST segment anomaly has been reported to be due to myocardial injury resulting from ischaemia with or without myocardial infarction and impaired myocardial function, due to patchy micro-vascular occlusion and hypo-perfusion that resulted from sickling of red blood cells in SCA (39) The finding of ST segment anomaly in children with SCA had been observed by several authors (13, 20, 30, 31, 36)

However, this was different from the study by Anjan et al,38 as they did not observe ST segment anomaly in children with SCA, possibly because some of the participants involved in the study were on chronic transfusion. The higher prevalence observed by Naburi et al (20) and Odia et al (36) might be as a result of inclusion of participants who were not in steady state; therefore, children who may have had a recent crisis may have been recruited, thus, increasing the chance of detecting ST anomaly.

This is because, Bode-Thomas et al (31) has shown that ST anomaly was significantly higher in children with SCA crisis than in those in steady state. The lower prevalence observed by Bode-Thomas et al (31) and Odike et al (13) may be due to the pattern of ST anomaly observed in their study, as they noted either ST segment elevation (13) or depression (31) in children with SCA, and not both. This pattern is unlike that, observed in the present study, which is also corroborated in other studies (20, 30). Bode-Thomas et al (31) noted that those in steady state had only ST segment depression.

Rather, ST segment elevation was observed in non-SCA children who had acute anaemia. Likewise, Odike et al (13) observed ST elevation more among non-SCA that had anaemia than in SCA children. These two findings may suggest that ST segment depression is seen more in chronic anaemia while ST elevation may be seen more in acute anaemia.

The finding of more ST segment depression (80%) in this study also supports this observation. Also, considering myocardial infarction, the observation of more ST segment depression may suggest that SCA may be associated with non-ST elevation myocardial infarction (NSTEMI) than ST elevation myocardial infarction (STEMI).

In contrast, though Odia et al (36) detected both anomalies, ST elevation was more prevalent than ST depression in children with SCA. This may be due to the fact that some of the subjects may have had acute anaemic crisis recently before recruitment, since the steady status was not an inclusion criterion in their study. The ST segment anomaly was significantly higher in female, unlike the previous ECG anomalies. The reason for this, is not clear, but it was in contrast to the observation made by Naburi et al, (20) where it was seen more males, though the difference was not significant. There was no significant difference among the ages of children with SCA, with regards to occurrence of ST anomaly, showing that the prevalence of the ischaemic changes is fairly distributed in both younger and older children.

In contrast, Naburi et al (20) observed that ST segment anomaly was seen more in older children; above 6 years of age. The mean Hb of those with ST anomaly was lower than that of those without ST anomaly across all ages, portraying the effect of the chronic anaemia of SCA on the myocardium.39 However, there was no significant difference among the different degrees of anaemia, with regards to ST anomaly, as moderate and severe anaemia were fairly distributed among the participants with ST anomaly. This is similar to the observation by Bode-Thomas et al, (31) and may be due to a possible adaptation of the child to the chronic anaemia.

Though Naburi et al (20) observed a significant relationship between degree of anaemia and ST anomaly (they observed that 83.3% of children with SCA who had ST anomaly had Hb concentration below 8g/dl), it may also be likened to the observation made in this study, as the mean Hb of those with ST anomaly for each age was also below 8g/dl.

CONCLUSION

Children with SCA have commonly demonstrated cardiac abnormality on an ECG. The haemoglobin concentration of those with cardiac abnormality was lower than the haemoglobin concentration of those without cardiac abnormality across all ages. Severe anaemia was a significant predictor of LVH, but there was no significant association between the degree of anaemia and the development of PR interval and ST segment anomalies.

Acknowledgment: We thank almighty God and also wish to express our profound gratitude to the management of the Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) and the staff of Sickle Cell Disease unit for their support and immense contributions towards the actualization of this work.

Conflict of interest: There was no conflict of interest.

Limitation: None

Funding: The research was solely funded by the research authors.

Patient and Public Involvement: There was no involvement of the patient/public during the design, or conduct, or reporting, or dissemination plans of our research.

REFERENCES

1. Adekile AD, Adeodu OO, Adegoke SA. Haemoglobinopathies. In Azubuike JC, Nkangineme KEO (editors). Paediatrics and Child Health in a Tropical Region, 3rd ed. Lagos: Educational printing and publishing; 2016. p.1051-1062

2. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull. World Health Organ. 2001;79:704–712

3. Anie KA, Egunjobi FE, Akinyanju OO. Psychosocial impact of sickle cell disorder: perspectives from a Nigerian setting. Global. Health. 2010;6:1–6.

4. Morris CR. Mechanisms of vasculopathy in sickle cell disease and thalassemia. Hematology Am Soc Hematol Educ Program. 2008;2008:177–185

5. Weatheral DJ. Genetic disorders of hemoglobin. In Hoffbrand AV, Lewis SM, Tuddenham EG, editors. Postgraduate Hematology, 14th ed: Oxford. Butterworth Heinemann; 1999. p.111-118

6. Gladwin MT, Sachdev V. Cardiovascular abnormalities in sickle cell disease. J Am Coll Cardiol 2012;59:1123-1133

7. Fitzhugh CD, Lauder N, Jonassaint JC,Telen MJ, Zhao X, Wright EC, et al. Cardiopulmonary Complications Leading to Premature Deaths in Adult Patients with Sickle Cell Disease. Am J Hematol. 2010;85:36-40

8. Makani J, Cox SE, Soka D, Komba AN, Oruo J, et al. Mortality in sickle cell anaemia in Africa: A prospective Cohort study in Tanzania. PLosONE 2011;6:e14699-e14704

9. Chacko P, Kraut EH, Zweier J, Hitchcock C, Raman SV. Myocardial Infarction in Sickle Cell Disease: Use of Translational Imaging to Diagnose an Under-Recognized Problem. J Cardiovasc Transl Res. 2013;6:752–761.

10. Gacon PH, Elhraiech A, Jourdain P, Heba N, Amara W. Transient atrioventricular block associated with sickle cell disease: a French survey.Eur. Heart J. 2013;34:5577 (https://doi.org/10.1093/eurheartj/eht310.P5577)

11. Kleber AG. ST-segment elevation in the electrocardiogram: a sign of myocardial ischemia. Cardiovasc. Res.2000;45:111-118

12. Schwartzman D. Atrioventricular block and atrioventricular dissociation. In Zipes D, Jalife J (editors.). Cardiac Electrophysiology: From cell to bedside, 4th ed. Philadelphia, PA: WB Saunders; 2004. p. 485-489

13. Odike AI, Omokhodion SI, Akpede GO. The electrocardiographic profile in non-sickle cell anaemia children and sickle cell anaemia children. Indian J Basic Appl Med Res 2018;7:68-76

14. Ali GOM. AbdalGader YS, Abuzeid ES, Attalla BAI. Cardiac manifestations of sickle cell anemia n Sudanese children. Sudan J Paediatr2012;12:70-78

15. Dosunmu A, Akinbami A, Uche E, Adediran A, John-Olabode S. Electrocardiographic Study in Adult Homozygous Sickle Cell Disease Patients in Lagos, Nigeria. J Trop Med 2016;2016:4214387-4214392

16. Serjeant GR, Serjeant BE. Cardiovascular system. In Serjeant GR, Serjeant BE (editors).Sickle Cell Disease, 3rd ed. Oxford: Oxford University Press; 2001. p.194-208

17. Adebayo RA, Balogun MO, Akinola NO, Akintomide AO, Asaleye C.M. Non-invasive assessment of cardiac function in patients with sickle cell anaemia. Trop Cardiol. 2004;30:51–55

18. Voskaridou E, Christoulas D, Terpos E. Sickle-cell disease and the heart: review of the current literature. Br. J Haematol 2012;157:664–673

19. Bluemke DA, Kronmal RA, Lima JA,Liu K, Olson J, Burke GL, et al. The relationship of left ventricular mass and geometry to incident cardiovascular events; the MESA (Multi- Ethnic study of Atherosclerosis) Study. J Am Coll Cardiol 2008;52:2148-2155

20. Naburi HED, Lwakatare JM, Kalokola FM, Mpembeni R, Kubojha S. Cardiovascular Abnormalities in Children and Adolescents with Sickle Cell Anemia at Muhimbili National
21. Hospital, Dar Es Salaam, Tanzania. Tanzania Medical Journal 2012;26:22-28

22. Kligfield P, Gettes LS, Bailey JJ, Childers R, Deal BJ, Hancock EW, et al. American Heart Association Committee Report: Recommendations for the Standardizationand interpretation of the electrocardiogram, Part1. J Am Coll Cardiol 2007;49:1109-1127

23. Afolabi JK, Oloko GYA. Pediatrics electrocardiography. Niger J Paed 2012;39:84-89

24. Afolabi JK, Omokhodion SI. Normal limits for pediatrics electrocardiogram in Ilorin, Nigeria. Nig J Cardiol 2014;11:112-123

25. Hampton JR. What the ECG is about. In Gale A (editor). The ECG made easy, 8th ed. Nottingham UK: Churchill Livingstone Elsevier; 2013. p.22-97

26. Bernstein D. Evaluation of the cardiovascular system. In Kliegman RM, Stanton BF, St. Geme JW, Schor NF, Behrman RE (editors). Nelson textbook of paediatrics, 20th ed. Philadelphia: Elsevier; 2016. p.2163-2178

27. Parab S, Bhalerao S. Choosing Statistical Test. Int J Ayurveda Res. 2010;1:187-191

28. Ratner B. The correlation Coefficient: its values range between +1/-1, or do they? J. Target Meas Anal Mark 2009;17:139-142

29. Janz KF, Dawson JD, Mahoney LT. Predicting heart growth during puberty: The Muscatine study. Pediatrics 2000;105:e63-e72

30. Hall A, Bobrow E, Brooker SJ, Jukes MCH, Nokes K, Lambo A, et al. Anemia in school children in eight countries in Africa and Asia. Public Health Nutr 2001;4:749-756

31. Adegoke SA, Okeniyi JAO, Akintunde AA. Electrocardiographic abnormalities and dyslipidaemic syndrome in children with sickle cell anaemia. Cardiovasc J Afr 2016;27:16-20.

32. Bode-Thomas F, Hyacinth HI, Ogunkunle O, Omotosa A. Myocardial ischemia in sickle cell anaemia: evaluation using a new scoring system. Ann Trop Paediatr 2011;31:67-74

33. Bode-Thomas F, Ogunkunle OO, Omotoso ABO. Cardiac arrhythmias in children with sickle cell anaemia. Niger J Paediatr 2003;30:13-17

34. Evans RW. The sickling phenomenon in the blood of the West African natives. Trans R Soc Trop Med Hyg. 1944;37:281-286

35. Davignon A, Rautaharju P, Boisselle E, Soumis F, Mégélas M, Choquette A. Normal ECG standards for infants and children. Pediatr Cardiol 1979;1:123-131

36. Dickson DF. The normal ECG in childhood and adolescence. Heart. 2005;91:1626–1630

37. Odia K, Dapper V, George I, Olorunfemi OJ. Changes in Some Electrocardiographic Parameters amongst Children with Sickle Cell Anemia in Port Harcourt, Nigeria. J Med Dent Sci 2016;15:69-72

38. Adewoyin AS. Management of Sickle Cell Disease: A Review for Physician Education in Nigeria (Sub-Saharan Africa). Anemia 2015;2015:1-21

39. Anjan SB, Ruben JA, Wing-yen W,Wood JC, Chan LS, Ramicone E, et al. Cardiac abnormalities in children with sickle cell anemia. Am J Hematol 2002;70:306-312

40. De Montalembert M, Maunoury C, Acar P, Brousse V, Sidi D, LenoirG. Myocardial ischaemia in children with sickle cell disease. Arch Dis Child 2004;89:359-362

Medical Science and Discovery
ISSN: 2148-6832

The Colistin-Related Nephrotoxicity and Risk Factors In The Intensive Care Unit; A Retrospective Study

Guler Eraslan Doganay[1]*, Mustafa Ozgur Cirik[1], Ali Alagoz[1]

1 University of Health Sciences, Atatürk Chest Diseases and Chest Surgery Training and Research Hospital Dept of Anesthesiology and Reanimation, Ankara, TR
*** Corresponding Author:** Guler Eraslan Doganay **E-mail:** gulerdoganay@hotmail.com.tr

ABSTRACT

Objective: Colistimethate sodium (CMS) which is salvage therapy in the management of infections caused by multi-drug resistance (MDR) gram-negative pathogens is eliminated by the kidneys and cause nephrotoxicity. Many factors may also contribute to this nephrotoxic effect. In this study we aimed to determine the risks for the development of nephrotoxicity patients who received CMS in the intensive care unit (ICU).

Materials and Methods: We evaluated retrospectively of the patients who have lung cancer or COPD, aged older than 18 years, and received intravenous CMS therapy at least 72 hours in ICU. Patients' age, comorbidities, C-reactive protein (CRP), procalcitonin, albumin, glomerular filtration rate (GFR), creatinine values on the 1st and 7th days of CMS treatment, positive inotropes, and nephrotoxic drugs used concurrently with CMS therapy, and renal replacement therapy (RRT) were recorded. RIFLE score , lenght of stay (LOS) in hospital and in the ICU, and 28-day mortality were also recorded.

Results: In this study, the GFR and creatinine level deteriorated significantly on the 7th day with CMS therapy patients who had preexisting lower GFR, hypoalbuminemia, and concomitant nephrotoxic drugs usage. The incidence of acute kidney injury was higher in malignant patients and 28-day mortality increased in patients with nephrotoxicity.

Conclusion: The CMS therapy with preexisting lower GFR, hypoalbuminemia, and concomitant nephrotoxic drugs usage significant risk factors to develop nephrotoxicity. It was also higher in malignant patients and increased 28-day mortality. Detailed clinical and laboratory evaluation of the patients is needed before CMS treatment.

Keywords: colistimethate sodium, nephrotoxicity, ICU, risk, mortality

Received 22-10-2020

Accepted 29-12-2020

Published 30-01-2021

INTRODUCTION

Colistin is an antibiotic belonging to the polymyxin group, which as salvage therapy in the management of infections caused by multi-drug resistance (MDR) gram-negative pathogens including Pseudomonas, Acinetobacter, Klebsiella, and Enterobacter species (1, 3). Due to the side effect of nephrotoxicity, its use was abandoned in the last quarter of the 20th century, but with the increase in infections due to multi-resistant gram-negative bacteria in the last two decades, it has been reintroduced due to the lack of a more reliable alternative (1, 4, 5). There are two commercial forms of colistin, colistin sulfate and colistimethate sodium (CMS). Colistin sulfate is eliminated by the extrarenal system, but CMS is eliminated by the kidneys. It is thought that the nephrotoxicity mechanism of CMS increases the cytoplasmic membrane permeability in proximal tubule cells, causing an excessive amount of water to pass into the cell and destroying the cell (6). Hypoalbuminemia, positive inotropes or nephrotoxic drugs use may also contribute to this nephrotoxic effect (7, 8). In clinical practice, CMS is often used as combination therapy, but data on whether combination therapy is superior to monotherapy is limited (9).

http://dx.doi.org/10.36472/msd.v8i1.433

This study aims to determine the risks for the development of nephrotoxicity according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria by retrospectively evaluating patients using CMS in intensive care unit (ICU) on malignancy and Chronic Obstructive Pulmonary Disease (COPD) patients (Table 1).

Table 1: Definition of RIFLE criteria (9).

Category	Criteria
Risk (R)	Increased creatinine level x 1.5 or GFR decrease >25%
Injury (I)	Increased creatinine level x 2 or GFR decrease >50%
Failure (F)	Increased creatinine level x 3, GFR decrease >75%, or creatinine level >4 mg/dL
Loss (L)	Persistent acute renal failure or complete loss of function for >4 weeks
ESKD (E)	End-stage Kidney Disease for >3 months

GFR:Glomerular Filtration Rate

MATERIALS AND METHODS

After obtaining the approval of the ethics committee (04/07/2019/634) a retrospective review of patients aged ⩾ 18 years who received intravenous CMS therapy for at least 72 hours while under treatment in the ICU January 2018 through December 2018. Patients who were pregnant or breastfeeding, patients receiving concurrent inhaler colistin therapy, patients receiving colistin therapy for less than 72 hours, receiving RRT before starting CMS treatment, and patients with chronic kidney disease were excluded from the study. Demographic data of patients who had COPD or lung cancer, comorbidities as Charlson Comorbidity Index (CCI), C-reactive protein (CRP), procalcitonin, albumin values, glomerular filtration rate (GFR), and creatinine values on the 1st and 7th days of CMS treatment, positive inotropes and nephrotoxic drugs (nonsteroidal anti-inflammatory drugs (NSAID), radiocontrast agents, diuretics, aminoglycoside, vancomycin, anticandidal) used concurrently with CMS therapy, and renal replacement therapy (RRT) was recorded retrospectively. RIFLE score (Table 1), lenght of stay (LOS) in hospital and in ICU, and 28-day mortality were also recorded. CMS therapy was started intravenously 300 mg loading dose and after maintenance dose was administered 5 mg/kg/day (twice-daily dosing regimen) at least 72 hours. Of the 39 patients who received CMS therapy, 2 patients were excluded from the study because one patient had a history of chronic kidney disease, was receiving RRT before starting CMS treatment and one patient died at the 48th hour of therapy. The study was planned with 37 patients (Figure 1).

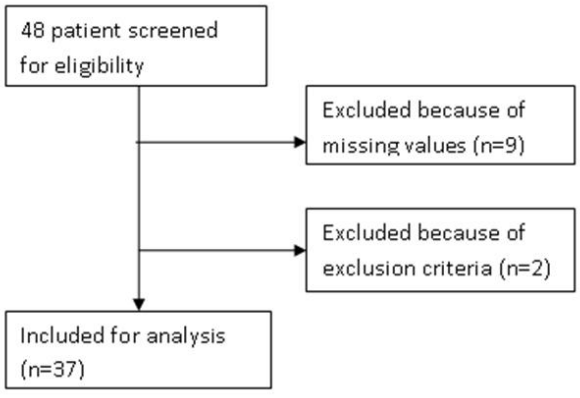


Figure 1: Flow diagram of the subjects.

Statistical analysis: Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables were normal or not was determined by Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean ± standard deviation for normal distributions, and median (minimum and maximum value) for skewed distributions.

Categorical data were described as the number of cases (%). Statistical analysis differences in normally distributed variables between two independent groups were compared by Student's t-test, Mann Whitney U test was applied for comparisons of the not normally distributed data. Categorical variables were compared using Pearson's chi-square test or fisher's exact test. It was accepted p-value < 0.05 as a significant level on all statistical analyses.

Logistic regression(LR) analysis was performed for age, APACHE II scores, SOFA scores, CRP levels, procalcitonin levels, albumin levels, concomitant use of positive inotropes and other nephrotoxic drugs, LOS in the ICU and hospital, which were considered as risk factors for acute kidney injury (AKI). The backward LR method was chosen as the method.

First of all, the patients were included in the multivariate analysis to investigate whether the age, albumin levels, concomittant use of positive inotropic drugs , concomitant use of other nephrotoxic drugs, LOS in hospital and in ICU if predictor of nephrotoxicity.

RESULTS

Data of 37 patients who met the inclusion criteria were analyzed. The 24 (65%) of them were male and 13 (35%) were female. Nephrotoxicity developed in 10 (27%) patients, 1 (2.7%) female and 9 (24.3%) male, after using CMS for at least 72 hours in ICU. There was no significant difference in terms of mean age, gender, APACHE II, CCI scores, CRP, and procalcitonin values (p > 0.05). The LOS in the hospital and in the ICU among the patient was not statistically significant groups with and without nephrotoxicity (p > 0.05). The patients' demographic and clinical characteristics are presented in table 2. In the group with nephrotoxicity; while albumin levels (p= 0.021) and GFR values in the 1st day (p= 0.028) of CMS treatment were statistically significantly lower than the group without nephrotoxicity, SOFA scores were higher (p= 0.008). On the 7th day of CMS use, creatinine(p= 0.001) values were statistically significantly higher and GFR values (p= 0.003) were statistically significantly lower in patients with nephrotoxicity compared to patients without nephrotoxicity. The 28-day mortality rate was also statistically significantly higher in patients with nephrotoxicity compared to patients without nephrotoxicity (p= 0.002), but there was no significant difference between the LOS in the hospital and in the ICU(p> 0.05). There was no statistically significant difference regarding the occurrence of nephrotoxicity in terms of concomitant positive inotropes use (p> 0.05). Using the Backward LR method, it was evaluated that in the last 5th step, hypoalbuminemia and the use of concomitant other nephrotoxic drugs were predictive for the development of nephrotoxicity.

http://dx.doi.org/10.36472/msd.v8i1.433

The risk of developing nephrotoxicity is higher in patients with hypoalbuminemia (p= 0.012) and the use of concomitant nephrotoxic drugs (p= 0.026), (Table 3).

ROC curve analysis was performed to determine the effect of albumin level on the development of nephrotoxicity and to determine the cut-off value. When the area under the curve was analyzed, it was determined that it was not statistically significant. (p>0,05), (Table 4), (Figure 2).

Malignancy (p= 0.035) was statistically significantly higher in patients with nephrotoxicity compared to patients without nephrotoxicity.

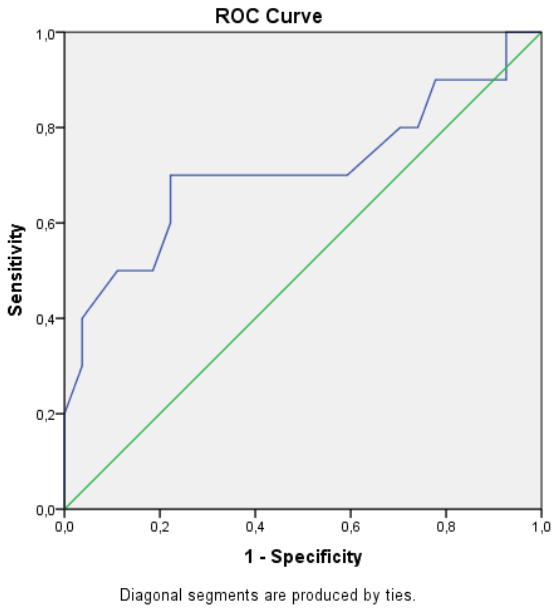
There were no statistically significant difference in the rates of Chronic Obstructive Pulmonary Disease(COPD) patients (p> 0.05) according to the development of nephrotoxicity (Table 5).

Table 2: Demographic and Clinical characteristic values of patients

	NEPHROTOXICITY				p
	Nephrotoxicity(+) (n:10)		Nephrotoxicity(-) (n:27)		
Age(year)	66.60	± 13.13	70.30	± 13.40	0.459
APACHE II	22.70	± 9.46	19.81	± 6.25	0.287
LOS in Hospital	35.80	± 10.02	38.26	± 25.17	0.767
LOS in ICU	10	(1-34)	8	(1-50)	0.775
Gender Male(n/%)	9	(90.0)	15	(55.6)	0.051
Female(n/%)	1	(10.0)	12	(44.4)	
Albumin(g/l)	2.55	± 0.72	3.05	± 0.50	**0.021**
1st day GFR(ml/min/1.73m^2)	95.5	(67-129)	91	(10-117)	**0.216**
1st day Creatinine(mg/dl)	0.6	(0.4-1.2)	0.8	(0.3-4.9)	0.139
7th day Creatinine(mg/dl)	1.95	(1.2-5.5)	0.9	(0.5-3.3)	**0.001**
7th day GFR(ml/min/1.73m^2)	30.5	(10-66)	81	(13-112)	**0.003**
CCI	6.00	± 3.02	5.33	± 1.90	0.427
Procalcitonin(ng/ml)	0.50	(0.14-9.7)	0.53	(0.01-14.5)	0.533
CRP(mg/l)	13.75	(1.1-23.1)	5.1	(0.1-34)	0.148
SOFA	7.5	(5-17)	5	(4-8)	**0.008**
Nephrotoxic drug					
Anticandidal(n/%)	1	(10.0)	-		
Vancomycin(n/%)	1	(10.0)	1	(3.7)	
Positive inotropes(n/%)	2	(20.0)	4	(14.8)	0.999
28-day mortality(n/%)	9	(90.0)	8	(29.6)	**0.002**
RRT (n/%)	2	(18.2)	-		0.068

Table 3: Variable excluded from multivariate analysis

	B	SE	Wald	P	Exp(B)	95% CI for Exp(B)
Albumin	2.340	0.932	6.299	**0.012**	0.096	(0.015-0.599)
Nephrotoxic drugs	3.484	1.560	4.987	**0.026**	32.581	(1.531-693.145)



 http://dx.doi.org/10.36472/msd.v8i1.433

Table 4: ROC Analysis for albumin results

Area	Std. Error	p	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.711	0.112	0.051	0.492	0.931

Table 5: Effects of COPD and Malignancy on Nephrotoxicity

	Nephrotoxicity(+) (n:10)	Nephrotoxicity(-) (n:27)	p
Malignancy	4 (40)	2 (7.4)	**0.035**
COPD	7 (70)	22 (81.5)	0.655

DISCUSSION

This study shown that hypoalbuminemia, CMS treatment, higher SOFA scores, and simultaneously the use of other nephrotoxic drugs were predictive for the development of nephrotoxicity. CMS treatment causes GFR and creatinine levels impairment approximately in 7 days and the development of nephrotoxicity is higher in patients who have lung cancer.

Nosocomial infections caused by MDR gram-negative microorganisms are an important cause of morbidity and mortality in ICU (10, 11). Due to the side effect of nephrotoxicity (approximately 5-55%) (12) its use was abandoned, but with the increase in infections due to MDR gram-negative bacteria, colistin has been reintroduced due to the lack of a more reliable alternative (1-5). Colistin nephrotoxicity generally develops in the first week of treatment (1).

In this study, we determine the risks for the development of nephrotoxicity according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria by retrospectively evaluating multi-parameter of patients using colistin in ICU.

In different studies, risk factors for nephrotoxicity include advanced age, pre-existing chronic renal disease, hypoalbuminemia, and the combined use of NSAIDs or vancomycin (1). Similarly, in some studies, among the reasons that increase the risk of acute renal failure due to CMS, male gender, advanced age, diabetes mellitus, obesity, hypoalbuminemia, use of nephrotoxic drugs (13, 14). In our study, in support of these publications, low albumin, low GFR at the beginning of the CMS therapy, simultaneous nephrotoxic drugs use and high SOFA scores increase the development of nephrotoxicity, but no difference was found in terms of comorbidity, advanced age, and gender. Although some risk factors are different from the studies mentioned, many risk factors are related to the development of nephrotoxicity as in our study. If CMS is used as an alternative therapy in MDR gram-negative bacteria, optimizing risk factors as much as possible and close monitoring may limit the development of nephrotoxicity.

It is thought that the nephrotoxicity mechanism of CMS increases the cytoplasmic membrane permeability in proximal tubule cells, causing an excessive amount of water to pass into the cell and destroying the cell (6).

Besides the pathophysiological mechanism of nephrotoxicity, the development of nephrotoxicity in a wide range (12) and ongoing discussions regarding the duration of treatment (1) may be due to the effect of nephrotoxic drugs and other risk factors used during colistin therapy.

Correlation between duration of colistin therapy and nephrotoxicity is controversial. Several studies have examined the dose-dependence of iv colistin-induced nephrotoxicity (15, 16). Some studies suggest that toxicity is related to the total dose and duration of therapy and the total cumulative dose (17, 18). Hartzell, et al found that, while the use of additional nephrotoxic drugs did not affect the development of colistin-related nephrotoxicity, the duration of colistin use was more than 14 days increased the risk of nephrotoxicity four times, and it was increased when this period was exceeded (19). On the contrary, Falagas, et al emphasizes that the duration of colistin therapy did not affect the nephrotoxicity (20).

In some studies older age, prolonged colistin administration, hypoalbuminemia, high CCI, and the presence of septic shock were reported to be related to nephrotoxicity (8, 13, 15, 16, 21). Similarly, a few studies older age, hypoalbunemia, and use of nephrotoxic drugs were identified as significant risk factors (22, 23).

In our study, we examined those who developed nephrotoxicity by looking at GFR and creatinine values on the 1st and 7th days of colistin therapy. We used at least 72 hours as a criterion, but it was not among our data on treatment time in our study; we analyzed 28-day mortality rates, the LOS in ICU and hospital. According to our study findings, 28-day mortality was significantly higher in patients with nephrotoxicity, while the effect of the LOS in ICU and hospital was insignificant.

In the literature, there is a publication that indicates to the development of nephrotoxicity in malignant patients is significantly higher (23). In our study, supporting this situation, patients who developed nephrotoxicity were more likely to be malignant than those who did not but we did not encounter any significant difference in the COPD patients.

We have several limitations for our study. First of all this study is retrospective, there is no control group in our study. In addition, it is a single-center study.

CONCLUSION

The risk of nephrotoxicity increases significantly in patients with hypoalbuminemia and use of concomitant nephrotoxic drugs and on the 7th day of colistin use, deterioration of GFR and creatinine is significant. The incidence of AKI is higher in malignant patients and 28-day mortality increases in patients with nephrotoxicity. For the early detection and prevention of colistin-induced nephrotoxicity with the elimination of possible risk factors crucial for the duration of colistin therapy. Larger-scale and prospective controlled studies are needed to determine possible risk factors and prevention of AKI during the colistin therapy.

Author Contributions: GED, MOC, AA: Project design, Review of the literature, Data collection and statistical analyzes **GED:** Writing and Revisions

Conflict of interest: No actual or potential conflicts of interest exist in relation to this article.

Ethical issues: All authors declare originality of research.

REFERENCES

1. Yahav D, Farbman L, Leibovici L, Paul M. Colistin: new lessons on an old antibiotic. Clin Microbiol Infect. 2012;18:18-29.
2. 2.Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40:1333-1341.
3. Velkov T, Roberts KD, Nation RL, Thompson PE, Li J. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. Future Microbiol. 2013;8:711-724.
4. Couet W, Grégoire N, Marchand S, Mimoz O. Colistin pharmacokinetics: the fog is lifting. Clin Microbiol Infect. 2012;18:30-39.
5. Falagas ME, Kasiakou SK. Toxicity of polymyxins:a systematic review of THA evidence from old and recent studies. Crit Care 2006;10:R27.
6. Omrani AS, Alfahad WA, Shoukri MM, Baadani AM, Aldalbahi S, Almitwazi AA et al. High dose intravenous colistin methanesulfonate therapy is associated with high rates of nephrotoxicity; a prospective cohort study from Saudi Arabia. Ann Clin Microbiol Antimicrob. 2015;14:3.
7. Kim J, Lee KH, Yoo S, Pai H. Clinical characteristics and risk factors of colistin induced nephrotoxicity. Int J Antimicrob Agents.2009;34:434-438.
8. Petrosillo N, Ioannidou E, Falagas ME. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. Clin Microbiol Infect. 2008; 14:816-827.
9. Joshua D. Hartzell, Robert Neff, Julie Ake, Robin Howard, Stephen Olson, Kristopher Paolino, Mark Vishnepolsky, Amy Weintrob, and Glenn Wortmann. Nephrotoxicity Associated with Intravenous Colistin (Colistimethate Sodium) Treatment at a Tertiary Care Medical Center. Clinical Infectious Diseases 2009; 48:1724-1728.
10. Fridkin SK Increasing prevalence of antimicrobial resistance in intensive care units. Crit Care Med. 2001; 29:64-68.
11. Pogue JM, Lee J, Marchaim D, Yee V, Zhao JJ, Chopra T, et al. Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system. Clin Infect Dis. 2011;53:879-884.
12. Gauthier TP, Wolowich WR, Reddy A, Cano E, Abbo L, Smith LB. Incidence and Predictors of Nephrotoxicity Associated with Intravenous Colistin in Overweight and Obese Patients. Antimicrob Agents and Chemother. 2012;56:2392-2396.
13. Rocco M, Montini L, Aleesandri E, Venditti M, Laderchi A, De Gennaro P,et al. Risk factors for acut kidney injury in critically ill patients receiving high doses of colistin methanesufonate and/or other nephrotoxic antibiotics:a retrospective cohort study.Crit Care 2013;17(4);R174.
14. Kwon KH, Oh JY, Yoon YS, Jeong YJ, Kim KS, Shin SJ, et al. Colistin treatment in carbapenem-resistant Acinetobacter baumannii pneumonia patients: Incidence of nephrotoxicity and outcomes. Int J Antimicrob Agents.2015;45:605-609.
15. Lee YJ, Wi YM, Kwon YJ, Kim SR, Chang SH, Cho S. Association between colistin dose and development of nephrotoxicity. Crit Care Med.2015;43:1187-1193.
16. Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC, Eaton AE. Adverse effects of sodium colistimethate: manifestations and specific reaction rates during 317 courses of therapy. Ann Intern Med 1970; 72:857-868.
17. Falagas ME, Fragoulis KN, Kasiakou SK, Sermaidis GJ, Michalopoulos A. Nephrotoxicity of intravenous colistin: a prospective evaluation. Int J Antimicrob Agents 2005; 26:504-507.
18. Hartzell JD, Neff R, Ake J, Howard R, Olson S,Paolino K, et al. Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. Clin Infect Dis 2009;48(12):1724-1728.
19. Falagas ME, Rizos M, Bliziotis IA, Rellos K, Kasiakou SK, Michalopoulos A. Toxicity after prolonged (more than four weeks) administration of intravenous colistin. BMC Infect Dis. 2005;5:1.
20. Rattanaumpawan P, Ungprasert P, Thamlikitkul V.Risk factors for colistin-associated nephrotoxicity.J Infect. 2011; 62:187-190.
21. Paul M, Bishara J, Levcovich A, Chowers M, Goldberg E, Singer P, et al. Effectiveness and safety of colistin:prospective comperative cohort study. J Antimicrob Chemother. 2010;65:1019-1027.
22. Doshi NM, Mount KL, Murphy CV. Nephrotoxicity associated with intravenous colistin in critically ill patients. Pharmacotherapy. 2011;31:1257-1264.
23. Kaya M, Tunçel YI, Kuru RN, Menteş S, Ünver S, Çeken S. Retrospective Evaluation of Colistin Associated Nephrotoxicity at Oncology Hospital Intensive Care Unit. J Turk Soc Intens Care. 2014;12:51-56.

Medical Science and Discovery
ISSN: 2148-6832

Autism spectrum disorder referrals to a rural hospital in the past two years – a retrospective evaluation

Mert Beşenek[1]*

1 Recep Tayyip Erdogan University School of Medicine, Department of Child and Adolescent Psychiatry, Rize, TR
*** Corresponding Author:** Mert Beşenek **E-mail:** mbesenek1989@gmail.com

ABSTRACT

Objective: In addition to the core symptoms of Autism Spectrum Disorder (ASD); symptoms such as aggression, self-harm, impulsivity, hyperactivity, anxiety, and mood problems are also often present. Medication use is frequent and studies report that 27-40% of ASD patients use at least one psychotropic medication. We aimed to examine the clinical and sociodemographic features and treatment modalities of ASD patients who were referred to a rural hospital in the last two years.

Material and Methods: Age, gender, mean diagnosis age (MDA), type of ASD, psychiatric symptoms, medication (if they use one) types, and doses were recorded for 200 children with ASD (who were referred between August 2018 – August 2020) were retrospectively evaluated. Also, patients who were diagnosed with "childhood autism (CA)" and "other ASD diagnoses" were compared.

Results: The majority of the patients were male, the MDA value of the all patients was 4.56 (±2.2) years and there were no significant differences between groups regarding MDA (p = 0.053). Most frequently seen psychiatric symptoms were behavioral (33%) and attention problems (21%) and 52.5% of patients (n=105) were using at least one psychotropic medication. Patients with CA had higher rates of psychotropic medication use (p=0.010) and the most frequently used medication group was antipsychotic drugs (92.4%).

Conclusion: Treatment approaches utilized in rural hospitals are in line with the universal trends. However, considerably higher MDA compared to previous studies show that; to provide early diagnosis and better prognosis for ASD patients who live in rural areas, new interventions should be promoted by the local and/or general authorities.

Keywords: Autism spectrum disorder; early diagnosis; prognosis; treatment

Received 09-12-2020

Accepted 09-01-2021

Available Online: 11-01-2021

Published 30-01-2021

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is defined by difficulties in social communication/interaction and repetitive and restricted behaviors and interests (1). Studies with large samples indicate that ASD has a prevalence of 1-2% in the general population (2). Although some social and communicational problems might be present before the diagnosis; ASD specific symptoms generally occur around the age of three and mean diagnosis age (MDA) is reported to be 3.1 years (3, 4). ASD is one of the major psychiatric diagnosis with gender dominance; it is more frequently seen in males and male/female ratio among general population is almost four (5).

In addition to the core symptoms of ASD (disturbances in social communication/interaction, repetitive/restricted behaviors and interests); symptoms such as aggression, self-harm, impulsivity, hyperactivity, anxiety and mood problems are also often present (6). With mental retardation being the most common comorbid psychiatric diagnosis; these children generally have other psychiatric comorbidities such as anxiety disorders, conduct disorder, attention deficit and hyperactivity disorder (ADHD) and sleep disorders (3).

http://dx.doi.org/10.36472/msd.v8i1.449

Medication use towards comorbid psychiatric symptoms and/or diagnoses is frequent among children with ASD diagnosis and studies report that 27-40% of these patients use at least one psychotropic medication (7). In the light of these findings; we aimed to retrospectively examine the clinical and sociodemographic features and treatment modalities of ASD patients who were referred to a rural hospital in the last two years.

MATERIALS AND METHODS

Participants: In this study; clinical, sociodemographic and treatment-related characteristics of 200 children with ASD diagnosis who were referred to Children and Adolescent Psychiatry Out-patient Unit of Rize Training and Research Hospital between the dates of August 2018 – August 2020 were retrospectively evaluated. There were no exclusion criteria and only patients who were under age of 18 and were referred to our department between the indicated dates were included. Age, gender, age of diagnosis, type of ASD, psychiatric symptoms, medications (if they use one) and doses of the medications were recorded for each individual. Furthermore, patients who were diagnosed with Childhood Autism (CA [F84.0]) and "Other ASD Diagnoses (OAD)" [which includes Atypical Autism (F84.1), Rett's Syndrome (F84.2), Other Childhood Disintegrative Disorder (F84.3), Overactive Disorder Associated with Mental Retardation and Stereotyped Movements (F84.4), Asperger's Syndrome (F84.5), Other Pervasive Developmental Disorders (F84.8), Pervasive Developmental Disorder – Unspecified (F84.9)] according to International Statistical Classification of Diseases and Related Health Problems 10th Edition were compared regarding their gender, medication and age of diagnosis (8). The study was conducted in accordance with the ethical guidelines, including the World Medical Association (1975) Declaration of Helsinki 2008, and the legal requirements of the Ethics Committee of the institution it was conducted in (approval no: 2020/213).

Statistical Analysis: The data was analyzed using Social Sciences software version 21.0 (9). A definitive analysis of categorical data was conducted and results were reported as frequencies and percentages. Categorical data were compared with Chi-square test (Fisher's Exact Chi-square test if needed). Kolmogorov-Smirnov test was used to determine if continuous data fitted normal distribution or not.

Mean (± standard deviation [SD]) values were given for normally distributed variables and median (interquartile range [IQR]) values were given for non-normally distributed variables. Continuous variables between dichotomous groups were compared with Independent T-test (if normally distributed) or Mann-Whitney-U test (if non-normally distributed). The value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Total of 114 patients (57%) had CA diagnosis, whereas number of patients with OAD was 86 (43%). The majority of the patients were male and they constituted 80.5% (n=161) of total ASD group. Ages of examined individuals were between 1 and 17 and mean age was 8.12 (±3.8) years. Ages of patients with CA diagnosis were between 1 and 11 and mean age was 8.67 (±3.8) years; while ages of patients with OAD were between 1 and 17 and mean age was 7.4 (±3.8) years. The MDA of all patients was 4.56 (±2.2) years, of patients with CA diagnosis was 4.3 (±1.8) years and of patients with OAD was 4.91 (±2.6) years (Table 1).

There were no statistically significant difference between CA and OAD groups regarding their MDA ($p = 0.053$, Independent T-test, Table 2). Most frequently seen psychiatric symptoms were behavioral (33%) and attention problems (21%) and 52.5% (n=105) of children with ASD were using at least one psychotropic medication. Gender, diagnosis type, medication, and psychiatric symptom characteristics of individuals included in our study were elaborated in Table 1.

Even though there were no significant differences between CA and OAD groups regarding their genders ($p=0.394$, Independent T-test); patients with CA diagnosis had higher rates of psychotropic medication use ($p=0.010$, Independent T-test, Table 2). The most frequently used medication group was antipsychotic drugs (92.4%), and most frequently chosen antipsychotics were risperidone (%46.7) and aripiprazole (35.2%).

In addition to antipsychotics, methylphenidate (MPH) was the second frequent drug choice and all of the medication treatments seen among our sample group and their mean doses were summarized in Table 3.

Table 1: Sociodemographic and clinical characteristics of the study sample

		Total *(n=200, 100%)*	Childhood Autism (F84.0) *(n=114, 57.0%)*	Other Autism Diagnoses* *(n=86, 43%)*
	Age	8.12±3.8	8.67±3.8	7.40±3.8
	Age of Diagnosis	4.56±2.2	4.30 ±1.8	4.91±2.6
Gender	**- Male**	161 (80.5%)	93 (81.6%)	68 (79.1%)
	- Female	39 (19.5%)	21 (18.4%)	18 (20.9%)
Medication Use	**- Yes**	105 (52.5%)	69 (60.5%)	36 (41.9%)
	- No	95 (47.5%)	45 (39.5%)	50 (58.1%)
Medication Reason	**Behavioral Problems**	66 (33.0%)	46 (40.4%)	20 (23.3%)
	Stereotypical Movements	17 (8.5%)	10 (8.8%)	7 (8.1%)
	Attention Problems / Hyperactivity	42 (21.0%)	24 (21.1%)	18 (20.9%)
	Fears / Anxiety	4 (2.0%)	2 (1.8%)	2 (2.3%)
	Agression	8 (4.0%)	7 (6.1%)	1 (1.2%)
	Sleep Disturbances	9 (4.5%)	7 (6.1%)	2 (2.3%)

http://dx.doi.org/10.36472/msd.v8i1.449

Table 2: Comparison of age, gender and medication status between diagnostic groups.

	Number of Cases			Mean (±SD)				
	CA *(n=114)*	OAD* *(n=86)*	p[a]	CA *(n=114)*	AA and Others* *(n=86)*	t	df	p[b]
Gender								
- Male	93	68	0.394					
- Female	21	18						
Medication Use								
- Yes	69	36	**0.010**					
- No	45	50						
Age of the Diagnosis				4.30±1.76	4.91±2.64	-1.9	198	0.053

CA, childhood autism (F84.0); OAD, Other Autism Diagnoses; SD, standart deviation. * (Atypical Autism [F84.1], Rett's Syndrome [F84.2], Other Childhood Disintegrative Disorder [F84.3], Overactive Disorder Associated with Mental Retardation and Stereotyped Movements [F84.4], Asperger's Syndrome [F84.5], Other Pervasive Developmental Disorders [F84.8], Pervasive Developmental Disorder – Unspecified [F84.9])
a Chi-Square test, statistically significant p values are written in bold. **b** Independent T-test, statistically significant p values are written in bold.

Table 3: Psychotropic medication profile in the study sample.

Total of cases that use medication *(n=105)*	Number (%)	Dose Range (mg/day)	Mean Dosage (±SD) (mg/day)
Risperidone	49 (46.7%)	0.25 - 4	1.20 (±1.01)
Aripiprazole	37 (35.2%)	20-Jan	4.93 (±4.52)
Quetiapine	3 (2.9%)	50 - 100	83.33 (±28.87)
Haloperidole	4 (3.8%)	0.5 - 16	5.63 (±7.20)
Olanzapine	3 (2.9%)	2.5 - 10	5.63 (±3.16)
Clozapine	1 (1.0%)	200	200.00 (±0.00)
Antipsychotic Total	97 (92.4%)	-	-
OROS-MPH	19 (18.1%)	Oct-54	28.42 (±14.97)
MPH	7 (6.7%)	10-May	7.86 (±2.67)
Atomoxetine	8 (7.6%)	Oct-80	38.25 (±22.74) ≈ 1.42 mg/kg/day *
ADHD Treatment Total	34 (32.4%)	-	-
Fluoxetine	6 (5.7%)	20-Feb	15.33 (±7.66)
Sertraline	1 (1.0%)	50	50.00 (±0.00)
SSRI Total	7 (6.5%)	-	-
Sodium Valproate	3 (2.9%)	500	500.00 (±0.00)
Carbamazepine	1 (1.0%)	400	400.00 (±0.00)
Mood Stabilisers Total	4 (3.8%)	-	-
Melatonin	3 (2.9%)	6-Mar	4.00 (±1.73)

SD, standart deviation; OROS, osmotic-controlled release oral delivery system; MPH, methylphenidate; ADHD, attention deficit and hyperactivity disorder; SSRI, selective serotonin re-uptake inhibitor. * Calculated according to mean age of study sample (8.12 years) and 50 percantile weight values of Turkish children for that particular age (27)

DISCUSSION

In this study, several sociodemographic and clinical features of children with ASD who were referred to a rural hospital child and adolescent out-patient unit were examined. It was found that; the majority of children with ASD were male, rates of CA diagnosis (57%) and OAD (43%) were almost similar, MDA of these patients who were under treatment in a rural hospital was fairly late (MDA = 4.56 [±2.2] years), around half of them used at least one psychotropic medication and most frequent psychiatric symptoms seen among these patients were behavioral and attention problems. ASD is a neurodevelopmental disorder that is more frequently seen in males and in line with this rates of male gender were significantly higher for all of the examined groups (total, CA, OAD) (5). In addition to this; with non-specific symptoms might be present before the age of three, ASD specific symptoms generally occur around that age period and patients are mostly diagnosed few months later the onset of these specific symptoms (MDA for ASD is reported to be around 3.1 years) (3, 4). In a recent study which was conducted in Ankara (capital city of Turkey); MDA was reported as 40.7 months (3.4 years) (10).

MDA which we found in our sample group is considerably greater than what both national and international studies reported. Sociodemographic characteristics of the city which this study was conducted in might explain this discrepancy: It is a fairly secluded city with low population and the socioeconomic and educational level of its population is quite low compared to major cities of Turkey. It can be postulated; that in cities further than the focus of central health care systems, patients might have difficulties in reaching out to a child and adolescent psychiatrist or families with lower socioeconomic/educational levels might overlook ASD specific symptoms which their children exhibit and do not seek help until it is quite late.

This situation should be particularly emphasized cause early diagnosis and intervention strategies are the major positive prognostic factors for ASD and necessary steps should be taken (such as proper facilitation of health care systems in small cities and psychoeducation of families living in these cities regarding early symptoms of ADS) in order to enable these (11,12).

Behavioral problems are seen in ASD account for the majority of the referrals to a child and adolescent psychiatry clinic (13). Disruptive behaviors such as irritability and aggression are common among children with ASD and they usually have negative impacts on the daily life of both child and family (14, 15). In a study done by Mazurek et al. (2013), behavioral problems at clinically significant levels were reported in 50% of ASD group (16). Similar to these results, we also found that behavioral problems were the most common psychiatric symptoms (33%) among individuals with ADS who were under follow-up in child and adolescent out-patient unit. In addition, attention problems were determined as the second most common psychiatric symptoms (21%) in our sample group. In fact, both genetic and epidemiologic studies underline that attention problems are frequent in autistic children and ASD and ADHD have high comorbidity rates, symptom overlaps and shared genetic and psychopathological mechanisms (17).

Regarding the relationship between psychiatric symptoms of ADS patients and drug prescriptions; Uğur and Göker (2018) found that 49.2% of children with ASD were using at least one psychotropic medication and among the patients who were using medication, behavioral problem scale (Aberrant Behavior Checklist and Autism Behavior Checklist) scores were significantly higher compared to patients who were not using any medications (10). CA can be taken into account as a more "severe" condition than OAD and behavioral problems among CA are expected to be more frequent. In this respect, higher rates of psychotropic medication we found in children with CA in our sample were in line with previous research done in this field. Our findings of antipsychotics (92.4%) and ADHD medications (32.4%) being the most frequently used medication groups in a disorder characterized by behavioral and attention problems are also anticipated. In a review done by Young and Robert (2015), antipsychotics are stated as the most commonly used drugs in patients with ASD (6). Antipsychotics, particularly risperidone and aripiprazole, extensively studied and found to have positive effects on irritability, hyperactivity and stereotypical symptoms (6). A recent study showed that, high dose (1.25-1.75 mg/day) risperidone treatment was more effective in reducing behavioral disturbances compared to low dose (0.125-0.175 mg/day) risperidone treatment (18). In our study, we also found that used risperidone doses (mean dose = 1.20 (±1.01) mg/day) were close to "high dose risperidone treatment" and among children with ASD, in order to control behavioral problems, risperidone doses might need to be increased. Studies examining the effects of aripiprazole in ASD sample reported that aripiprazole was effective in reducing irritability on all doses (5 – 10 – 15 mg/day) (19, 20). Nevertheless, we found that symptoms of individuals who were using aripiprazole treatment were under control on 4.93 (±4.52) mg/day mean dose and this may indicate that relatively lower doses might be sufficient and increasing aripiprazole doses might not be necessary in the follow-ups. Research on the effectiveness of olanzapine (7.5 – 12.5 mg/day) and haloperidol (0.25 – 4 mg/day) in autistic children state that; haloperidol was effective in reducing behavioral problems whereas olanzapine was not and negative side effect profiles (extrapyramidal side effects, weight gain) limit the clinical usage of these drugs (21, 22). Furthermore there are no randomized double-blind placebo-controlled drug trials conducted for determining the efficacy of quetiapine, ziprasidone or newer agents (such as paliperidone, iloperidone, acenapine or lurasidone) in ASD sample (6). In line with these, we found that haloperidole (3.8%), quetiapine (2.9%), olanzapine (2.9%) or clozapine (1%) choices among other antipsychotics for the treatment of behavioral problems seen in ASD were rare.

MPH treatment (7,5 – 50 mg/day) is effective for the treatment of ADHD comorbidity in ASD patients; but likelihood of experiencing side effects which reported to be higher in children with autism compared to their normally developing peers (23, 24). In our sample, we found that clinicians generally kept the dose of MPH rather low (mean dosages are 28.42 [±14.97] mg/day for extended-release [osmotic-controlled release oral delivery system] MPH and 7.86 [±2.67] mg/day for normal MPH) and this might be due to the efforts on preventing the possible side effects which seems to be more frequent among these patients. Atomoxetine (ATX) which is another agent used in the treatment of ADHD has also been found to be effective in ASD on mean dose of 1.2 mg/kg/day which is similar to the mean ATX dose (1.42 mg/kg/day) that we determined in our sample (25, 26, 27).

Selective serotonin re-uptake inhibitors (SSRI) are used in order to reduce repetitive/stereotypical behaviors and movements or anxiety symptoms seen in autism (6). Studies with large sample sizes examining the effectiveness of citalopram and fluoxetine reported that, efficacy of these medications on repetitive/stereotypical behaviors did not differ from placebo and they had negative side effect profiles including energy increase, impulsivity and insomnia (28, 29). There are no randomized double-blind placebo-controlled drug trials done among ASD patients for other SSRI drugs such as sertraline, paroxetine or escitalopram. Moreover in their review, Williams et al. (2013) underline that, "there is not enough evidence proving the efficacy of SSRI drugs for children with autism" (30). Respectively, we also observed that SSRI drugs are rarely used (6.5% among all children who use at least one psychotropic medication) in the clinical management of ASD.

Mood stabilizers are often used to reduce behavioral symptoms and sodium valproate has been found to be effective in children with ASD (31, 32). However, one study showed that sodium valproate had no significant effect on aggression among ASD patients (33). Our results indicate that, even though most commonly selected mood stabilizer for the treatment of problematic behaviors of children with ASD is sodium valproate; mood stabilizers are not frequently preferred in these patients. Another delicate issue to mention about is the sleep disorders of autistic children and unfortunately, our accumulated knowledge about melatonin use in order to treat sleep disturbances seen in ASD is quite limited. One study compared the efficacy of cognitive-behavioral therapy (CBT) and melatonin in the treatment of

insomnia symptoms of children with ASD, and they found that melatonin plus CBT group was the most effective followed by melatonin alone and then the CBT alone group compared with the placebo group (34). While 9 cases in our study had sleep disturbances only 3 of them (%33.33) were using melatonin; so we think that further research and scientific evidence are highly needed in this field.

The major strength of this research is the comprehensive nature of how we examined the of wide range of characteristics of ASD patients such as the diagnosis age, psychiatric symptoms, medications and medication doses; and to our knowledge, this is also the first study to compare different ASD types regarding these parameters. However, it still has some limitations. The major limitation is the retrospective nature in which patients were evaluated through their medical records, so we were not able to confirm the psychiatric comorbidities via (semi)structured psychiatric interviews. This approach reflects the major symptom clusters which can be present in ASD, rather than specific psychiatric diagnoses; so it is difficult to establish a direct relationship between psychiatric comorbidities and treatment modalities/medication doses. Furthermore, even though our study has fairly large sample size (n=200); it is still quite small compared to other studies done in this field with larger sample sizes. In addition, retrospective examination of last two years' referrals of ASD patients might provide us with a cross-sectional definition; but our results cannot be used to infer causality due to the cross-sectional design.

CONCLUSION

In conclusion, we investigated the treatment accessibility and used treatment modalities among children with ASD who live in rural areas/non-central districts. Results indicate that, treatment approaches utilized in rural hospitals are in line with the universal trends. However, considerably higher MDA in the study sample compared to other studies in the literature show that, in order to ensure early diagnosis and better prognosis for the children with ASD who live in rural areas/non-central districts new interventions should be promoted and proper steps should be taken by the local and/or general authorities.

Acknowledgments: None.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: MB: Project design, Review of the literature, Data collection and statistical analyzes **MB:** Writing and Revisions

Ethical issues: All authors declare originality of research.

REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). Diagnostic Stat Man Ment Disord 4th Ed TR. 2013;280.
2. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of autism spectrum disorder among children aged 8 Years - Autism and developmental disabilities monitoring network, 11 Sites, United States, 2014. MMWR Surveill Summ. 2018;67(6):1-23. DOI: 10.15585/mmwr.ss6706a1
3. Mandell DS, Novak M. The role of culture in families' treatment decisions for children with autism spectrum disorders. Mental Retardation and Developmental Disabilities Research Reviews. 2005;11:110–5.
4. Committee to Evaluate the Supplemental Security Income Disability Program for Children with Mental Disorders; Board on the Health of Select Populations; Board on Children, Youth, and Families; Institute of Medicine; Division of Behavioral and Social Scien and M. Clinical Characteristics of Autism Spectrum Disorder. In: Boat T, Wu J, editors. Mental Disorders and Disabilities Among Low-Income Children. Washington DC: National Academies Press; 2015.
5. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. J Clin Psychiatry. 2005;66(10):3–8.
6. Young N, Findling RL. An update on pharmacotherapy for autism spectrum disorder in children and adolescents. Current Opinion in Psychiatry. 2015;28(2):91–101. DOI: 10.1097/YCO.0000000000000132
7. Coury DL, Anagnostou E, Manning-Courtney P, Reynolds A, Cole L, McCoy R, et al. Use of psychotropic medication in children and adolescents with autism spectrum disorders. Pediatrics. 2012;130(SUPPL. 2):69-76. DOI: 10.1542/peds.2012-0900D
8. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th Revis. 2004.
9. IBM SPSS statistics for Windows. IBM Corporation; 2018.
10. Uğur Ç, Göker Z. Autism Spectrum Disorders in Cases Presenting to a Child Psychiatry Department: Demographics and Clinical Features. Turkish J Pediatr Dis. 2019;3:177–83.
11. Helt M, Kelley E, Kinsbourne M, Pandey J, Boorstein H, Herbert M, et al. Can children with autism recover? If so, how? Neuropsychol Rev. 2008;18(4):339–66. DOI: 10.1007/s11065-008-9075-9
12. Posar A, Visconti P. Long-term outcome of autism spectrum disorder. Turkish Achives Pediatr. 2019;54(4):207–12.
13. Arnold LE, Vitiello B, McDougle C, Scahill L, Shah B, Gonzalez NM, et al. Parent-defined target symptoms respond to risperidone in RUPP Autism study: Customer approach to clinical trials. J Am Acad Child Adolesc Psychiatry. 2003;42(12):1443–50. DOI: 10.1097/00004583-200312000-00011
14. Kanne SM, Mazurek MO. Aggression in children and adolescents with ASD: Prevalence and risk factors. J Autism Dev Disord. 2011;41(7):926–37. DOI: 10.1007/s10803-010-1118-4
15. Ibrahim K, Eilbott JA, Ventola P, He G, Pelphrey KA, McCarthy G, et al. Reduced Amygdala–Prefrontal Functional Connectivity in Children With Autism Spectrum Disorder and Co-occurring Disruptive Behavior. Biol Psychiatry Cogn Neurosci Neuroimaging. 2019;4(12):1031–41. DOI: 10.1016/j.bpsc.2019.01.009
16. Mazurek MO, Kanne SM, Wodka EL. Physical aggression in children and adolescents with autism spectrum disorders. Res Autism Spectr Disord. 2013;7(3):455–65.
17. Taurines R, Schwenck C, Westerwald E, Sachse M, Siniatchkin M, Freitag C. ADHD and autism: Differential diagnosis or overlapping traits? A selective review. Atten Defic Hyperact Disord. 2012;4(3):115–39. DOI: 10.1007/s12402-012-0086-2

18. Kent JM, Kushner S, Ning X, Karcher K, Ness S, Aman M, et al. Risperidone dosing in children and adolescents with autistic disorder: A double-blind, placebo-controlled study. J Autism Dev Disord. 2013;43(8):1773–83. DOI: 10.1007/s10803-012-1723-5

19. Marcus RN, Owen R, Kamen L, Manos G, McQuade RD, Carson WH, et al. A Placebo-Controlled, Fixed-Dose Study of Aripiprazole in Children and Adolescents With Irritability Associated With Autistic Disorder. J Am Acad Child Adolesc Psychiatry. 2009;48(11):1110–9. DOI: 10.1097/CHI.0b013e3181b76658

20. Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, et al. Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatrics. 2009;124(6):1533–40. DOI: 10.1542/peds.2008-3782

21. Hollander E, Wasserman S, Swanson EN, Chaplin W, Schapiro ML, Zagursky K, et al. A double-blind placebo-controlled pilot study of olanzapine in childhood/adolescent pervasive developmental disorder. J Child Adolesc Psychopharmacol. 2006;16(5):541–8. DOI: 10.1089/cap.2006.16.541

22. Anderson LT, Campbell M, Adams P, Small AM, Perry R, Shell J. The effects of haloperidol on discrimination learning and behavioral symptoms in autistic children. J Autism Dev Disord. 1989;19(2):227–39. DOI: 10.1007/BF02211843

23. Posey DJ, Aman MG, Arnold LE, Ramadan Y, Witwer A, Lindsay R, et al. Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders with hyperactivity. Arch Gen Psychiatry. 2005;62(11):1266–74. DOI: 10.1001/archpsyc.62.11.1266

24. Jahromi LB, Kasari CL, McCracken JT, Lee LSY, Aman MG, McDougle CJ, et al. Positive effects of methylphenidate on social communication and self-regulation in children with pervasive developmental disorders and hyperactivity. J Autism Dev Disord. 2009;39(3):395–404. DOI: 10.1007/s10803-008-0636-9

25. Handen BL, Aman MG, Arnold LE, Hyman SL, Tumuluru R V., Lecavalier L, et al. Atomoxetine, Parent Training, and Their Combination in Children With Autism Spectrum Disorder and Attention-Deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatry. 2015;54(11):905–15. DOI: 10.1016/j.jaac.2015.08.013

26. Harfterkamp M, Van De Loo-Neus G, Minderaa RB, Van Der Gaag RJ, Escobar R, Schacht A, et al. A randomized double-blind study of atomoxetine versus placebo for attention-deficit/hyperactivity disorder symptoms in children with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry. 2012;51(7):733–41. DOI: 10.1016/j.jaac.2012.04.011

27. Neyzi O, Günöz H, Furman A, Bundak R, Gökçay G, Darendeliler F, et al. Türk çocuklarında vücut ağırlığı, boy uzunluğu, baş çevresi ve vücut kitle indeksi referans değerleri. Çocuk Sağlığı ve Hast Derg. 2008;51:1–14.

28. King BH, Hollander E, Sikich L, McCracken JT, Scahill L, Bregman JD, et al. Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: Citalopram ineffective in children with autism. Arch Gen Psychiatry. 2009;66(6):583–90. DOI: 10.1001/archgenpsychiatry.2009.30

29. Autism Speaks. Autism Speaks Announces Results Reported for the Study of Fluoxetine in Autism (SOFIA) [Internet]. 2009. Available from: http://www.autism-speaks.org/about-us/press-release/autism-speaks-announces-results-reported-study-fluoxetine-austism-sofia

30. Williams K, Brignell A, Randall M, Silove N, Hazell P. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). Cochrane Database of Systematic Reviews. 2013;20(8):CD004677. DOI: 10.1002/14651858.CD004677.pub3

31. Hollander E, Soorya L, Wasserman S, Esposito K, Chaplin W, Anagnostou E. Divalproex sodium vs. placebo in the treatment of repetitive behaviours in autism spectrum disorder. Int J Neuropsychopharmacol. 2006;9(2):209–13. DOI: 10.1017/S1461145705005791

32. Hollander E, Chaplin W, Soorya L, Wasserman S, Novotny S, Rusoff J, et al. Divalproex sodium vs placebo for the treatment of irritability in children and adolescents with autism spectrum disorders. Neuropsychopharmacology. 2010;35(4):990–8.

33. Hellings JA, Weckbaugh M, Nickel EJ, Cain SE, Zarcone JR, Reese RM, et al. A double-blind, placebo-controlled study of valproate for aggression in youth with pervasive developmental disorders. J Child Adolesc Psychopharmacol. 2005;15(4):682–92. DOI: 10.1089/cap.2005.15.682

34. Cortesi F, Giannotti F, Sebastiani T, Panunzi S, Valente D. Controlled-release melatonin, singly and combined with cognitive behavioural therapy, for persistent insomnia in children with autism spectrum disorders: A randomized placebo-controlled trial. J Sleep Res. 2012;21(6):700–9. DOI: 10.1111/j.1365-2869.2012.01021.x

MSD

Medical Science and Discovery
ISSN: 2148-6832

The significance of Serum Vitamin D Levels on Changes in Hematological Parameters After Chemotherapy in Patients with Breast Cancer

Fatih İnci[1]*, Fatih Karataş[1]

1 Karabuk University, Faculty of Medicine, Department of Internal Medicine and Medical Oncology, Karabuk, TR

*** Corresponding Author:** Fatih İnci **E-mail:** fatihinci65@hotmail.com

ABSTRACT

Objective: To investigate the relationship between serum Vitamin D (VD) level and the change of hematological parameters after chemotherapy in patients with breast cancer (BC) who received adjuvant Adriamycin and Cyclophosphamide (AC).

Material and Methods: A total of 74 BC patients who were treated with adjuvant 60 mg/m2 Adriamycin and 600 mg/m2 Cyclophosphamide (AC) were included in the study. VD levels, complete blood count (CBC) findings after 1st cycle AC were retrospectively recorded. The relationship between changes in CBC parameters according to VD levels and the presence of hematological toxicity was examined.

Results: The mean age was 55.11±9.97 years and the median VD level was 12.78 (4-53.40) ng/mL. In all patient groups, there was a significant decrease in the values of lymphocytes and monocytes after chemotherapy (p=0.030, p=0.024 respectively). In the correlation analysis, there was no correlation between VD levels and hemoglobin levels, the number of cells in CBC-1, and the amount of change in the number of cells in after chemotherapy. However, there was a negative correlation between VD level and platelet/lymphocyte ratio-1 (PLR-1), monocyte/lymphocyte ratio-1 (MLR-1) (p=0.025, r:-0.237; p=0.001, r:-0.370, respectively), but there was no correlation with PLR-2 and MLR-2 (p>0.05 all).

Conclusion: There was no relationship between VD levels and changes in hematological parameters and hematological toxicity related to AC chemotherapy in the patient with BC. VD level was inversely correlated with PLR-1 and MLR-1, which is generally accepted as inflammatory markers. This result showed that the levels of VD do not have a significant role in the development of hematological toxicity after AC chemotherapy in BC.

Keywords: Breast cancer, serum VD level, hematological toxicity, PLR, NLR, MLR

Received 03-01-2021

Accepted 11-01-2021

Available Online: 13-01-2021

Published 30-01-2021

INTRODUCTION

Vitamin D (VD) is a steroid hormone that regulates calcium and phosphorus metabolism (1). VD exerted important functions in tumor development by regulating cell proliferation, facilitating apoptosis, promoting cell differentiation, and inhibiting angiogenesis (2). Also, VD regulates the function of immune cells and the differentiation and proliferation of hematopoietic cells (3). Vitamin D Receptor (VDR) is expressed in many cell types, playing that it may have additional roles in other organs, including the hematopoietic system (4). 1,25(OH)2D3 promotes monocyte/macrophage as opposed to neutrophil development of normal myeloid progenitors. Moreover, it influences the early development of monocytes and invariant natural killer T cells and the further maturation of some immune cell types. Findings regarding the regulation of gene expression have revealed that there are links between the actions of VD and cytokines. These include influences on the production/action of the hematopoietic cytokines that are essential to the development and function of the blood cells (5).

http://dx.doi.org/10.36472/msd.v8i1.456

The 1,25(OH)2D3, the most biological active metabolite of VD, acts on T and B lymphocytes to modulate both the cytotoxic and antibody-producing functions of lymphocytes (6). Hematopoietic defects such as anemia, extramedullary hematopoiesis, thrombocytopenia, myelofibrosis and myelodysplasia were exhibited by children with VD deficiency-associated rickets (7). There is a strong linkage between inflammation and cancer. Cancer-related inflammation causes suppression of antitumor immunity by recruiting regulatory T cells and activating chemokines, which results in tumor growth and metastasis (8). Although the relationship between chronic inflammatory diseases and VD deficiency has been described in the literature (9). VD has now been convincingly shown both in vitro and in preclinical animal models to alter the differentiation, proliferation, and apoptosis of cancer cells. VD also shows anti-inflammatory, anti-oxidative, and immunomodulatory effects (10).

VD level is evaluated with serum 25(OH)D level. 25(OH)D indicates intake of VD and endogenous production. If 25(OH)D level is lower than 20 ng/mL, it is defined as VD deficiency, and between 21 and 29 ng/mL is defined as VD insufficiency. If it is higher than 30 ng/mL, it is defined as normal VD level and if it is higher than 150 ng/mL, it is defined as VD intoxication (11).

Neoplastic cells contain a VDR. When the 25(OH)D level exceeds 30 ng/mL, cancer cells make 1.25(OH)D via 1 alpha hydroxylase enzyme. 1.25(OH)D has a reducing effect on proliferation, invasion, angiogenesis, metastasis, and has an enhancing effect on differentiation and apoptosis. 1.25(OH)D collapses after it has completed its function in the cancer cell and cannot enter the circulation. Therefore, it does not affect calcium metabolism (12).

The platelet/lymphocyte ratio (PLR) and neutrophil/lymphocyte ratio (NLR) were used to determine inflammation in different types of malignancies, metabolic syndrome, infectious diseases, cardiovascular disease, end-stage renal disease, and other inflammatory diseases (13). Increased NLR, PLR levels indicate increased inflammation and are used as predictors of morbidity and mortality in systemic inflammation. NLR, PLR, and monocytes/lymphocyte ratio (MLR) are shown as new markers in the evaluation of systemic inflammatory response and are used to monitor the prognosis, morbidity, and mortality of many diseases (14). The NLR may act as a marker for the evaluation of the systemic balance between pro-tumor inflammation associated with neutrophils and antitumor immune response associated with lymphocytes. An increased NLR may indicate a trend for decreased antitumor immune capacity and increased pro-tumor inflammation. In another study, a higher NLR was associated with more advanced disease stage in younger patients. They found that an elevated NLR was associated with lymph node metastases and the depth of stromal infiltration (15). In a study, In patients with pancreatic cancer, NLR was found to be a more sensitive marker than PLR in determining the response to chemotherapy (16).

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among females (17). 60 mg/m2 Adriamycin and 600 mg/m2 Cyclophosphamide (AC) is widely used in the adjuvant treatment of breast cancer. Cyclophosphamide is an alkylating agent. As cyclophosphamide is in clinical use for more than 40 years, there is a lot of experience using this drug for the treatment of cancer and as an immunosuppressive agent for the treatment of autoimmune and immune-mediated diseases (18). After using cyclophosphamide leukopenia develops 8 to 14 days after administration. Thrombocytopenia occurs but is rarely significant. Adriamycin is an antitumor antibiotic and effective in a large variety of tumors. One of the dose-limiting side effects of adriamycin is myelosuppression, especially leukopenia. The combination of adriamycin and cyclophosphamide has a greater myelosuppression effect (19). The occurrence of cytopenia's can cause delay or discontinuation of treatment. This situation affects cure rates negatively.

In light of the above-mentioned information's about the essential role of VD in the hematopoietic system and we investigated whether VD has any effects on hematological toxicity and changes in hematological parameters due to AC chemotherapy.

MATERIAL AND METHODS

The study included 74 women with BC who had surgery [(mastectomy or lumpectomy) + (sentinel lymph node dissection or axillary lymph node dissection)]. Sociodemographic data and blood tests of the individuals were obtained from the hospital automation system retrospectively. VD values are measurements within the last one month.

Complete blood count (CBC) values are measurements taken before the first chemotherapy cycle and before the second chemotherapy. It was investigated whether there were any changes that would make statistical difference in CBC results, NLR, PLR and MLR levels before and after treatment.

The relationship between these changes and the VD level was evaluated. VD status was classified as VD deficiency (<20 ng/mL) and non-deficiency (>20 ng/mL) according to Holick et all (20). Exclusion criteria were determined as follows, male patients, pregnant patients, patients with metastases, and parathyroid and thyroid diseases.

In the statistical analysis of the data, v.22.0 version of the IBM SPSS package program was used. In comparison between groups, paired samples test was used for normally distributed values and Wilcoxon signed rank test was used for non-parametric data. Categorical data were compared with chi-square test. Values with $p<0.05$ were considered statistically significant.

Approval was obtained from the local ethics committee for this study (Decision No: 2020/193).

http://dx.doi.org/10.36472/msd.v8i1.456

RESULTS

The mean age of the patients was 55.11 ± 9.97 years. Average diseases free survival was 26.3 (min:5-max:50) months. When VD level is considered as 20 ng/mL as cut off, in the group with low and high VD level, leukocyte-1, neutrophil-1, platelets-1, eosinophils-1, basophils-1, mean platelet volume-1, leukocyte-2, neutrophil-2, platelets-2, eosinophils-2, basophils-2, mean platelet volume-2 levels were similar (p>0.05; all). There was a significant decrease between lymphocyte-1, monocyte-1, and lymphocyte-2, monocyte-2 values after chemotherapy in all patient groups (p=0.030, p=0.024) respectively (Table 1).

There was no correlation between VD level, age, hemoglobin level, and the number of cells on CBC. However, there was a negative correlation between VD level and PLR-1, MLR-1 (p=0.025, r:-0.237; p=0.001, r:-0.370, respectively), there was no correlation with PLR-2 and MLR-2 (p>0.05 all) (Table 2).

There was no significant relationship between the amount of changes in CBC parameters after chemotherapy and VD level (p>0.05 all) (Table 3).

Table 1. The demographic features and complete blood count parameters of patients

	Variables	All patients (N= 74)	p*
Age (years), mean ± SD		55.11 ± 9.97	N/A
VD, ng/mL median (min – max)		12.78 (4 – 53.40)	N/A
Hemoglobin (g/dL), median (min- max)	Hb1 Hb2	12.61 ± 1.44 12.21 ± 1.43	0.000#
Leucocyte(10^3/mm^3) mean ± SD	Leu1 Leu2	7.28 ± 2 7.20 ± 2.98	0.801#
Lymphocyte(10^3/mm^3) median (min – max)	Ly1 Ly2	2.15 (0.6 – 4.8) 1.93 (0.35 – 4.54)	0.030*
Neutrophil(10^3/mm^3) median (min – max)	Neu1 Neu2	4.21 (1.67 – 8.83) 4.09 (0.42 – 17.6)	0.848*
Platelet(10^3/mm^3) mean ± SD	Plt1 Plt2	261.18 ± 67.81 259.71 ± 70.61	0.408#
Eosinophil(10^3/mm^3) median (min – max)	Eos1 Eos2	0.11 (0 – 2.61) 0.09 (0 – 0.58)	0.173*
Basophil(10^3/mm^3) median (min – max)	Baso1 Baso2	0.03 (0 – 4) 0.02 (0 – 0.11)	0.020*
Monocyte(10^3/mm^3) median (min – max)	Mono1 Mono2	0.43 (0.09 – 1.91) 0.37 (0.02 – 1.19)	0.024*
Mean Platelet Volume(fL) mean ± SD	MPV1 MPV2	10.34 ± 1.03 10.18 ± 1	0.063#
NLR (%) (min – max)	Neu1/Ly1 Neu2/Ly2	2.011 (0.76 – 5.93) 2.103 (0.26 – 16.94)	0.108
PLR (%) median (min – max)	Plt1/Ly1 Plt2/Ly2	120.76 (39.73 – 461.29) 132.75 (37.01 – 607.69)	0.024
MLR (%) median (min – max)	Mono1/Ly1 Mono2/Ly2	0.208 (0.03 – 0.81) 0.181 (0.01 – 0.98)	0.307
Stage, n (%)	II III	32 (43.2) 42 (56.8)	N/A

*Wilcoxon test, #Paired samples T test. SD, standard deviation; Hb1, basal hemoglobin level; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; MLR, monocyte/lymphocyte ratio; MPV, mean platelet volume; PLT, platelet count; ELR, eosinophil/lymphocyte ratio; MER, monocyte/eosinophil ratio; Leu1, basal leucocyte count; Ly1, basal lymphocyte count; Neu1, basal neutrophil count; Plt1, basal platelet count; Eos1, basal eosinophil count; Baso1, basal basophil count; Mono1, basal monocyte count; MPV1, basal mean platelet volume Hb2, hemoglobin level after chemotherapy; Leu2, leucocyte count after chemotherapy; Ly2, lymphocyte count after chemotherapy; Neu2, neutrophil count after chemotherapy; Plt2, platelet count after chemotherapy; Eos2, eosinophil count after chemotherapy; Baso2, basophil count after chemotherapy; Mono2, monocyte count after chemotherapy; MPV2, mean platelet volume after chemotherapy.

Table 2. Changes in cytopenia and complete blood count according to VD deficiency

Variables		VD<20 ng/mL (n=58)	VD≥20 ng/mL (n=16)	p*
Decrease in Hemoglobin, n (%)	Yes No	36 (62) 22 (38)	8 (50) 8 (50)	0.278
Decrease in Basophil, n (%)	Yes No	32 (55) 26 (45)	8 (50) 8 (50)	0.465
Decrease in Eosinophil, n (%)	Yes No	31 (53.5) 27 (46.5)	10 (62.5) 6 (37.5)	0.362
Decrease in Lymphocyte, n (%)	Yes No	38 (65.5) 20 (34.5)	9 (56) 7 (44)	0.344
PLR Increase, n (%)	Yes No	25 (43) 33 (57)	6 (37.5) 10 (62.5)	0.458
Decrease in Monocyte, n (%)	Yes No	25 (43) 33 (57)	5 (31) 11 (69)	0.288

*Pearson Correlation test.

http://dx.doi.org/10.36472/msd.v8i1.456

Table 3. Relationship between VD levels patients with and without significant reduction after chemotherapy

Variables		N	VD levels median (min – max)	p*
Decrease in Eosinophil, n (%)	Yes	41 (55.4)	13.56 (4 – 53.40)	0.341
	No	33 (44.6)	12.18 (4.50 – 50.16)	
Decrease in Basophil, n (%)	Yes	34 (45.9)	12.47 (4 – 53.4)	0.854
	No	40 (54.1)	13.10 (4.50 – 50.16)	
Decrease in Hemoglobin, n (%)	Yes	44 (59.5)	12.78 (4 – 53.4)	0.467
	No	30 (40.5)	12.89 (4.50 – 50.16)	
Decrease in Lymphocyte, n (%)	Yes	47 (63.5)	12.8 (4 – 53.40)	0.225
	No	27 (36.5)	14.13 (6.45 – 50.16)	
Decrease in Monocyte, n (%)	Yes	44 (59.5)	13.78 (4 – 53.40)	0.269
	No	30 (40.5)	11.78 (4 – 35.05)	
PLR Increase, n (%)	Yes	43 (58.1)	12.23 (4 – 53.4)	0.437
	No	31 (41.9)	12.85 (6.85 – 50.16)	

* Mann-Whitney U test; PLR, platelet/lymphocyte ratio

DISCUSSION

This is the first study investigating the significance of serum VD levels in changes in hematological parameters after anthracycline-based chemotherapy in breast cancer patients. According to the data obtained in our study, the mean VD level in patients was determined as 12.78 (4-53.40) ng/mL and was under 20 ng/mL reported as a deficiency. The role of exposure to sunlight in VD synthesis is very important. Our study was conducted in locations situated at 41.20 north latitude and 32.60 east longitude in the north of Turkey. Studies have shown that those living in the north pole have a higher risk of getting certain types of cancer than those living in the south pole (**21**).

In a study, with 103 patients with BC, the average VD level was determined as 17 ng/mL. These results were similar to the results of our study (**22**). A meta-analysis showed a direct relationship between VD deficiency and breast cancer (**23**).

A negative correlation between VD level and PLR 1, MLR 1 (p=0.025, r: -0.237; p=0.001, r: -0.370; respectively) may indicate the presence of chronic high inflammation in patients with low VD levels. This may indicate that chronic increased inflammation is a risk factor for the development of malignancy. Cancer-related inflammation contributed to the proliferation of tumor cells, destroyed the adaptive immune response, and changed the effect of chemotherapeutics. Lymphocytes can eliminate tumor cells by inhibiting cell-induced cytotoxicity, proliferation and migration (**24**).

In a study, patients with breast cancer preoperative increased PLR found that worsening the prognosis (**25**). In the retrospective study in 2016, PLR and NLR in the general population were significantly higher in patients with low 25(OH)D levels. PLR and NLR were significantly associated with 25(OH)D levels, and PLR was found to be an independent predictor of 25(OH)D levels (**26**).

Increased PLR is a negative inflammatory marker in cardiovascular diseases and malignant conditions (**27**). PLR is seen as a predictor of mortality in heart, lung and some oncological diseases (**28**). There was no correlation between PLR-2, MLR-2 (p>0.05 all). This situation can be explained by the normalization of inflammatory markers due to the AC effect independent of VD level after AC administration. The change in CBC parameters after chemotherapy was found to be significantly lower between Lymphocyte-1, Monocyte-1 and Lymphocyte-2, Monocyte-2 values (p>0.030, p>0.024 respectively) and there was no significant difference in these parameters between low and high VD groups. This may have caused a significant difference in the study group, as the mean VD levels were close and the number of patients in the high VD group was low. In the correlation analysis, there was no relationship between VD levels between the PLR-1, PLR-2; NLR-1, NLR-2, and MLR-1, MLR-2 levels before and after chemotherapy. Narien et al. reported that the use of VD analogs before the application of antineoplastic agents can reduce hematological toxicity by modulating bone marrow and stromal cells (**29**). However in a study investigating the effect of NLR, PLR and MLR on the prognosis of BC, increased NLR and PLR were found to be important in prognosis, while MLR was not effective in prognosis (**30**). NLR and PLR are valuable prognostic biomarkers in most solid tumors but their value in guiding treatment management needs further research. Moreover, the prognostic values of these systemic inflammatory biomarkers need to be further confirmed in prospective clinical trials for various malignancies (**31**). Since death did not occur, we were unable to provide information about the effects of these parameters on overall survival. Further studies are also needed to explore the driving and regulatory mechanisms of the cancer-related systemic inflammatory response and to search for potential therapeutic targets for cancer population (**32**). Our study has some limitations. First, only one session of chemotherapy was used. In order to clearly understand the effect of vitamin D levels on hematological parameters and hematological toxity, it is necessary to see the changes in the following chemotherapy sessions. In addition, the study could be done with more patient participation.

CONCLUSION

There was no relationship between VD levels and changes in hematological parameters and hematological toxicity related to AC chemotherapy in patient with BC. VD level at the time of diagnosis was found to correlate negatively with PLR and MLR. This result showed that the levels of VD do not have a significant role in the development of hematological toxity after AC chemotherapy in BC. In order to understand the importance of VD levels in breast cancer prospective studies are needed.

Acknowledgments: None.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: Fİ, FK: Project design, Review of the literature, Data collection and statistical analyzes **Fİ:** Writing and Revisions

Ethical issues: All authors declare originality of research.

REFERENCES

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American journal of clinical nutrition. 2004;80(6):1678S-88S.

2. Zhan R, Shen X, Ma Z, Li S, Liang J, Zhang W. Dietary vitamin D intake and risk of non-small cell lung cancer: a matched case-control study. Translational Cancer Research. 2016;5(4):470-5.

3. Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: effect on haematopoiesis and immune system and clinical applications. International journal of molecular sciences. 2018;19(9):2663.

4. Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, et al. Vitamin D: beyond bone. Annals of the New York Academy of Sciences. 2013;1287(1):45.

5. Brown G, Kutner A, Marcinkowska E. Vitamin D and Haematopoiesis. Current Tissue Microenvironment Reports. 2020:1-11.

6. Mao X, Hu B, Zhou Z, Xing X, Wu Y, Gao J, et al. Vitamin D levels correlate with lymphocyte subsets in elderly patients with age-related diseases. Scientific reports. 2018;8(1):1-9.

7. Cortes M, Chen MJ, Stachura DL, Liu SY, Kwan W, Wright F, et al. Developmental vitamin D availability impacts hematopoietic stem cell production. Cell reports. 2016;17(2):458-68.

8. Qu X, Tang Y, Hua S. Immunological approaches towards cancer and inflammation: a cross talk. Frontiers in immunology. 2018;9:563.

9. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Internal and emergency medicine. 2013;8(1):33-40.

10. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nature reviews cancer. 2014;14(5):342-57.

11. Holick MF. Vitamin D deficiency. New England Journal of Medicine. 2007;357(3):266-81.

12. Larsen ER, Mosekilde L, Foldspang A. Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. Journal of Bone and Mineral Research. 2004;19(3):370-8.

13. Liu CL, Lee JJ, Liu TP, Chang YC, Hsu YC, Cheng SP. Blood neutrophil-to-lymphocyte ratio correlates with tumor size in patients with differentiated thyroid cancer. Journal of surgical oncology. 2013;107(5):493-7.

14. Yucel C, Keskin M, Cakmak O, Ergani B, Kose C, Celik O, et al. Predictive value of pre-operative inflammation-based prognostic scores (neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and monocyte-to-eosinophil ratio) in testicular sperm extraction: a pilot study. Andrology. 2017;5(6):1100-4.

15. Jonska-Gmyrek J, Gmyrek L, Zolciak-Siwinska A, Kowalska M, Fuksiewicz M, Kotowicz B. Pretreatment neutrophil to lymphocyte and platelet to lymphocyte ratios as predictive factors for the survival of cervical adenocarcinoma patients. Cancer management and research. 2018;10:6029.

16. Gao Y, Wang WJ, Zhi Q, Shen M, Jiang M, Bian X, et al. Neutrophil/lymphocyte ratio is a more sensitive systemic inflammatory response biomarker than platelet/lymphocyte ratio in the prognosis evaluation of unresectable pancreatic cancer. Oncotarget. 2017;8(51):88835-44.

17. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a cancer journal for clinicians. 2011;61(2):69-90.

18. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemotherapy and Pharmacology. 2016;78.

19. Ferguson JE, Dodwell DJ, Seymour AM, Richards MA, Howell A. High dose, dose-intensive chemotherapy with doxorubicin and cyclophosphamide for the treatment of advanced breast cancer. British journal of cancer. 1993;67(4):825-9.

20. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96(7):1911-30.

21. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer causes & control. 2005;16(2):83-95.

22. Crew KD, Shane E, Cremers S, McMahon DJ, Irani D, Hershman DL. High prevalence of vitamin D deficiency despite supplementation in premenopausal women with breast cancer undergoing adjuvant chemotherapy. Journal of Clinical Oncology. 2009;27(13):2151.

23. Hossain S, Beydoun MA, Beydoun HA, Chen X, Zonderman AB, Wood RJ. Vitamin D and breast cancer: A systematic review and meta-analysis of observational studies. Clinical nutrition ESPEN. 2019;30:170-84.

24. Huang Y, Zhou C, Zhao R, Cui Y, Wu X. The relationship between vitamin D, ratio of neutrophil to lymphocyte, and ratio of lymphocyte to monocyte in preoperative serum and prognosis of patients with breast conserving surgery in breast cancer. INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL MEDICINE. 2019;12(8):10537-48.

25. Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, et al. The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients. British journal of cancer. 2014;110(10):2524-30.

26. Akbas EM, Gungor A, Ozcicek A, Akbas N, Askin S, Polat M. Vitamin D and inflammation: evaluation with neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio. Archives of medical science: AMS. 2016;12(4):721.

27. Akboga MK, Canpolat U, Yayla C, Ozcan F, Ozeke O, Topaloglu S, et al. Association of platelet to lymphocyte ratio with inflammation and severity of coronary atherosclerosis in patients with stable coronary artery disease. Angiology. 2016;67(1):89-95.

28. Kim DS, Shin D, Lee MS, Kim HJ, Kim DY, Kim SM, et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. The Journal of dermatology. 2016;43(3):305-10.

29. Narain Niven R, Jimenez Joaquin J, McCook John P, inventors; BERG BIOSYSTEMS LLC, assignee. VITAMIN D3 AND ANALOGS THEREOF FOR ALLEVIATING SIDE EFFECTS ASSOCIATED WITH CHEMOTHERAPY2010 2010/01/27/Application date.

30. Huszno J, Kolosza Z. Prognostic value of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratio in breast cancer patients. Oncology Letters. 2019;18.

31. Kumarasamy C, Sabarimurugan S, Madurantakam RM, Lakhotiya K, Samiappan S, Baxi S, et al. Prognostic significance of blood inflammatory biomarkers NLR, PLR, and LMR in cancer—A protocol for systematic review and meta-analysis. Medicine. 2019;98(24).

32. Ni L, Tao J, Xu J, Yuan X, Long Y, Yu N, et al. Prognostic values of pretreatment neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in endometrial cancer: a systematic review and meta-analysis. Archives of gynecology and obstetrics. 2020;301(1):251-61.

Medical Science and Discovery
ISSN: 2148-6832

Prognostic significance of ferritin, D-dimer, lymphocyte monocyte ratio and some biochemical markers in patients with SARS-CoV-2

Ali Eser[1]*, Nang Hseng Kyio[2]

1 Bezmialem Vakif University, Department of Hematology, Istanbul, TR
2 Bezmialem Vakif University, Department of Internal Medicine, Istanbul,TR
*** Corresponding Author:** Ali Eser **E-mail:** dralieser@gmail.com

ABSTRACT

Objective: The disease caused by COVID-19 that progress with severe acute respiratory distress syndrome (SARS) and can result in death, spread all over the world emerging from China. It is important to know the cases that expected to show a fatal course beforehand due to the cases resulting in death. In this study we analyzed the changes observed in ferritin, D-dimer, lymphocyte and monocyte levels, which are easily measured in patients, and evaluated how these determinants can be used as prognostic factors of the disease.

Material and Methods: One hundred patients who applied to Bezmialem Vakif University Hospital between April 2020 – May 2020, who were COVID-19 PCR positive, and had infiltration in their pulmonary computerized tomography scan, were included in the study. These patients were divided into two groups as normal inpatient and intensive care unit patients. Ferritin, D-dimer, lymphocyte and monocyte levels, ALT, AST, LDH, and CRP levels were recorded at the time of diagnosis. Lymphocyte/monocyte ratio (LMR) was calculated.

Results: Ferritin and D-dimer levels, ALT, AST, LDH, and CRP levels were found to be statistically and significantly higher in the exitus group compared to alive group ($p<0.05$). LMR, on the other hand, was found to be statistically and significantly lower in the mortality group ($p<0.05$).

Conclusion: Ferritin, D-dimer levels and LMR can be determinant laboratory findings in the prognosis of the disease that are detected in the Covid-19 patients at the dime of diagnosis. More studies should be conducted to objectively evaluate disease-related prognostic factors.

Key words: Ferritin, D-dimer, LMR, SARS-CoV-2, COVID-19

Received 03-01-2021

Accepted 11-01-2021

Available Online: 13-01-2021

Published 30-01-2021

INTRODUCTION

COVID-19 (SARS-CoV 2) infection have become a world pandemic in March 2019, emerging from China's state Wuhan, in December 2019 (1). SARS-CoV2 can primarily cause respiratory tract infection as well as a clinical picture affecting hematopoietic, gastrointestinal, cardiovascular, neurological and immune systems (2,3). Though the outbreak is likely to spread to humans as a result of a zoonotic transmission, there is evidence of person-to-person transmission through direct contact or droplets from an infected person (4). The common symptoms of SARS-CoV-2 are as follows; fever, cough, back and joint pain, dyspnea, hemoptysis, and diarrhea. The clinical spectrum of the disease can range from asymptomatic or a mild upper respiratory tract symptom to acute pneumonia, respiratory failure, and death (5).

Various hematological and biochemical parameters related to lymphopenia, monocytosis, hyperferritinemia, high lactate dehydrogenase (LDH), C-reactive protein (CRP), fibrinogen and d-dimer levels have been reported in Covid-19 patients (6-8). The lymphocyte/ monocyte ratio (LMR) is an appropriate index that can be calculated from a complete blood count, and many studies have shown a prognostic value in a variety of conditions such as sepsis, lymphoma and malignant tumors (9-11).

http://dx.doi.org/10.36472/msd.v8i1.457

The purpose of this study is to retrospectively examine some of the hematological and biochemical parameters of the patients hospitalized due to Covid-19 infection (inpatient and intensive care patients), and is to evaluate the effect of these parameters on the course of the disease and prognosis.

MATERIAL AND METHODS

One hundred patients who applied to our center between April 2020 – May 2020, who were Covid-19 PCR positive, and had infiltration in their thorax computed tomography (CT) scan, were included in the study.

These patients were divided into two groups such as alive and exitus according to their last status. Hematological and biochemical parameters such as ferritin, d-dimer, CRP, absolute lymphocyte count (ALC), absolute monocyte count (AMC), alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP), and LDH were checked from the peripheral blood.

The baseline hematological and biochemical values of all patients were retrospectively analyzed in the following phases; when they were first hospitalized after treatment and before they were discharged (in patients who survived) and before death (in patients who died). The ratios of lymphocyte and monocyte absolute numbers (LMR) were calculated.

Statistical Analysis

Mean, standard deviation, median, minimum, maximum value frequency and percentage were used for descriptive statistics. The distribution of variables was checked with Kolmogorov-Simirnov test. Independent Samples T test and Mann-Whitney U test were used for the comparison of quantitative data. Wilcoxon test were used for the repeated measurement analysis. Chi-Square test was used for the comparison of the comparison of qualitative data. Logistic Regression was used to show the effect level. SPSS 27.0 was used for statistical analyses.

RESULTS

One hundred patients who resulted as positive for Covid-19 PCR test were included in the study. All the patients had thorax CT scans consistent with typical viral pneumonia. The follow-up and treatment of the half of the patients who were included in the study were carried out in inpatient clinic, the other half were followed in the intensive care unit (ICU). Fifty nine of the patients were male and 41 of the patients were female.

Median age of the patients was 64,5 (range: 47-89). The laboratory findings of the patients at the time of diagnosis are as follows; ferritin level median 379 (range:19-21130) ng/dl, D-dimer level median 307 (140-6422) ng/ml, ALC median 1,1 (range:0,2-7)x103/ul, AMC median 0,5 (range:0,1-2)x103/ul, LMR median 2,38 (range: 0,34-8,54), CRP median 81,5 (range:2-325) mg/L, ALT median 30 (range:12-930) U/L, AST: median 26 (range: 9-414) U/L, ALP median 62,5 (range:27-555) U/L, LDH median 322 (range:159-870) U/L.

The demographic characteristics, and laboratory findings of the patients at the time of diagnosis are summarized in Table 1.

The age of the patients was significantly higher in exitus group than in the alive group ($p<0.05$) (Figure 1). There was no significant difference regarding the gender in both groups. The rate of mortality in ICU patients were significantly higher than inpatient clinic patients ($p<0.05$) (Figure 2).

Ferritin levels before and after treatment were significantly higher in the exitus group than in the alive group ($p<0.05$). After treatment, ferritin values have shown a significant decrease in the alive group, and increase in the exitus group ($p<0.05$) (Figure 3).

D-dimer levels before and after treatment were significantly higher in the exitus group than in the alive group ($p<0.05$). After treatment, D-dimer values have shown a significant decrease in the alive group, and increase in the exitus group ($p<0.05$). The increase of d-dimer, after treatment, was significantly higher in the exitus group than in the alive group ($p<0.05$) (Figure 4).

The ALC level before and after treatment was significantly lower in the exitus group when compared to the alive group (0.05). After treatment, the ALC level has shown a significant increase in the alive group, and decrease in the exitus group ($p<0.05$). After treatment, the change in the ALC level in the exitus significantly differed from the alive group ($p<0.05$).

The AMC level before and after treatment did not differ significantly between the two groups ($p<0.05$). After treatment, the AMC level has shown a significant increase in the alive group, and decrease in the exitus ($p<0.05$). After treatment, the change in the AMC level in the exitus group significantly different from the alive group ($p<0.05$).

LMR before and after treatment was significantly lower in the exitus group than in the alive group ($p<0.05$). After treatment, LMR did not show a significant difference in both groups ($p>0.05$). After treatment, the change in LMR in the exitus group significantly differed from the alive group ($p<0.05$).

CRP, ALT, AST, and LDH levels before and after treatment were significantly higher in the exitus group when compared to the alive group ($p<0.05$). ALP level did not show a significant difference in both groups.

In the univariate model, a significant ($p<0.05$) difference was observed between the exitus and alive groups in terms of age, pre-treatment and post-treatment ferritin, D dimer, LMR, CRP, ALT, AST and LDH values (Table 3).

In the multivariate model, a significant difference ($p <0.05$) was observed between CRP and post-treatment ferritin and LMR between exitus and alive groups (Table 3).

http://dx.doi.org/10.36472/msd.v8i1.457

Table 1: Patient characteristics

		Min-Max		Median	Mean±sd/n-%
Age		47,0	89,0	64,5	66,3±10,6
Gender	**Female**				41 (41,0%)
	Male				59 (59,0%)
Intensive Care Hospitalization	**(-)**				50 (50,0%)
	(+)				50 (50,0%)
Ferritin		19,0	21130	379,0	1196,8±2527,0
D-Dimer		140,0	6422	307,0	887,3±1218,7
ALC		0,2	7	1,1	1,4±1,1
AMC		0,1	2	0,5	0,5±0,3
LMR		0,34	8,54	2,38	2,88±1,73
CRP		2,0	325,0	81,5	94,1±76,0
ALT		12,0	930,0	30,0	62,1±116,5
AST		9,0	414,0	26,0	45,0±61,7
ALP		27,0	555,0	62,5	86,2±78,9
LDH		159,0	870,0	322,0	357,9±159,4

ALC: Absolute lymphocyte count, AMC: Absolute monocyte count, LMR: Lymphocyte/Monocyte ratio , CRP: C-reaktif protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: alkaline phosphataseLDH: Lactate dehydrogenase
Normal laboratory values: Ferritin: 21-274 mg/dl, D-Dimer: 0-300 ng/ml, ALC: 0,6-3,4x103/ul, AMC: 0,19-0,77x103/ul, ALT: 0-55 U/L, AST: 5-34 U/L, ALP: 40-150 U/L, LDH: 125-220 U/L

Table 2: Pre-treatment and post-treatment laboratory values of patients

			Mortality (-) Mean±sd			Mortality (-) Median	Mortality (+) Mean±sd			Mortality (+) Median	p	
	Age		62,3	±	9,2	61,0	70,5	±	10,5	71,0	***0,000***	m
	Gender	Female	20		39,2%		21		42,9%		0,711	x²
		Male	31		60,8%		28		57,1%			
	Intensive Care Hospitalization	(-)	50		98,0%		0		0,0%		***0,000***	x²
		(+)	1		2,0%		49		100,0%			
Ferritin	Before Treatment		375,4	±	506,5	271,0	2051,7	±	3382,3	886,0	***0,000***	m
	After Treatment		268,6	±	220,2	222,0	5909,3	±	11928,2	2560,0	***0,000***	m
	Intra Goup Difference		-106,8	±	409,5	-19,0	3857,6	±	11212,7	400,0	***0,000***	m
	Intra Group p				***0,034***	w			***0,000***	w		
D-Dimer	Before Treatment		644,9	±	1049,4	262,0	1139,7	±	1337,5	497,0	***0,001***	m
	After Treatment		435,5	±	535,3	263,0	2500,6	±	4070,0	1568,0	***0,000***	m
	Intra Goup Difference		-209,4	±	911,0	-14,0	1360,9	±	3907,0	525,0	***0,000***	m
	Intra Group p				***0,253***	w			***0,000***	w		
ALC	Before Treatment		1,4	±	0,7	1,3	1,4	±	1,4	0,9	***0,008***	m
	After Treatment		1,6	±	0,6	1,6	1,3	±	1,1	1,0	***0,001***	m
	Intra Goup Difference		0,2	±	0,7	0,3	-0,1	±	1,5	-0,1	0,122	m
	Intra Group p				***0,051***	w			***0,612***	w		
AMC	Before Treatment		0,5	±	0,2	0,5	0,6	±	0,4	0,6	0,069	m
	After Treatment		0,5	±	0,2	0,5	0,7	±	0,5	0,5	0,141	m
	Intra Goup Difference		0,03	±	0,19	0,01	0,12	±	0,47	0,07	0,456	m
	Intra Group p				0,370	w			0,258	w		
LMR	Before Treatment		3,37	±	1,74	2,97	2,36	±	1,57	1,86	***0,000***	m
	After Treatment		3,70	±	1,78	3,41	2,16	±	1,42	1,82	***0,000***	m
	Intra Goup Difference		0,32	±	1,90	0,40	-0,20	±	1,73	-0,21	0,063	m
	Intra Group p				0,097	w			0,395	w		
	CRP		58,8	±	50,4	42,0	130,8	±	81,0	125,0	***0,000***	m
	ALT		31,0	±	17,5	26,0	94,4	±	160,0	42,0	***0,000***	m
	AST		29,5	±	22,2	23,0	61,2	±	82,5	38,0	***0,008***	m
	ALP		80,0	±	78,0	60,0	92,6	±	80,0	72,0	0,345	m
	LDH		292,2	±	93,2	289,0	426,2	±	184,4	379,0	***0,000***	m

[m]: Mann-whitney u test, **x^2:** Chi-square test **ALC:** Absolute lymphocyte count, A**MC:** Absolute monocyte count**, LMR:** Lymphocyte/Monocyte ratio**, CRP:** C-reactive protein**, ALT:** Alanine aminotransferase, **AST:** Aspartate aminotransferase, **ALP:** Alkaline phosphatase, **LDH:** Lactate dehydrogenase

http://dx.doi.org/10.36472/msd.v8i1.457

Table 3: Univariate and multivariate logistic regression of risk factors associated with mortality

	Univariate Model				Multivariate Model			
	OR	% 95 CI		p	OR	% 95 CI		p
Age	1,086	1,039 -	1,135	***0,000***				
Ferritin (BT)	1,002	1,001 -	1,002	***0,001***				
Ferritin (AT)	1,004	1,002 -	1,006	***0,001***	1,006	1,002 -	1,009	***0,002***
D-Dimer (BT)	1,000	1,000 -	1,001	***0,052***				
D-Dimer (AT)	1,002	1,001 -	1,003	***0,000***				
LMR (BT)	0,678	0,516 -	0,891	***0,005***				
LMR (AT)	0,524	0,379 -	0,724	***0,000***	0,223	0,087 -	0,571	***0,002***
CRP	1,017	1,009 -	1,025	***0,000***	1,016	1,002 -	1,031	***0,027***
ALT	1,031	1,010 -	1,053	***0,004***				
AST	1,017	1,001 -	1,034	***0,041***				
LDH	1,007	1,003 -	1,010	***0,000***				

Logistic Regression (Forward LR), **BT:** Before treatment, **AT:** After treatment, **LMR:** Lymphocyte/Monocyte ratio**, CRP:** C-reactive protein**, ALT:** Alanine aminotransferase, **AST:** Aspartate aminotransferase, **ALP:** Alkaline phosphatase, **LDH:** Lactate dehydrogenase

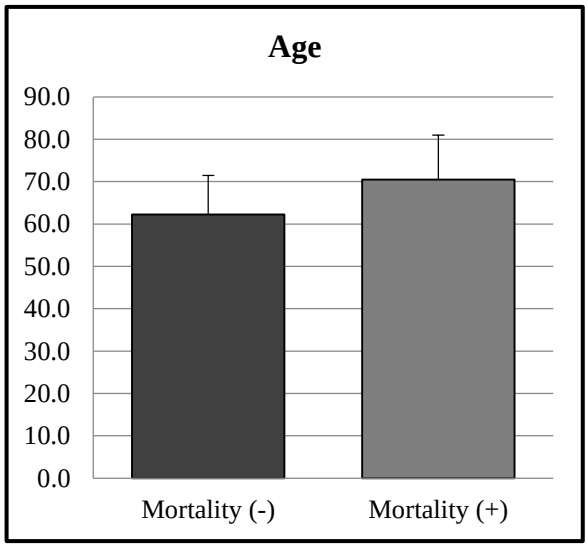


Figure 1: Age and mortality relationship in C-19 patients

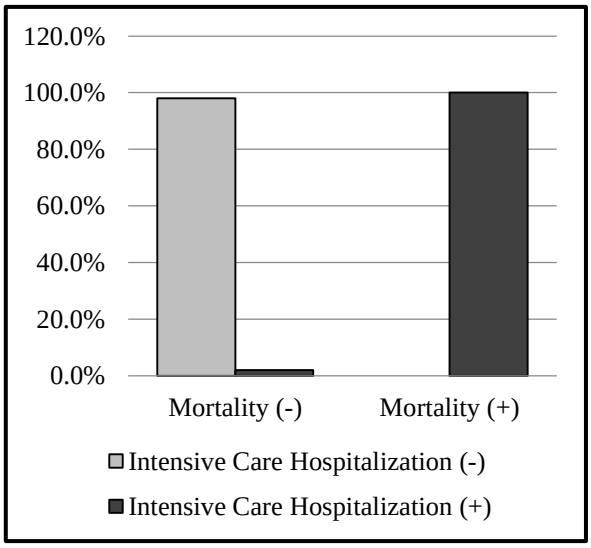


Figure 2: Intensive care and mortality relationship in C-19 patients

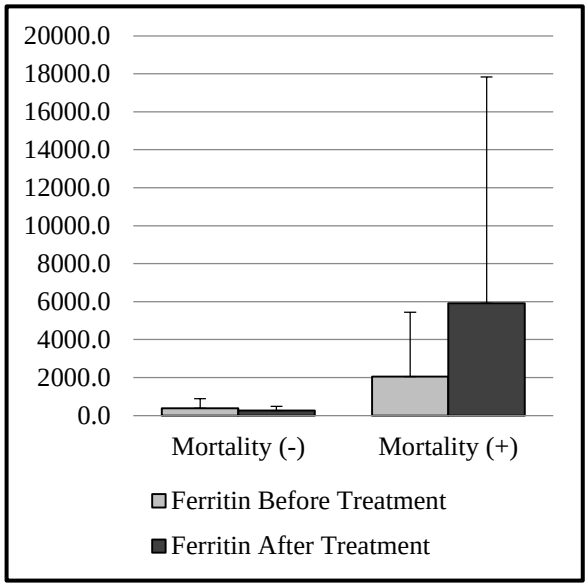


Figure 3: Ferritin level and mortality relationship in C-19 patients

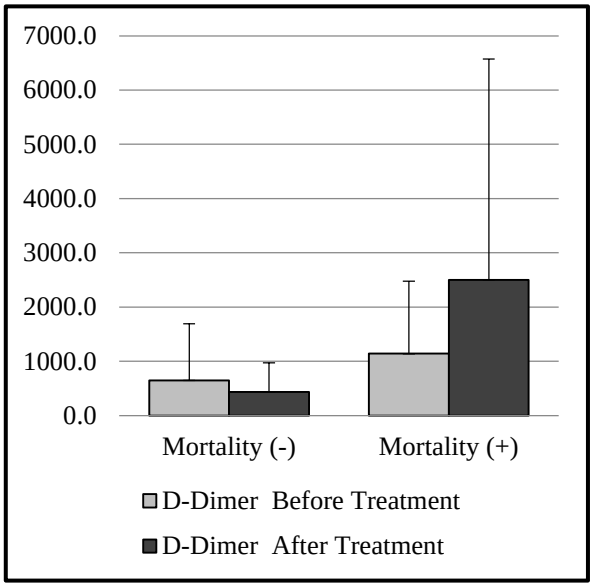


Figure 4: D-Dimer level and mortality relationship in C-19 patients

DISCUSSION

In Covid-19 patients, cytokine storm occurs in 7-14 days after the first symptoms begin, with an increase in cytokine and inflammatory mediators (12). Cytokine secretion in actively infected areas, especially in the macrophages in pulmonary parenchyma, can cause serum ferritin secretion (13). Previous studies show that ferritin is associated with poor prognosis in severe Covid-19 patients and hyperferritinemia may be a new parameter in SARS-CoV-2(6,14).

In this study, we examined CRP and ferritin levels between two groups as inflammatory parameters. There was a significant difference in CRP and Ferritin levels in the groups with and without mortality. Mean ferritin levels in the alive group were found to be two times the normal at the initial examination. In the exitus group, basal mean ferritin levels were > 2000 ng/dl. So, it was 10 times higher compared to the alive group. The prognosis and mortality of the patience is related to high level of ferritin. Early and intensive anti-inflammatory treatments can be planned by looking at basal ferritin levels. Plasma exchange that decreases cytokine and ferritin levels, can be useful for severe Covid-19 patients.

D-dimer level in COVID-19 patients can be high due to reasons such as intense inflammation, insufficient anti-inflammatory response, endothelial damage, sepsis, and disseminated intravascular coagulation (DIC) (15). High D-dimer level indicates hypercoagulability or thrombosis. The study by Litao and ark. showed that patients with mortality had > four times the increase in D-dimer, in hospital admissions (16). In our study, D-dimer level showed a mean two times the increase in the alive group as well, but in the exitus group the increase was four times. This increase was statistically significant ($p<0.05$). While there was a significant decrease in the D-dimer level after treatment in the alive group, there was a significant increase in the exitus group. Severe Covid-19 patients may develop increased coagulability and thrombosis together with DIC. This situation causes high risk of venous thromboembolism (VTE) (17). D-dimer levels in predicting VTE in patients with severe SARS-CoV-2 (18).

If 1.5 µg/mL was used as the cut-off value for D-dimer to predict VTE, the sensitivity, specificity, Positive predictive value, and Negative predictive value were 85.0%, 88.5%, 70.8%, and 94.7%, respectively (18). In severe Covid-19 patients who present with high D-dimer value, administration of anticoagulants at therapeutic dose instead of prophylaxis dose may have a positive effect on mortality. Covid-19 can directly infect angiotensin converting enzyme-2 (ACE-2) receptors on lymphocytes' surfaces. As a result of the cytokine storm that develops secondary to excessive inflammation with the lysis of these cells, the increase in interleukins such as IL-1, IL-6, TNF-alpha induce apoptosis and develop lymphopenia (19,20). It was detected in our study that in the alive group, the lymphopenia the treatment, improved after treatment. In the exitus group lymphopenia was significantly lower than in the alive group, and higher decrease was detected in the follow-up period. It was thought that the degree of lymphopenia may be directly related to mortality, therefore, early initiation of anticytokines such as IL-1 receptor blocker anakinra and IL-6 receptor blocker tocilizumab may improve the prognosis of the disease.

Though LMR has been shown as a parameter related to prognosis in patients with sepsis, lymphoma and cancer, there is not enough information regarding Covid-19 patients (9,10). In a study conducted by Russel et al., LMR was detected to be lower in patients who, previously, had respiratory tract involvement due to influenza virus (9). In our study, although ALC was lower in the exitus group than in the alive group, no significant difference was found between the two groups in AMC. Therefore, LMR in exitus group was detected to be significantly lower than the alive group. In the exitus group, the mean LMR as 2,36 cut-off value is acceptable. It was thought that the LMR at initial diagnosis could be used as a predictive value for prognosis and mortality of the patients.

COVID-19 virus can connect to ACE-2 receptors on cholangiocytes, leading to liver damage secondary to cholangiocyte dysfunction and systemic inflammatory response (21). The liver postmortem biopsy of a patient who deceased due to COVID-19 indicated microvesicular steatosis that showed liver damage, mild lobular and portal activity (22). In our study, ALT, AST, LDH levels in the exitus group were found to be significantly higher ($p<0.05$) compared to the alive group. Liver test abnormalities can be used as a determinant for disease severity, and mortality risk. A recent study revealed that patients with elevated AST level were at great risk of progressing to severe disease those require close monitoring (23). Studies conducted in patients with symptomatic COVID-19 showed a decrease in interferon (IFN) expression in both human bronchial cells and circulating mononuclear blood cells (24). Therefore, interferon therapy may be life-saving in patients with low LMR, high ferritin and D-dimer levels at the time of diagnosis.

CONCLUSION

Covid-19 is a new entity for everybody and there are still many things unknown. It is essential to know the biochemical indicators that show poor prognosis beforehand, as some of the cases result in death with a rapid course. In patients with high ferritin and D-dimer levels and low LMR at the time of diagnosis, early initiation of treatments such as immune plasma therapy, IL-1 and IL-2 inhibitors, and interferon may reduce mortality rates.

Acknowledgments: None.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: AE, NHK: Project design, Review of the literature, Data collection and statistical analyzes **AE:** Writing and Revisions

Ethical issues: All authors declare originality of research.

REFERENCES

1. WHO, World Health Organization. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19-11 March 2020. 2020.

2. Chen, G., D. Wu, W. Guo, Y. Cao, D. Huang, et al., Clinical and immunological features of severe and moderate coronavirus disease 2019. 2020. 130(5).

3. Jiang, L., K. Tang, M. Levin, O. Irfan, S.K. Morris, et al., COVID-19 and multisystem inflammatory syndrome in children and adolescents. 2020.

4. Rothan, H.A. and S.N.J.J.o.a. Byrareddy, The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. 2020: p. 102433.

5. Kannan, S., P.S.S. Ali, A. Sheeza, and K.J.E.R.M.P.S. Hemalatha, COVID-19 (Novel Coronavirus 2019)-recent trends. 2020. 24(4): p. 2006-2011.

6. Gomez-Pastora, J., M. Weigand, J. Kim, X. Wu, J. Strayer, et al., Hyperferritinemia in critically ill COVID-19 patients - Is ferritin the product of inflammation or a pathogenic mediator? Clin Chim Acta, 2020. 509: p. 249-251.

7. Lagadinou, M., E.E. Solomou, N. Zareifopoulos, M. Marangos, C. Gogos, et al., Prognosis of COVID-19: Changes in laboratory parameters. 2020. 62(13.4): p. 47-16.2.

8. YARALI, H.N. and A.K.J.T.Ç.H.D. YOZGAT, Hematologic Parameters of Patients with COVID-19 Infection. 2020. 14(COVID-19): p. 48-50.

9. Russell, C.D., A. Parajuli, H.J. Gale, N.S. Bulteel, P. Schuetz, et al., The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: A systematic review and meta-analysis. 2019. 78(5): p. 339-348.

10. Feng, X., L. Li, J. Wu, L. Zhang, Z. Sun, et al., Complete Blood Count Score Model Integrating Reduced Lymphocyte-Monocyte Ratio, Elevated Neutrophil-Lymphocyte Ratio, and Elevated Platelet-Lymphocyte Ratio Predicts Inferior Clinical Outcomes in Adult T-Lymphoblastic Lymphoma. 2019. 24(11): p. e1123.

11. Feng, F., G. Zheng, Q. Wang, S. Liu, Z. Liu, et al., Low lymphocyte count and high monocyte count predicts poor prognosis of gastric cancer. 2018. 18(1): p. 1-7.

12. Li, T., H. Lu, W.J.E.M. Zhang, and Infections, Clinical observation and management of COVID-19 patients. 2020. 9(1): p. 687-690.

13. Rosário, C., G. Zandman-Goddard, E.G. Meyron-Holtz, D.P. D'Cruz, and Y.J.B.m. Shoenfeld, The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. 2013. 11(1): p. 185.

14. Ruscitti, P., O. Berardicurti, P. Di Benedetto, P. Cipriani, A. Iagnocco, et al., Severe COVID-19, another piece in the puzzle of the hyperferritinemic syndrome. An immunomodulatory perspective to alleviate the storm. 2020. 11.

15. Levi, M. and T.J.T.r. van der Poll, Coagulation and sepsis. 2017. 149: p. 38-44.

16. Zhang, L., X. Yan, Q. Fan, H. Liu, X. Liu, et al., D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. 2020. 18(6): p. 1324-1329.

17. Kollias, A., K.G. Kyriakoulis, E. Dimakakos, G. Poulakou, G.S. Stergiou, et al., Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. 2020. 189(5): p. 846-847.

18. Cui S., S. Chen, X. Li, S. Liu, F.J.J.o.T. Wang, et al., Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. 2020.

19. Xu, H., L. Zhong, J. Deng, J. Peng, H. Dan, et al., High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. 2020. 12(1): p. 1-5.

20. Fathi, N. and N.J.C.B.I. Rezaei, Lymphopenia in COVID-19: Therapeutic opportunities. 2020.

21. Chai, X., L. Hu, Y. Zhang, W. Han, Z. Lu, et al., & Cai, J.(2020). Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection.

22. Xu, Z., L. Shi, Y. Wang, J. Zhang, L. Huang, et al., Pathological findings of COVID-19 associated with acute respiratory distress syndrome. 2020. 8(4): p. 420-422.

23. Demir HA, Changes in liver function in patients with COVID-19 and the demographics of the disease in a mid-sized city of Turkey: A retrospectiveanalysis, Medical Science and Discovery 2020;7(12):739-44

24. Hadjadj, J.; Yatim, N.; Barnabei, L.; Corneau, A.; Boussier, J.; Smith, N.; Péré, H.; Charbit, B.; Bondet, V.; Chenevier-Gobeaux, C.; et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020, 369, 718–724.

MSD

Medical Science and Discovery
ISSN: 2148-6832

Is traditional herbal mix composition effective on wound healing?

Serap Gokce Eskin[1]*, Yucel Basimoglu Koca[2], Serdal Ogut[1]

1 Adnan Menderes University, Faculty of Health Science, Nutrition and Dietetic, Aydin, TR
2 Adnan Menderes University, Faculty of Art and Science, Dept. of Biology, Aydin, TR

*** Corresponding Author:** Serap Gokce Eskin **E-mail:** serap.gokce@adu.edu.tr

ABSTRACT

Objective: Wound healing is a dynamic process that includes biochemical and physiological phenomena. Studies confirm that plants are used in wound healing to a great extent. Antique oil (HBX 2371) has been produced as a traditional herbal mixed. This herbal mixture contains sesame oil, thyme oil, olive leaf, fig seed, grape seed, turmeric, and cinnamon. We evaluated traditional plant mixed oil (Antique oil) for its wound-healing activity using an excision wound model in rats.

Methods: The study used a preclinical, in vivo experimental, and analytical design. In the study, a total of 21 Sprague-Dawley rats, including 7 as experimental group, 7 as negative-control group, and 7 as positive-control group, were used. After the ischemic wound was created in experimental animals, the research data relating to the histochemical changes and biochemical parameters of the wound healing parameters were collected

Results: A to research in experimental group fibroblast count, collagen density, fat cells, epithelization scores higher than the other groups and inflammatory cell density lower than the others. According to the results of the study, the highest TAC value (3.94 ± 0.21) was determined in the experimental group and results showed that antique oil administration decreased the TOC value.

Conclusion: In conclusion, it was observed that antique oil prevented the wound changes induced by the incision, increased the repair of the epidermal and dermal structure in a short time, increased the antioxidant level, and decreased the oxidation level. Clinical studies are recommended.

Key words: Wound healing, fig, olive, grape, thyme, turmeric, cinnamon, sesame oil

Received 22-12-2020

Accepted 25-01-2021

Available Online: 28-01-2021

Published 30-01-2021

INTRODUCTION

A wound is defined as the disruption of tissue continuity, interruption of, or damage to tissues. Wound healing is a dynamic process that includes biochemical and physiological phenomena and works in harmony with each other to guarantee tissue restoration, starting from the moment of injury and lasting days, months, or even years (**1, 2**). Wound healing, which is a physiological process under normal conditions, is interrupted by reasons such as diabetes, infection, and radiation. It is important to have smooth wound edges and oxygen input in healing. If the wound edge is smooth, wound healing takes a short time, and epithelization is completed; however, large wounds with irregular edges take more time to heal, and epithelization cannot be completed. The presence of infection is another factor that prolongs the wound healing period (**3**). The wound healing process involves adipose tissue, platelets, macrophages, and lymphocytes and Epidermal Growth Factor secreted from them (EGF), Growth Factor (PDGF) released by platelets, Fibroblast Growth Factor (FGF), Interleukin l (IL-1), Interleukin 2 (IL-2), Tumor necrosis factor-alpha (TNF-α), Transforming Growth Factor (TGF), and melatonin (**2, 4, 5, 6**). It is important that the wound care material used for normal wound healing should have antibacterial, antifungal, antioxidant effects, and increase the number of growth factors, collagens, and fibroblasts.

http://dx.doi.org/10.36472/msd.v8i1.455

For these effects to occur, more than one product or agent is used together. It is thought that approaches that strengthen the antioxidant system, especially against oxidative stress, during wound healing contribute to the healing. Studies confirm that plants are used in wound healing to a great extent (2,3,5).

Antique oil HBX 2371 has been produced as an herbal dietary supplement, which has been approved by Yıldız Technical University, Faculty of Chemistry-Metallurgy Department of Food Engineering, to have an antibacterial, antimycotic, antiviral, antiparasitic, anticarcinogenic, and antioxidant effect and to strengthen the immune system. This herbal mixture contains sesame oil, thyme oil, olive leaf, fig seed, grape seed, turmeric, and cinnamon. These are medicinal plants that have been used by the public for many years, either separately or as a mixture, for various ailments. Sesame oil (Sesamum indicum) is rich in oleic acid, linoleic acid and tocopherol. So it has an antioxidant effect and is among the traditional methods used in wound healing (**7,8,9**). Thyme (Thymus vulgaris) is an antioxidant, antibacterial, antiviral, antibiofilm, and antifungal herb. It contains especially phenol compounds (thymol, carvacrol) (**10,11,12,13).**

In experimental studies, it has been shown to have an enhancer effect on collagen synthesis, mast cell, and fibroblast proliferation when applied on the wound (**14,15,16**). Studies have shown that grape (Vitis vinifera L. Vitaceae) also has anti-inflammatory and antioxidant effects and that it shortens the epithelization time and increases wound contraction, thus positively affecting wound healing when applied on the wound. Because it contains minerals, vitamins (provitamin A carotenoids, vitamin C and E), and phytochemical compounds including phenolic acids, flavonoids, and anthocyanins (**16,17,18**). Grape-seed extracts contain rich flavonoids. Ma et al showed that in their research; grape seed supported the proliferation and migration of human dermal fibroblasts as well as promoted angiogenesis and skin regeneration in chronic wounds (**19**). Fig (Ficus carica) leaf is known as an effective pharmacological agent in wound healing. Ficus carica has anti-inflammatory antiangiogenesis and anti-VEGF effects. The major minerals in fig seeds were found as Ca, K and P and also it contains oleic asit, lionoleik acid and palmitic asit (**20, 21**).

According to research, the therapeutical properties of the Ficus carica may be related with its significant antioxidant potentials (**22**). Studies have revealed that turmeric (Curcuma longa) accelerates wound healing (**23, 24).** Cinnamon (Cinnamomum zeylanicum) prepared in ethanol extract accelerates epithelization and stimulates wound closure when applied to the wound (**25**). Also, cinnamon has an antioxidant effect when used as a dietary supplement (**26**). Olive leaf extract (Olea europaea) a rich source of bioactive compounds, particularly phenolic and flavanoids (gallic asid, rutin, quercetin, ellagic acid) on the other hand, shows an antioxidant effect when applied on both diabetic and clean wounds, increases collagen density and fibroblast migration, and positively affects wound healing (**27,28,29, 30**).

When viewed one by one, it is thought that this effect can occur more powerfully and faster with the mixture of plants that have a positive effect on wound healing. As a result, despite scientific studies conducted on all medical, pharmacological, and alternative-complementary approaches, wound treatment and care remains as an important health problem. For this reason, there is a need to identify new, low-cost, and effective wound care materials.

MATERIAL AND METHODS

The study used a preclinical, in vivo experimental and analytical design. The experiments were conducted between March 01, 2017 and March 01, 2018 in the Experimental Animals Unit of Adnan Menderes University Faculty of Medicine. In the study, a total of 21 Sprague-Dawley male rats. Each rat averaged between 250-300 g. Studies on animal experiments show that a minimum of 5 and a maximum of 10 animals should be used in each group (**31**). So in this study including 7 as the experimental group (antique oil), 7 as negative-control group (any wound care), and 7 as positive-control group (salin solution) were used. Didn't used any criteria used for including and excluding animals (or experimental units) and randomization during the experiment. The animals were placed in individual cages, fed with the same food in a room with standardized temperature and humidity, and given tap water. The rats were anesthetized by the researcher by administering 50 mg/kg Ketamine Hydrochloride (Ketamine HCL, Ketalar, EWL Eczacıbaşı Warner-Lambert Pharmaceutical Industry and Trade Inc.) and 5 mg/kg xylazine intraperitoneally, the back skin of rats was shaved with an electric shaver, and skin cleansing was done with batticon solution. Three full-thickness skin incisions with a diameter of 15 mm were made in the cephalic area in the midline of the back with a size 15 scalpel. On the day when the wound was opened, 500 mg paracetamol was added to the drinking water of the rats. The day when the wound was formed was accepted as day 0 in two groups, and the wound care was carried out between 17:00 and 18:00 every day for 21 days. Wound care of the rats was applied regularly by the researcher every day.

In this study we use antique oil extract. Antique oil extract is a product prepared by traditional methods, consisting of Antique oil extract, sesame oil, thyme oil, olive leaf, fig seed, grape seed, turmeric, and cinnamon mixture. It is sold with Antique oil HBX name in Turkey (TIAP Group).The essential oil composition of the product was made in Yıldız Technical University. According to this it contains oleic asid (59.23%), linoleic asid (15.77%), palmitic asid (8.48%), stearic asid (2.53%), linoleic asid (2.30%), arachidic acid (0.28%), (E)-cinnamoldehid (26.12%),carvaclol, cyclohexanol (16.9%).Antique oil extract was spread as 1 ml thin layer on the wound of the experimental group every day between 17:00 and 18:00 hours for 21 days and it was covered with sterile sponge saline solution was applied onto the wound of the positive control group and then it was covered. Any care wasn't performed for the wound of negative control groups; normal wound healing was observed in these rats (**28,32**).

After the ischemic wound was created in experimental animals, the research data relating to the histochemical changes and biochemical parameters of the wound healing parameters were collected in line with the literature by evaluating Total antioxidant capacity (TAC) and Total oxidant capacity (TOC).

http://dx.doi.org/10.36472/msd.v8i1.455

Histological preparation procedure was applied to the tissue samples taken on the 3rd, 10th, and 21st days to determine the healing status of all wounds. Tissue samples taken on the specified days were fixed in Saint Marie's fixative and neutral buffered formalin (NBF) at +4°C for 24-48 hours. Then the detected tissues were dehydrated by passing through graded alcohol series and made transparent with xylol. They were blocked after waiting in liquid paraffin at 58°C overnight. They were blocked after waiting in liquid paraffin at 58°C overnight. Skin tissue blocks were sectioned on a rotary microtome (Leica RM-2145) at 5 µm thickness. Sections were stained with Hematoxylin-Eosin (H&E) for general histological structure and Gomori's trichrome (GT) (Artisan Gomori's Blue Trichrome Kit ,Artisan Elastic [Verhoeff - Van Gieson Technique - VVG] Kit, Dako North America, Inc.) to show collagen (**33**). Also, a semi-quantitative evaluation over 0-3 points was done in terms of inflammatory cell density, fibroblast count, collagen density, fat cells, and epithelization in the tissue samples examined. Accordingly, the average of the counts in each area at x40 magnification was applied as in the table below For this evaluation, a histoscore prepared according to the literature was used (**32**) (Table 1).

Total Antioxidant and Total Oxidant Capacity Measurements

Total antioxidant and total oxidant capacity were measured with ready-built kits developed by Erel (Total Antioxidant Status (TAS) kit and Total oxidant Status (TOS) kit – Rel Assay - Turkey). Antioxidants may have the capacity to protect organisms against free radical damage. Measurement of total antioxidant capacity (TAC) is important to evaluate the effects of bio-chemicals as indicated in many studies. TAC shows the oxidant buffering potential of a tissue or biological fluid. Many methods have been developed to measure TAC (34). In this study, the TAC measurement method developed by Erel (2004) was employed (35). According to this method, the reduced 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) molecule is oxidized to ABTS ● + with hydrogen peroxide in acidic medium (30 mM acetate buffer pH 3.6). ABTS ● + molecules (dark green in color) can remain stable for a long time in acetate buffer. In the method developed by Erel, if it is diluted with more concentrated acetate buffer (400 mM pH 5.8), the color spontaneously will lighten slowly. The antioxidants present in the sample accelerate the lightening of the color in proportion to their concentration. This reaction is followed in the spectrophotometer. The rate of lightening is inversely related to the total antioxidant capacity of the sample. Analyses were carried out in 2 repetitions. On the day of analysis, after the blood samples taken from -80°C medium were dissolved in a hot water bath, spectro-photometric analyses were done in the blood serum.

$$\text{Result} = \frac{\text{AbsStd1-Abs}}{\text{AbsStd1-AbsStd2}} \text{ X Standard 2 Value}$$

Oxidative stress index (OSI) is an indicator parameter of the degree of oxidative stress, and its formulation is as follows.

Wound care material used in the study

In the study, negative control group wounds were not administered any procedure. In the positive control group, 09% sterile isotonic solution was used for wound care. On the other hand, "antique oil HBX 2371", a herbal composition, which was approved by Yıldız Technical University Chemistry-Metallurgy Faculty Food Engineering Department to have an antibacterial, antimycotic, antiviral, antiparasitic, anticarcinogenic, and antioxidant effect and to strengthen the immune system, was used in the experimental group wounds.

Sacrifice process: All animals were sacrificed with high dose ether inhalation on postoperative 21st day.

Data analysis: The data obtained in this study were analyzed using the SPSS 19 software package. The frequency and percentage distributions of the data were examined. ANOVA and Tukey tests were employed for further analysis.

Dependent and independent variables of the study

Dependent Variable: Wound healing status

Independent Variables: Wound dressing with 09% isotonic and "antique oil HBX 2371" solution

RESULTS

Histological results

Control group: In the sections prepared from the back skin of the control group animals, the layers of the skin were distinguished. Regarding the general histological structure, the epidermis at the outermost layer, hair follicles and shafts, and sebaceous glands in the dermis below it were observed. The deep dermis was observed to contain fat and muscle tissues (shown in Fig. 1a-h).

The epidermis was observed to consist of stratum corneum, stratum granulosum, stratum spinosum, and stratum basale layers The dermis has a connective tissue structure and contains many hair follicles (shown in Fig. 1a-h).

.In the transverse section hair follicles, there was a transparent hair cortex as a thin layer around the central hair shaft. The hair cuticle was observed to surround the hair cortex in the form of a dark band. The outer root sheath was in the form of a round-core layer with clear cell borders. There was a layer of connective tissues outside the hair (shown in Fig. 1b, d, f, h). Basal cells in the sebaceous gland structure closely located to the hair follicles and cells secreting sebum on the basal cells were distinguished (shown in Fig. 1c, g).

Experimental group: No significant difference was observed in the general histological structure in all groups. In all groups, the outermost epidermis layer and the hair follicles and sweat glands in the dermis below it were distinguished in a normal structure. There is fat and muscle tissue under the dermis layer (shown in Fig. 2a, b, c, e).

http://dx.doi.org/10.36472/msd.v8i1.455

Inflammatory cell density in positive and negative control groups was severe on the 3rd day, moderate on the 10th day, and mild on 21st day. In the antique oil-wound-care group, while the inflammatory cell count score was 3 in the examination done on the 3rd day and 2 on the10thday, it decreased to 0 on the 21st day (Table 2).

The fibroblast count and collagen density measurements, performed for the evaluation of wound healing, indicated mild levels on the 3rd day and moderate on the 10th and 21st days in the positive and negative control groups. They were mild on the 3rd day and severe on the 10th and 21st days in the antique oil-applied experimental group (Table 2). Generally, the collagen Gomori trichrome showed a positive reaction in all groups and was stained intensely bright green. On the 3rd day, collagen bundles were observed in the dermis in a scattered and fragmented fashion. On the 10th, and especially on the 21st day, highly dense collagen fibrils were distinguished parallel to the increasing number of fibroblasts (shown in Fig. 2, 3).

The number of fat cells in the sections taken from the skin tissues did not show much difference between the positive and negative control groups; while it was observed similar to the controls on the 3rd and 10th days in the experimental groups, it was found to be severe on the 21st day (shown in Fig. 1, 2, 3,4- Table 2).

Another parameter considered in the study used to evaluate wound healing was epithelization. Epithelization was not observed on the 3rd day in the control groups, but it was found as mild on the 10th day and moderate on the 21st day. In the experimental groups, it was observed that epithelization increased slightly on the 3rd day and markedly on the 10th and 21st days (shown in Fig. 1, 2, 3,4- Table 2).

Biochemical results: According to the findings of our study, the mean TAC scores in the experimental group and control groups were 3.94 and 2.33, respectively. According to the statistical analysis, a statistically significant difference was found between the groups in terms of TAC According to the Tukey analysis, conducted to determine the group causing the difference, there was no difference between the negative and positive control groups, but there was a statistically significant difference between the experimental and control groups (Graphic 2).

The mean TOC score, on the other hand, was 1.65 in the experimental group and 3.32 in the control groups. The difference between groups was statistically significant, and this difference originated from the experimental group, similar to the results relating to TAC (Table 3, Graphic 2).

Table 1. Histological Scoring

Score	Inflammatory cell density	Fibroblast count	Level of collagen density	Fat cells	Epithelization
0	None (0)	None (0)	None (0)	None (0)	None
1	Mild (1-5)	Mild (1-5)	Mild (1-5)	Mild (1-5)	Mild
2	Moderate(6-10)	Moderate (6-10)	Moderate (6-10)	Moderate (6-10)	Moderate
3	Severe (10>)	Severe (10>)	Severe (10>)	Severe (10>)	Full

Table 2. Inflammatory cell density, fibroblast count, collagen density, fat cells, and epithelization scoring of the wound tissues.

Parameter	Positive control group			Negative control group			Experimental group		
	3rd day	10th day	21st day	3rd day	10th day	21st day	3rd day	10th day	21st day
Inflammatory cell density	3	2	1	3	2	1	3	1	0
Fibroblast count	1	2	2	1	2	2	1	3	3
Collagen density	1	2	2	1	2	2	1	3	3
Fat cells	1	2	2	1	2	2	2	2	3
Epithelization	0	1	2	0	1	2	1	3	3

Table 3. Intergroup comparison of TAC and TOC Values

	TAC				TOC		
	n	Min.-Max.	Mean		Min.-Max.	Mean	
Experimental	7	3,68-4,25	3,94 ± 0,21	F=30,102	1,65-2,43	2,09 ± 0,27	F=77,439
Positive control	7	2,33-3,24	2,83 + 0,34	p=0.00	3,32-3,98	3,62+ 0,25	p=0.00
Negative control	7	2,33-3,24	1,91 ± 013		3,32-3,98	3,62 ± 0,25	
Total	21	2,33-4,25	3,20 ± 0,13		1,65-3,98	3,11 ± 0,77	

http://dx.doi.org/10.36472/msd.v8i1.455

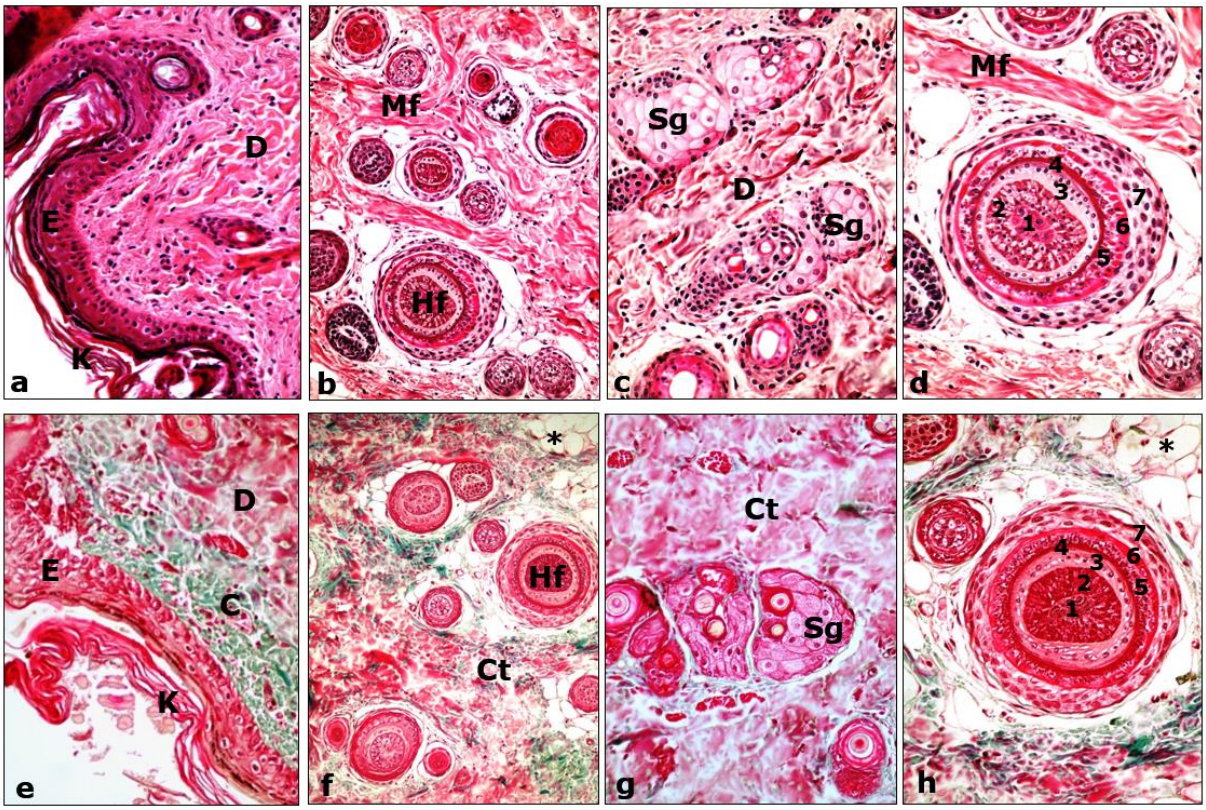


Figure 1. Skin sections belonging to the control groups. E: Epidermis, D: Dermis, Sg: Sebaceous gland, Hf: Hair follicle, C: collagen, K: keratin, Mf: muscle fibres, Ct: connective tissue, *: adipose cells, hair follicle: 1. hair follicle medulla, 2. cortex, 3. inner root sheath (Huxley), 4. inner root sheath (Henle), 5. outer root sheath, 6. vitreous layer, 7. dermis connective tissue sheath. Staining: a, b, c, d H&E - e, f, g, h Gomori trichrome. Magnification: a, b, c, e, f, g x20 - d, h x40

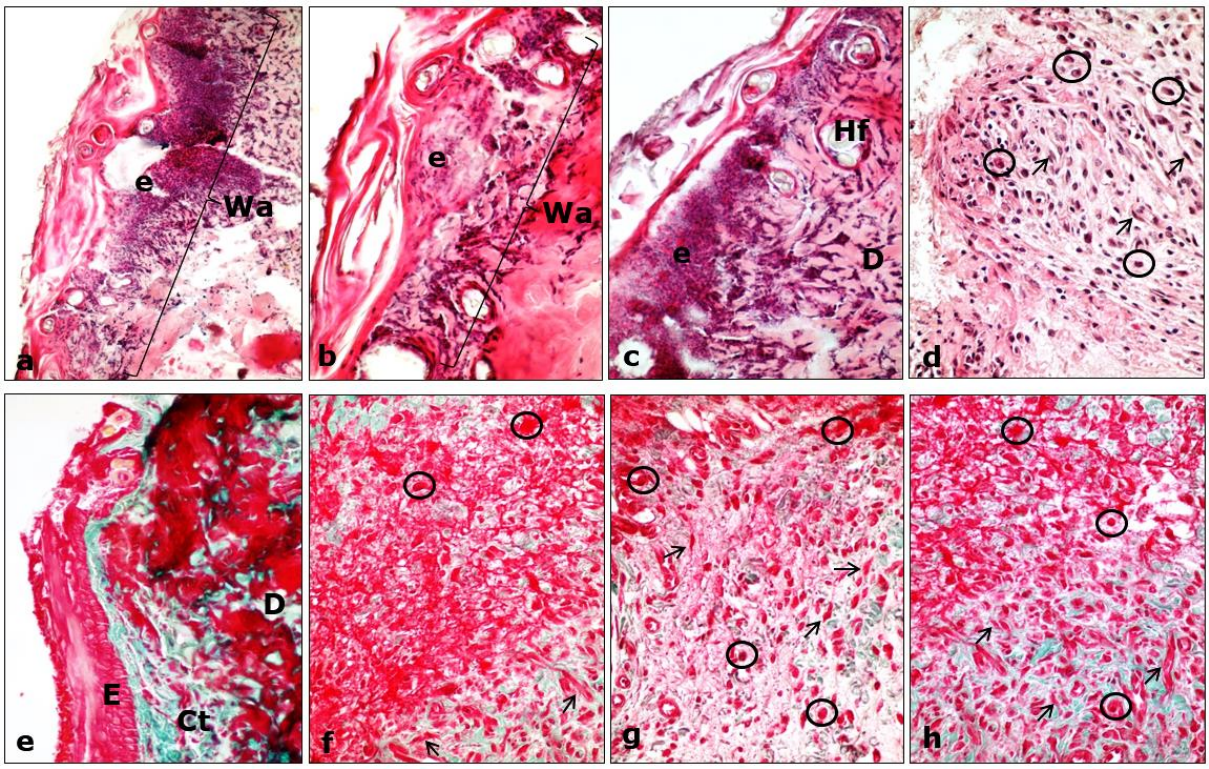


Figure 2. Inflammation in the wound area and numerous mast cells and fibroblasts in the connective tissue were noted in the skin sections of the group that was treated with antique oil for 3 days. E: Epidermis, D: Dermis, Wa: wound area, e: inflammation, Ct: connective tissue, Hf: Hair follicle, □: fibroblast, □: mast cells. Staining: a, b, c, d H&E - e, f, g, h Gomori trichrome. Magnification: a, b, c, e x20 - d, f, g, h x40

http://dx.doi.org/10.36472/msd.v8i1.455

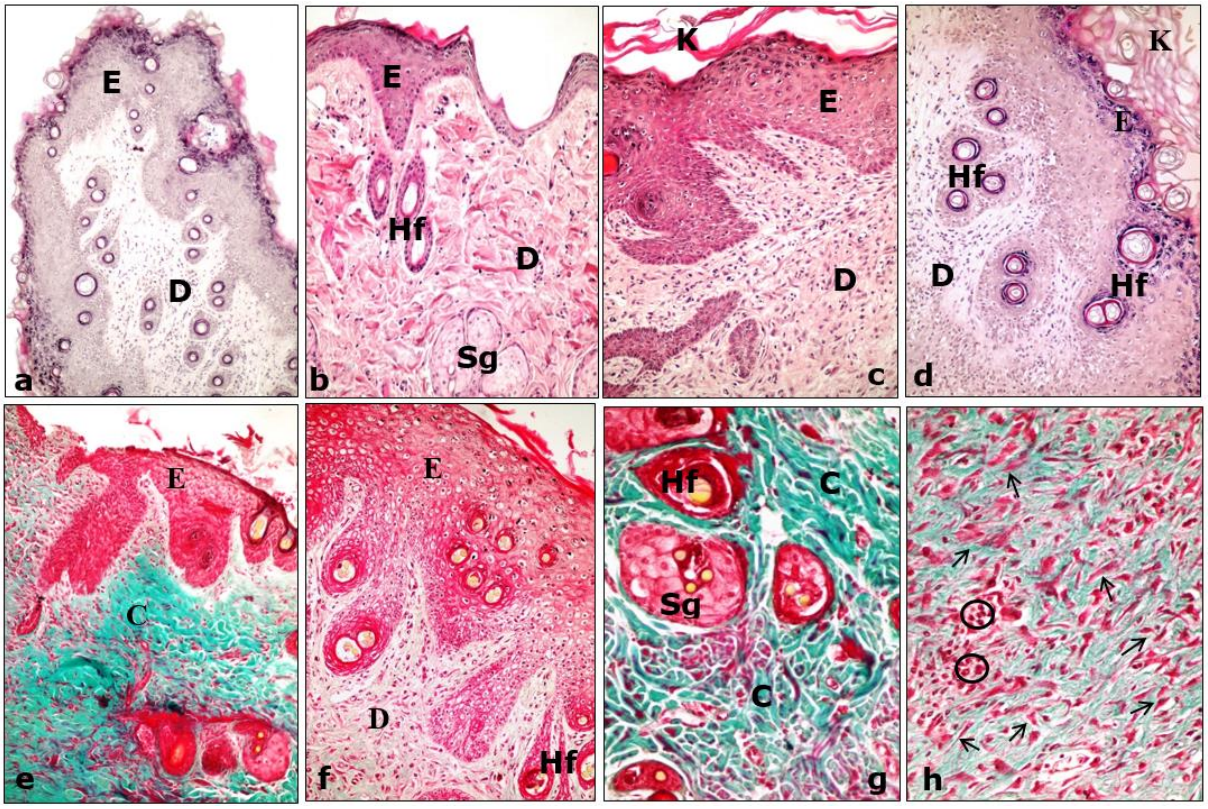


Figure 3. In the skin sections of the group treated with antique oil for 10 days, inflammation decreased, and epithelial thickness, and fibroblast and collagen fibers in the connective tissue increased. E: Epidermis, D: Dermis, Hf: Hair follicle, □: fibroblast, □: mast cell. Sg: Sebaceous gland, Kf: Hair follicle, C: collagen (green areas). Staining: a, b, c, d H&E - e, f, g, h Gomori trichrome. Magnification: a, x10 - b, c, d, e, f x20 - g, h x40

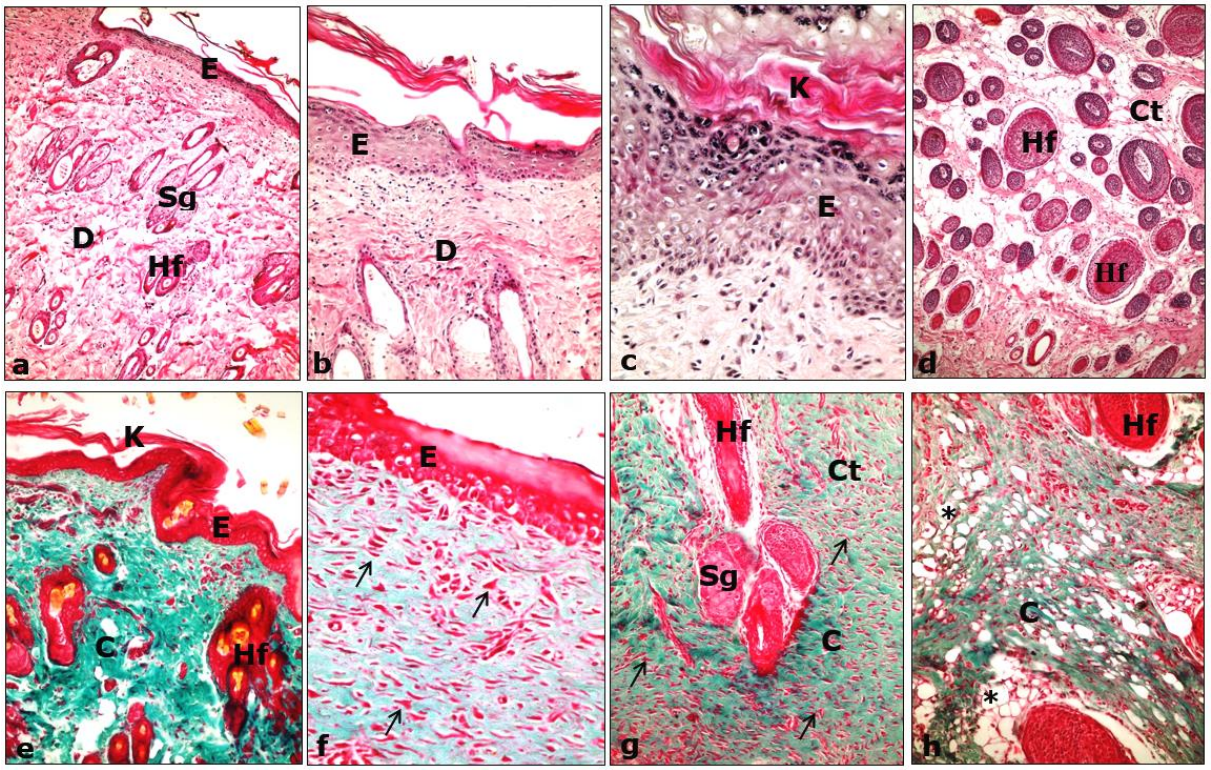
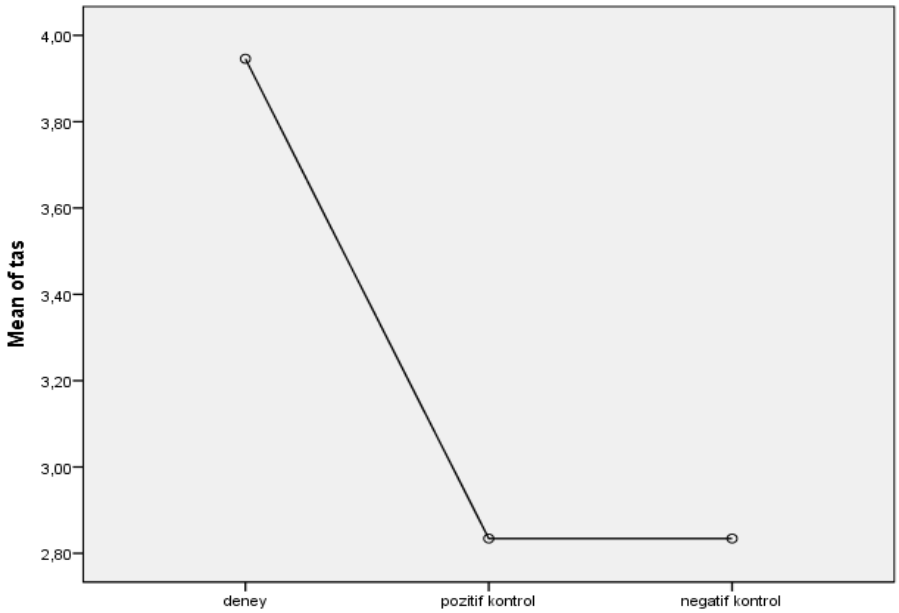
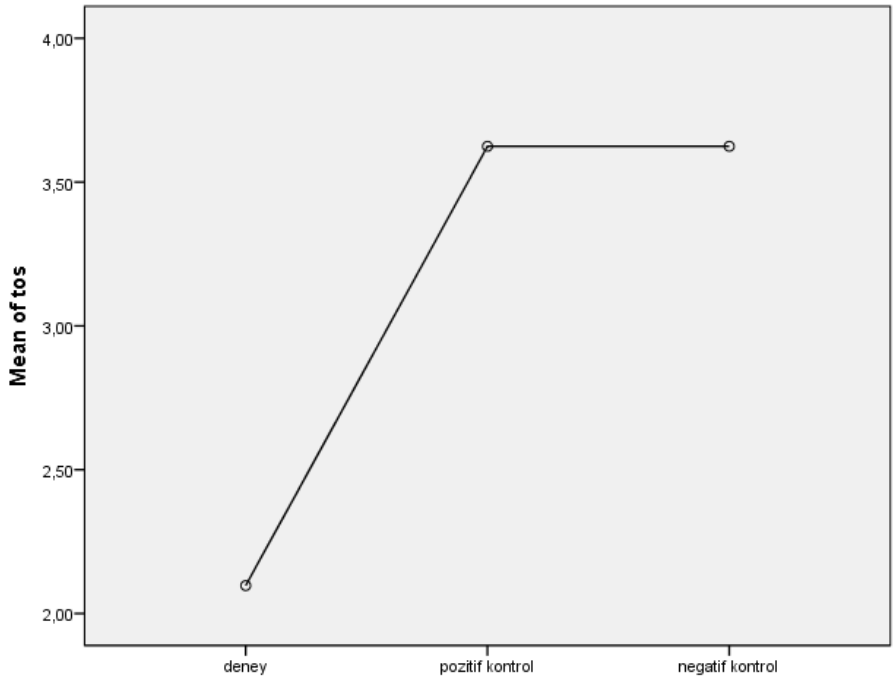


Figure 4. In the skin sections of the group treated with antique oil for 21 days, there was an increase in epithelial thickness, and numerous fibroblasts and dense collagen fibril bundles were observed in the connective tissue. E: Epidermis, D: Dermis, *: fat cells, □: fibroblast, Sg: Sebaceous gland, Hf: Hair follicle, C: collagen (green areas). Staining: a, b, c, d H&E - e, f, g, h Gomori trichrome. Magnification: a, b, d, e, g, h x20 - c, f x40

http://dx.doi.org/10.36472/msd.v8i1.455



Graphic 1. Evaluation of TAC values with Tukey analysis



Graphic 2. Evaluation of TOC values with Tukey analysis

DISCUSSION

Antique oil is a mixture containing sesame oil, thyme oil, olive leaf, fig seed, grape seed, turmeric, and cinnamon. This mixture is expected to have a positive effect on wound healing. In our study, the amount of inflammatory cells, fibroblast count, the level of collagen density, and epithelization status were evaluated. The first phase of wound healing is inflammatory cell migration (**3, 5**). According to the results of our study, while the inflammatory cell density in the experimental group was the highest on the 3rd day, it was not observed on the 21st day. In the control groups, on the other hand, while it was found to be severe on the 3rd day, it decreased to a mild level on the 21st day.

Since ingredients, such as grape seed and thyme, in antique oil had anti-inflammatory effects, the inflammation phase was expected to shorten in the experimental group (**16,17, 18**). Our study finding was consistent with the literature.

Regarding the number of fibroblasts, the mild level of fibroblasts detected on the 3rd day in the positive and negative groups increased to a moderate level on the last day. However, the mild level of fibroblast observed in the experimental group on the 3rd day reached a severe level on the 21st day.

A fibroblast count increase was expected in the second and third phases of the wound healing process (10th and 21st days) (**3, 5**). For this reason, achieving an increase in fibroblasts is an important feature in products that will accelerate wound healing. It has been shown that the olive leaf and thyme found in the antique oil content accelerate the fibroblast migration (**14,15,27,28**). Collagen is another important molecule that provides wound healing. It is expected that the use of the wound care product will have the ability to enhance collagen production. According to the results of our study, a mild level of collagen was detected in the experimental and control groups on the 3rd day, and it increased to a moderate level in the last two measurements. In the experimental group, the collagen density starting at a mild level was found at a severe level on the 10th and 21st day samples. It is known that the increase in collagen density accelerates wound healing. Samancıoğlu (2016) revealed that olive leaf extract increased collagen production (**28**). Research results examining the effect of thyme on wound healing also revealed its effect on increasing collagen production (**14,15,16**). This finding was also consistent with the literature.

Epithelization is one of the last stages of wound healing. It is expected to occur especially on the 21st and the following days. Early-onset of epithelization will accelerate wound healing (**2**). In our study, epithelization did not start on the 3rd day in the control groups; it was observed at a moderate level on the 21st day. In the experimental group, on the other hand, it started at a mild level on the 3rd day of wound healing and reached a severe level on the 21st day. Various studies have shown that the grape seed in the antique oil content shortens the epithelization period (**16, 17, 18**). Farahpor and Habibibi stated that cinnamon accelerated epithelization (**25**). Similarly, it was found that the epithelization period shortened in the antique oil dressing group in our study.

Antioxidants may have the capacity to protect organisms against free radical damage. Measurement of total antioxidant capacity (TAC) is important to evaluate the effects of bio-chemicals as indicated in many studies. TAC shows the oxidant buffering potential of a tissue or biological fluid. Many methods have been developed to measure TAC (34). In this study, the TAC measurement method developed by Erel (2004) was employed (**35**). This method determines the antioxidant effect of the sample against strong free radical reactions initiated by the hydroxyl radical product. The reaction was calibrated with Trolox (**35**). According to the results of the study, the highest TAC value (3.94 ± 0.21) was determined in the experimental group, that is, the group in which the wounds were treated with antique oil. This value indicated that antique oil administration increased the antioxidant capacity in the experimental group. As mentioned before, the antique oil composition has high antioxidant content, and in this application, it may have increased the antioxidant capacity in rats. NCG group with only wound opening and with no intervention had the lowest TAC value. Not applying any treatment to wounds in this group explained the low TAC values. The higher TAC values in the positive control group (PCG) compared to NCG may have been due to the application of 0.9% sterile isotonic solution to the wounds. Ficus carica fruits contain numerous seeds that vary according to their maturity and size. The largest portion of the fatty acid composition of these seeds consists of linolenic acid, which is an omega-3 derivative and contains alpha-linolenic acid (ALA). Following linolenic acid, it also contains linoleic, oleic, palmitic, and stearic acid to a less extent, respectively (**20**). One of the main ingredients in antique oil is fig seed oil. The important effects of omega-3 oils found particularly in fig seeds on wound healing are known. It is stated that omega-3 fatty acids are the precursors of some substances that play an active role in the resolution of inflammation and support wound healing by preventing chronic inflammation. It is thought that fig seed oil has an important effect on wound healing in rats. Besides, it has been determined that the oral use of fig seed oil reversed the biochemical and histopathological findings in ischemia-reperfusion injury developing in rats due to the acute mesenteric ischemia model, possibly due to its antioxidant and anti-inflammatory properties (**21**). The antioxidants found in sesame oil detoxify the liver, may reduce the incidence of some tumors, and protect against oxidative stress (**8, 9**). These findings in the literature support our research. In addition to its use for many purposes (appetite stimulator, cardiovascular diseases, intestinal parasites, biliary tract diseases), thyme is also utilized in wound care. The wound healing property of thyme oil stems from its active ingredient of Carvacrol (**12**). The antioxidant properties of carvacrol and thymol essential oil, which is also found in thyme, are known (**13**). In our study, we consider that one reason for the increase in wound healing and antioxidant capacity is due to carvacrol and thymol. In their rat experiment, Gürbüz and Öğüt (2020) determined that when compared with the control group, the levels of total thiol and native thiol, which are antioxidant parameters, increased in rats given olive leaf extract. It was stated that the effective substance of oleuropein found in olive leaves may have played an important role in this effect (**29**). The effect of olive leaf extract was compared with classical dressing material used in clinical treatment and care in diabetic rats in which an ischemic wound model was developed. According to the results of the study, wound dressing with olive leaf extract was found to be more effective than conventional wound dressing in diabetic and non-diabetic wounds (**21**). Similar results were found in the antique oil applied rats in our study compared to the control group.

Reactive oxygen species can form in metabolic and physiological reactions in biological systems, and these harmful effects maybe neutralized by enzymatic or non-enzymatic antioxidant systems. In some adverse conditions, an increase in oxidants and a decrease in antioxidants may adversely affect the organism. This causes the oxidative / antioxidative balance to shift towards the oxidative state, which causes oxidative stress. Total oxidant capacity (TOC) is the indicator of in vivo total oxidant status (**36**).

TOC measurement method developed by Erel was used in this study (**37**). According to the results of the study, the highest TOC value (3.95 ± 0.42) was determined in NCG with only wound opening and with no intervention. The lowest TOC value (2.09 + 0.27) was determined in the experimental group. These results showed that antique oil administration decreased the TOC value. Antioxidant

substances in the antique oil content may have decreased the TOC values.

Serum lipid hydroperoxide (LOOH) levels were measured with thiocyanate ion using a commercial kit. The method is based on the principle that hydroperoxides oxidize to ferric ion (Fe + 3) and ferrous ion (Fe + 2) in an acidic environment. The LOOH level is an indicator of oxidative damage in systems. The most common reactive oxygen species in the organism are lipid radicals formed by the removal of one hydrogen from the allyl group of unsaturated fatty acids. Unsaturated fatty acids first react with oxygen to form the lipid peroxy radical and then form LOOH by chain reaction with lipids (**38**).

The evaluation of the LOOH findings in the scope of the study indicated that the highest value was in NCG. The high value of LOOH, which is an oxidant marker, in this group with only wound opening and with no intervention was also in line with the hypothesis of the study. On the other hand, the lowest LOOH values were determined in the experimental group. The administration of the high antioxidant compound to the wound decreased the LOOH values in rats. This result was also consistent with the hypothesis of the study.

Thiols (RSH), also called mercaptan, are functional groups that are in the class of organic compounds and are found in the structure of the main proteins in living organisms and contain a sulfhydryl group (-SH). Thiols with antioxidant properties are formed as a result of one sulfur atom and one hydrogen atom bonding to a carbon atom (**39, 40**). Thiol disulfide balance has been measured in only one direction since 1979, but with the new automatic method developed by Erel and Neşelioğlu, the levels of both variables can be measured separately and collectively (**41**). This method was also used in this study.

According to the results of the study, the highest total thiol (µmol / L) value (151.548 ± 24.644) was determined in the experimental group. This may have been due to the antioxidant content of the antique oil, as well. The lowest total thiol value (145.684 ± 23.217) was determined in NCG.

CONCLUSION

In conclusion, antique oil reduced the inflammation and increase in mast cell count observed severely in groups with short-term (3 days) treatment gradually in moderate (10 days) and especially long term (21 days) treatment applications. It also increased collagen fiber synthesis arising from fibroblast increase in the epithelization and dermis. It was observed that antique oil prevented the wound changes induced by the incision, increased the repair of the epidermal and dermal structure in a short time, increased the antioxidant level, and decreased the oxidation level.

To support the findings, it is thought that it will be useful to compare them to the findings of similar studies to be conducted with antique oil and the findings of further molecular analyses. We can suggest testing the effects of all herbal products on the tissue of animal models with various parameters.

Ethical Permissions: To conduct this study, permission was obtained from the local ethics committee of Adnan Menderes University Animal Experiments. (decision number: 2016-045)

Funding Sources: The research was supported by Aydin Adnan Menderes University Scientific Research Project Office. (SBF-17003)

Author Contributions: Serap GOKCE ESKIN: Conceptualization, Methodology, Software , Writing, Formal Analysis, Visualization, Experiment, **Yucel KOCA:** Histological analyze Data curation, Formal analysis, Writing-Original draft preparation. **Serdal OGUT:** Biochemical analyze, Methodology, Visualization, Investigation. Resources, Supervision.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

REFERENCES

1. Ellis S, Lin E, Tartar D. Immunology of Wound Healing. Curr Derm Rep.2018; 7:350–358. https://doi.org/10.1007/s13671-018-0234-9

2. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A Cellular Perspective. Physiol Rev.2019; 99: 665–706. doi: 10.1152/physrev.00067.2017

3. Singh S, Young A, Mcnaught CE. The physiology of wound healing. Surgery (Oxford). 2017;35(9): 473-477

4. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regeneration. 2014; 22: 569–578.

5. Quing C. The molecular biology in wound healing & non-healing wound. Chinese Journal of Traumatology. 2017;20(4):189-193

6. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U: Skin wound healing: An update on the current knowledge and concepts. Eur Surg Res 2017;58:81-94. doi: 10.1159/000454919

7. Alam G, Singh MP, Singh A. Wound healing potential of some medicinal plants. International Journal of Pharmaceutical Sciences Review and Research. 2011; 9(1): 136-145.

8. Abdou HM, Hussien HM, Yousef MI. Deleterious effects of cypermethrin on rat liver and kidney: protective role of sesame oil. J Environ Sci Heal B.2012; 47(4): 306-14.

9. Kumar M. Hepatoprotective activity of Sesamum Indicum Linn. against CCl4-induced hepatic damage in rats. Int J Pharm Biol Arch.2011; 2(2): 710-5.

10. Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and thyme essential oil—new insights into selected therapeutic applications. Molecules. 2020;25:4125. https://doi.org/10.3390/molecules25184125.

11. Salehi B, Mıshra AP, Shukla I, Sharifi-Rad M, ContrerasMM, Segura - Carretero A, Fathi H, Nasrabadi NN, Kobarfard F, Sharifi-Rad J. Thymol, thyme, and other plant sources: Health and potential uses. Phytotheraphy Research. 2018; 32(9): 1688-1706 https://doi.org/10.1002/ptr.6109

12. Yaman S, Özdemir Z , Şit M , Özer B, Çatal O. Effects of oregano oil carvacrol on human health. SETSCI Conference Indexing System. 2018; (2): 391-392.

13. Coban EÖ, Patır B. Use of some spices and herbs antioxidant Affected in foods. Electronic Journal of Food Technologies. 2010; 5(2): (7-19).

14. Mariam AK. The effect of oıl extract of thymus vulgarıs on full thıckness ıncısıon of wound healıng in rabbits. Bas.J.Vet.Research. 2015; 14(2): 103-109.

15. Panah G K, Hesaraki S and Farahpour MR. Histopathological evaluation of thymus vulgaris on wound healing. Indian Journal of Fundamental and Applied Life Sciences. 2014; 4 (S4): 3538-3544.

16. Tümen G, Malyer H, Başer KHC, Özaydın S. Plant used in Anatolia for wound healing. Proceedings of the IVth International Congress of Ethnobotany (ICEB 2005). 2006; 217-221.

17. Hemmati AA, Aghel N, Rashidi I, Gholampur-Aghdami A. Topical grape (Vitis vinifera) seed extract promotes repair of full thickness wound in rabbit.Int Wound Journal. 2011; 8:514–520.

18. Nayak SD, Dan Ramdath DB, Marshall JR, Isitor GN, Eversley M, Xue S, Shi J. Wound-healing activity of the skin of the common grape (vitis vinifera) variant, cabernet sauvignon. Phytotherapy Research.2010; 24: 1151–1157 DOI: 10.1002/ptr.2999

19. Ma H, Quan Zhou Q, Chang J, Wu C. ACS Nano. 2019; 13 (4): 4302-4311. DOI: 10.1021/acsnano.8b09496

20. Nakilcioğlu Taş E. Biochemical characterization of fig (Ficus carica L.) seeds. Journal of Agricultural Sciences.2019;25: 232-237.

21. Orak C. Possıble effects of fıg (Ficus carica) seed oıl on ıschemıareperfusıon damage, resultıng from experımental mesenterıc arterıal ıschemıa ın small ıntestıne. Aydin Adnan Menderes University Health Sciences Institute of Physiology.(Medical) Program, Doctoral Thesis, Aydin, 2020.

22. Eteraf-Oskouei T, Allahyari S, Akbarzadeh-Atashkhosrow A, Delazar A, Pashaii M, Gan Sh, Najafi M. Methanolic extract of ficus carica linn. leaves exerts antiangiogenesis effects based on the rat air pouch model of inflammation. Evidence-Based Complementary and Alternative Medicine. 2015. Article ID 760405 | https://doi.org/10.1155/2015/760405

23. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res Int. 2014; 1–12. doi: http://dx.doi.org/10.1155/2014/186864

24. Guimarães AF,Vinhas ACA, Gomes AF, Souza, LH, Krepsky PB. Essentıal oıl of Lurcuma Longa L. Rhızomes chemıcal composıtıon, yıeld varıatıon and stabılıty. Química Nova.2020;43(7): 909-913. https://dx.doi.org/10.21577/0100-4042.20170547

25. Farahpour MR, Habibi M. Evaluation of the wound healing activity of an ethanolicextract of ceylon cinnamon in mice. Veterinarni Medicina.2012; 57(1): 53–57.

26. Faix S, Faixová z, Plachá I, Koppel J. Effect of cinnamomum zeylanicum essential oil on antioxidative status in broiler chickens. Acta Vet. Brno.2009; 78: 411-417; doi:10.2754/avb200978030411.

27. Koca U, Süntar I, Akkol EK, Yilmazer D, Alper M. Wound repair potential of Olea europaea L. leaf extracts revealed by in vivo experimental models and comparative evaluation of the extracts' antioxidant activity. J Med Food. 2011;14(1-2):140-6. doi: 10.1089/jmf.2010.0039.

28. Samancıoglu S, Esen A, Ercan G, Mansoub NH, Vatansever S, İnce İ. A new dressing material in diabetic wounds: Wound healing activity of oleuropein-rich olive leaf extract in diabetic rats. Gaziantep Medical Journal 2016;22(1):14-21 • DOI: 10.5578/GMJ.27960

29. Gurbuz M, Ogut S. 2020. Antidiabetic effect of olive leaf extract on streptozotocin-induced diabetes mellitus in experimental animals. Nutr Hosp. 2020:37(5): 1012-1021.

30. Guo, Z., Jia, X., Zheng, Z. et al. Chemical composition and nutritional function of olive (Olea europaea L.): a review. Phytochem Rev.2018; 17: 1091–1110 https://doi.org/10.1007/s11101-017-9526-0

31. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 2002;43(4):244–258.

32. Gokce S, Tureyen, Gokcimen A. Effects of hypericum perforatum, clinoptilolite and hydrocolloid dressing on wound healing in diabetic rats. Wound Medicine. 2018; 22: 14-22

33. Bancroft JD, Cook HC. Manual of Histological Techniques and Their Diagnostic Application. Churchill livingstone. London, 1994.

34. Magalhaes, L.s.M., et al., Methodological aspects about in vitro evaluation of antioxidant properties. Analytica chimica acta, 2008; 613(1): 1-19.

35. Erel O, A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical biochemistry, 2004; 37(2):112-119.

36. Ogut S, Kucukoner E, Gultekin F, Gurbuz N. (2014). Study of long-term pesticide application amongst agricultural workers: total antioxidant status, total oxidant status and acetylcholinesterase activity in blood. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. DOI 10.1007/s40011-013-0291-6.

37. Erel Ö. A new automated colorimetric method for measuring total oxidant status, Clinical Biochemistry. 2005;38:1103–111.

38. Sezer K, Keskin M. Role of the free oxygen radicals on the pathogenesis of the diseases. Firat University Journal of Health Science Veterinary 2014, 28 (1), 49 – 56.

39. Sen CK, Packer L, Thiol homeostasis and supplements in physical exercise. The American journal of clinical nutrition, 2000; 72(2): 653-69.

40. Omma A, Sandikci SC, Kucuksahin O, Alisik M, Erel O, Can the Thiol/Disulfide Imbalance Be a Predictor of Colchicine Resistance in Familial Mediterranean Fever? Journal of Korean medical science, 2017;32, 10: 1588-94.

41. Erel O, Neselioglu S . A novel and automated assay for thiol/disulphide homeostasis. Clinical Biochemistry 2014; 47(18): 326-332.

MSD

Medical Science and Discovery
ISSN: 2148-6832

Effect of systemic immune-inflammation index on prognostic parameters and survival in patients with breast cancer under the age of 40 years

Hacer Demir[1]*, İsmail Beypınar[2]

1 Afyon Health Sciences University School of Medicine, Department of Internal Medicine and Medical Oncology, Afyonkarahisar, TR
2 Eskişehir City Hospital, Department of Internal Medicine and Medical Oncology, Eskişehir, TR
*** Corresponding Author:** Hacer Demir **E-mail:** drhacerdemir@gmail.com

ABSTRACT

Objective: Systemic immune inflammation index, which is one of the systemic inflammatory markers obtained by using peripheral blood cells, neutrophils, lymphocyte and platelet counts, has been previously shown to be prognostic in many types of cancer, and it has been also shown in previous studies that SII was associated with prognosis in patients who received adjuvant and neoadjuvant therapy in breast cancer. In our study, the evaluation of the potential prognostic importance of SII in patients with breast cancer diagnosed before the age of 40 was aimed.

Material and method: For the study, demographic, histopathological, clinical and file data of 129 patients who were diagnosed with breast cancer in the tertiary medical oncology outpatient clinic and were 40 years old and younger at the time of diagnosis were recorded retrospectively. SII was calculated according to the neutrophil count x platelet number/lymphocyte (NxP / L) formula, and those below the optimal cut-off value obtained by ROC analysis were classified as low SII, and those above it as High SII. The relationship between breast cancer clinicopathological variables and SII was evaluated by Chi-Square test. While the effect of SII on survival was evaluated by Kaplan Meiermethod, the Logrank test was used to evaluate survival in low and high groups.

Results: For the study, 1400 patients diagnosed with breast cancer were reviewed and 129 patients who were under the age of 40 at the time of diagnosis were included. Patients who had insufficient follow-up or whose pre-treatment hemogram values could not be reached, who had medication use that could affect their hemogram parameters, and those with inflammatory diseases were not included. The median age in the study was 35, and the youngest patient was 21 years old. In the study group, based on the SII cut-off value of 720 calculated according to the roc analysis, 73 patients were in the low SII group and 56 patients were in the high SII group. When the relationship between prognostic factors of the patients and SII was examined, no statistically significant relationship was observed between age, hormone receptor status, Her-2 status, histological subtype, clinical stage, grade, Ki 67 status, lymph node involvement and SII. However, in the survival analysis, although the median value could not be reached between the two groups, there was a significant difference in overall survival with SII ($p = 0.051$) and it was observed that survival was worse in the high SII group, and the 3 and 5-year survival rates were worse in the high group compared to the low ones.

Conclusion: In our study, we reached the conclusion that SII can be an independent prognostic factor for survival in patients with breast cancer diagnosed at 40 years of age or younger. Considering the SII status together with other prognostic factors in diagnosis, a more intensive treatment plan can be made for the patients. However, well-designed prospective studies including more patients are needed for the routine use of SII.

Keywords: Breast cancer, prognosis, systemic immune inflammation index, SII

Received 18-12-2020

Accepted 15-01-2021

Available Online: 18-01-2021

Published 30-01-2021

http://dx.doi.org/10.36472/msd.v8i1.452

INTRODUCTION

Breast cancer is the most common cancer among women all over the world, ranks 2nd among cancer deaths in women. (1). In the United States, it is estimated that 279,000 patients will be diagnosed with breast cancer in 2020 and approximately 43,000 patients will die due to breast cancer (1).

Mortality rates tend to decrease due to the development of examinations that allow early diagnosis of breast cancer and the successes achieved in systemic treatment over the past years. The risk of developing breast cancer increases with age; however, although breast cancer can also develop in young women, those diagnosed before the age of 40 are 6.6% of all cases, while those under 35 years old constitute 2.4% (2).

Breast cancer has a more aggressive course in cases under the age of 40, and the tumor generally tends to be larger, higher grade, more hormone receptor (HR) negative and epidermal growth factor 2 (HER-2) being positive. In younger patients, the frequency of triple-negative (ER (-) PR (-) HER2 (-)) tumors also increases (3).

Since breast cancer is a heterogeneous tumor with different genomic subtypes, it differs in prognosis. Tumor size, stage, histological subtype, lymph node involvement, hormone receptor (HR) status, epidermal growth factor 2 (HER2) status, grade survival, and prognosis are histopathological factors used to determine.

Also, age is accepted as an independent prognostic factor in breast cancer patients (4).

However, up-to-date and reliable prognostic parameters are still needed to personalize the treatment of breast cancer patients and improve survival.

Tumor microenvironment and cancer-associated inflammation play an important role in tumor development and prognosis (5).

Tumor micro environment includes neutrophils, monocytes, lymphocytes, and platelets, and in recent years studies evaluating the prognostic effect of inflammatory biomarkers in breast cancer patients, parameters such as neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR) have been confirmed to be an independent prognostic factor (6).

The systemic immune-inflammation index (SII) is a parameter calculated using platelet, neutrophil, and lymphocyte counts, reflecting the balance between host immune and inflammation status.

SII has previously been studied in colorectal, gastric, and pancreatic cancers and has been shown to be prognostic. The prognostic effect of SII has been investigated in patients with breast cancer before neoadjuvant therapy and in the adjuvant period in various subtypes, and the results are controversial (7, 8, 9, 10).

In our study, we aimed to evaluate the relationship of SII with other prognostic parameters and the effect on survival in patients diagnosed with breast cancer before the age of 40.

MATERIAL AND METHODS

The study, which was designed retrospectively, included patients who were diagnosed with breast cancer between 2006 and 2020, aged 40 years and younger, and admitted to the Medical Oncology Outpatient Clinic. The study was initiated after obtaining approval from the ethics committee of Afyon Health Sciences University. After obtaining written consents from the patients, histopathological, clinical and file data were recorded retrospectively.

Patients who were diagnosed with confirmed breast cancer histopathologically, over the age of 18, in the age of 40 years and younger, with regular file data and regular follow-up were included in the study.

Study exclusion criteria were determined as;

1) Patients with active infections or using steroids at the time of the hemogram
2) Ductal or Lobulercarcinoma in-situ
3) Patients who do not have sufficient follow-up and file data cannot be reached
4) Those with acute or chronic inflammatory diseases
5) Those diagnosed with hematological disease
6) Without hemogram data at the time of diagnosis
7) Male breast cancer.

Patients' age, histology, tumor size, lymph node metastasis status, histological grade, ER, PR, HER-2 status, Ki-67 index, operation type and treatment characteristics were obtained from the file and by reviewing the hospital information system. SII was calculated with the formula (neutrophil x platelet / lymphocyte) using the platelet (10^3/ µL), neutrophil (10^3/ µL), and lymphocyte (10^3/ µL) counts obtained from the preoperative hemogram examinations. The SII cut-off value was calculated by performing ROC analysis. The value obtained in max sensitivity and specificity was used as the SII value cut-off value.

After the treatment was completed, the patients were followed up every 3 months for the first 2 years, every 6 months between the 2nd and 5th years, and once a year after 5 years. Disease-free survival (DFS) in patients was calculated according to the time from diagnosis until the development of first disease recurrence, Overall survival (OS) was calculated according to the date of death from any cause or the last control date.

Statistical Analysis

The SPSS v. 20.0 Software (SPSS; Chicago, IL, USA) program was used in all analyses and a p value of < 0.05 was considered statistically significant. Descriptive statistics including patient age, tumor stage, clinical presentation, histopathological type, grade, immune histochemical findings, Ki 67 status were presented as frequencies and percentages of categorical variables and means and standard deviations of quantitative variables. Chi-square or Fisher's exact tests were employed for categorical variables. The relationship between SII and pathological parameters was evaluated by Roc curves, The Kaplan Meier method was used for OS and log-rank test was used to evaluate the survival differences between patients divided into two groups according to the optimal cut-off point.

http://dx.doi.org/10.36472/msd.v8i1.452

RESULTS

For the study, 1400 patients diagnosed with breast cancer in the tertiary medical oncology outpatient clinic were reviewed and 129 patients aged 40 and under at the time of diagnosis were included. The clinic-pathological characteristics of the patients are shown in Table 1.

The median age in the study was 35, and the youngest patient was 21 years old. Median body mass index (BMI) was 26.3 (19-40), smoking history was present in 7.8% (10 patients), 14 patients (10.9%) had a family history of breast cancer and only 1 patient was in the postmenopausal period. The most common clinical presentation was a palpable mass (86%), pain was the reason for the clinical presentation in 10 patients. Breast cancer was diagnosed in 5 (3.9%) patients during the controls performed for any reason. Considering histological subtypes, 115 (89%) patients had invasive ductal carcinoma, 5 patients (3.9%) had medullary carcinoma, 2 (1.6%) patients had invasive lobular carcinoma, and 7 patients had other histological subtypes.

It was seen that the right/left breast placement (64/65) was equal. Pathologically, in immuno histochemical evaluation, 105 (81.4%) of the patients were ER (+), 97 (75 %) were PR (+) and 34 (26.4%) were HER-2 (+). The number of triple-negative patients was 17 (13.2%). When the histological grades were examined, Grade 2 (39.5%) disease was the most common. Lymphovascular invasion was present in 38.8% of the patients, the perineural invasion was detected in 19% of the patients. When evaluated according to the stages of T, the most common clinical was T2 (37.2% 48 patients) while the most common with N1 patient ratio (32% 41 patients) was the lymph node involvement. According to the AJCC 7th staging system, the ratios of stage 1/2/3 patients were 26 (21.7%) / 58 (45%) / 26 (20.2%), respectively, and 13.2% of the patients were at the metastatic stage at the time of diagnosis. The most common type of surgery performed in patients who underwent surgery was breast-conserving surgery (50.4%). Adjuvant radiotherapy (RT) and chemotherapy (CT) were applied in 73.6% of patients, and 93 patients (72.1%) were given adjuvant hormonotherapy. The most commonly used hormonotherapy was determined as tamoxifen and LHRH (61.2) treatment. Neoadjuvant therapy was given to 12 (9.3%) patients. The median Ki67 level was 30 (2-90) in 87 patients whose Ki 67 data were available, while there were 47 (54%) patients below 30 and 40 (46%) patients above 30. Recurrence was observed in 26 patients during follow-up, and recurrence/metastasis development was served in 9 patients after adjuvant therapy. While 2 patients had second primary breast cancer, only 8 patients died in the study group. The diagnostic stages of those who died consisted of stage 3 and stage 4 patients (75%) at most.

In the study group, based on the SII cut-off value of 720 calculated according to the ROC analysis, 73 patients were in the low SII group and 56 patients were in the high SII group. When the relationship between prognostic factors of the patients and SII was examined, no statistically significant relationship was observed between age, hormone receptor status, Her-2 status, histological subtype, clinical-stage, grade, Ki 67 status, lymph node involvement and SII. Although it did not reach statistical significance according to SII levels, it was observed that the patients in the higher group had more advanced clinical stage and T stage and were younger patients. (Table 2)

However, in the survival analysis, although the median value could not be reached between the two groups, there was a difference between the two groups with SII in overall survival but statistical significance could not be reached ($p = 0.051$) and it was observed that survival was worse in the SII high group (figure 1 Kaplan-Meier).

Considering the 3-year and 5-year survival rates of the patients, it was seen that it was 98% and 98% in the Low SII group, respectively, while it was 89% and 69% in the high SII group. The 3 and 5-year survival rates were worse in the high group than in the low group. (Figure 2)

Table 1. General characteristics of the study group

		Number	%
Age	≤35	78	60.5%
	>35	51	39.5%
Family history	Present	14	10.9%
	Absent	111	86.0%
Histological Type	Invasive Ductal	115	89.1%
	Invasive lobuler	2	1.6%
	Medullary	5	3.9%
	Other	7	5.4%
Breast side	Right	64	49.6%
	Left	65	50.4%
Hormone receptor status (HR)	HR +	106	82.2%
	HR -	23	17.8%
HER-2 status	Her-2 +	34	26.4%
	Her-2 -	95	73.6%
AJCC Stage at Diagnosis	I	28	21.7%
	II	58	45.0%
	III	26	20.2%
	IV	17	13.2%
Type of Surgery	BCS	65	50.4%
	MRM	54	41.9%
Adjuvant Radiotherapy	Present	95	73.6%
	Absent	32	24.8%
Adjuvant Chemotherapy	Present	95	73.6%
	Absent	34	26.4%
Recurrence	Present	26	20.2%
	Absent	100	77.5%
Grade	I	15	11.6%
	II	51	39.5%
	III	46	35.7%
T stage	T1	32	24.8%
	T2	75	58.1%
	T3	13	10.1%
	T4	5	3.9%
SII	≤720	73	56.6%
	>720	56	43.4%
Ki67	≤30	44	50.5 %
	>30	43	49.5%

BCS (breast conservative surgery), **MRM** (modified radical mastectomy)

http://dx.doi.org/10.36472/msd.v8i1.452

Table 2. Clinicopathologic characteristics of the patients according to SII groups

	Category	SII 720 (73)	SII> 720 (56)	P value
Age (n/%)	≤35	38 (59.4)	40 (61.5)	0.472
	> 35	26 (40.6)	25 (38.5)	
ER Status (n/%)	Negative	16 (25)	8 (12.3)	0.510
	Positive	48 (75)	57 (87.7)	
PR status (n/%)	Negative	20 (31.3)	12 (18.5)	0.690
	Positive	44 (68.7)	53 (81.5%)	
HER-2 status (n/%)	Negative	45 (70.3)	50 (76.9)	0.257
	Positive	19 (29.7)	15 (23.1)	
AJCC stage (n/%)	Stage I	15 (53.6)	13 (46.4)	0.680
	Stage II	38 (65.5%)	20 (34.5)	
	Stage III	15 (57.7)	11 (42.3)	
	Stage IV	5 (29.4)	12 (70.6)	
Grade (n/%)	Good	11 (73.3)	4 (26.7)	0.164
	Moderate	25 (49)	26 (51)	
	Poor	29 (63)	17 (37))	
Lymph Node Status (n/%)	N0	32 (61.5)	20 (38.5)	0.880
	N1	26 (59.1)	18 (40.9)	
	N2	8 (61.5)	5 (38.5)	
	N3	7 (50)	7 (50)	
Breast side (n/%)	Right	27 (42.2)	37 (56.9)	0.670
	Left	37 (57.8)	28 (43.1)	
Surgery Type (n/%)	BCS	39 (60.9)	26 (41.3)	0.210
	MRM	24 (37.5)	30 (47.6)	
Ki 67 Status (n/%)	≤30	19 (43.2)	25 (56.8)	0.124
	>30	28 (65.1)	15 (34.9)	
Histological Type (n/%)	Invasive ductal carcinoma	55 (85.9)	60 (92.3)	0.245
	Invasive lobular carcinoma	2 (3.1)	0 (0)	
	Other	7 (11.1)	5 (7.7)	

SII: systemicimmune-inflammatoryindex, **ER:** estrogenreceptor, **PR:** progesterone receptor, **BCC:** breast , conservative surgery, **MRM:** modified radical mastectomy,

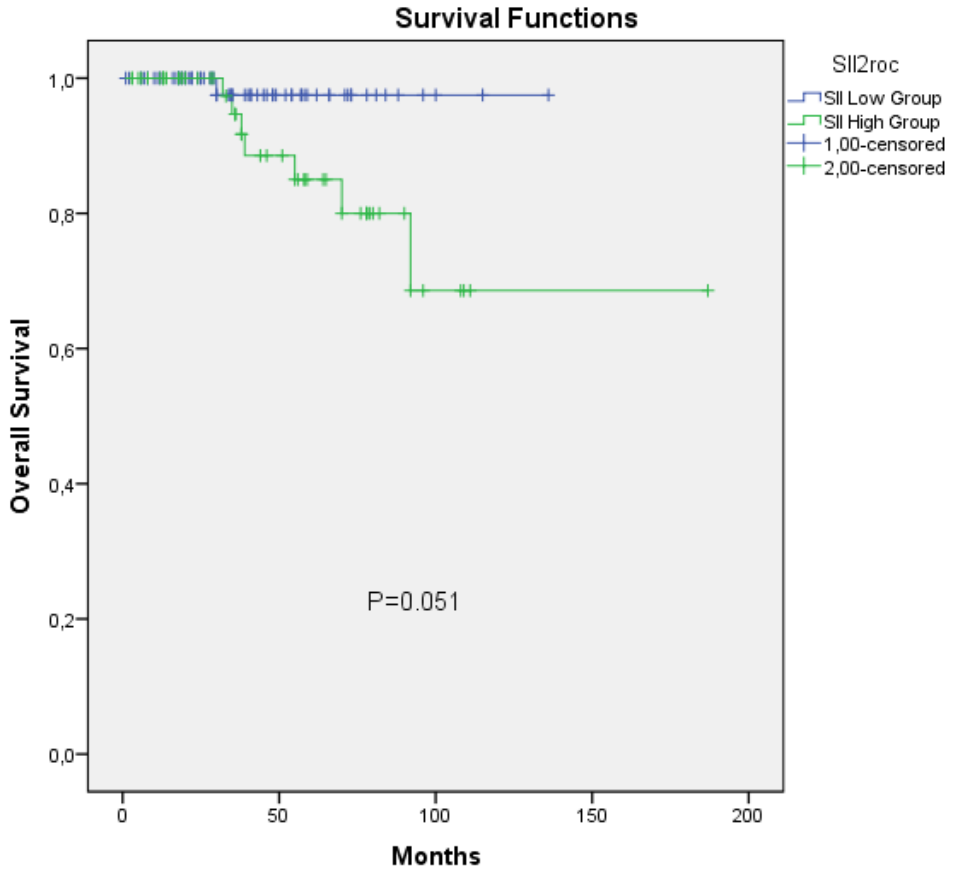


Figure 1: The effect of SII on OS in breast cancer under 40 years old female

http://dx.doi.org/10.36472/msd.v8i1.452

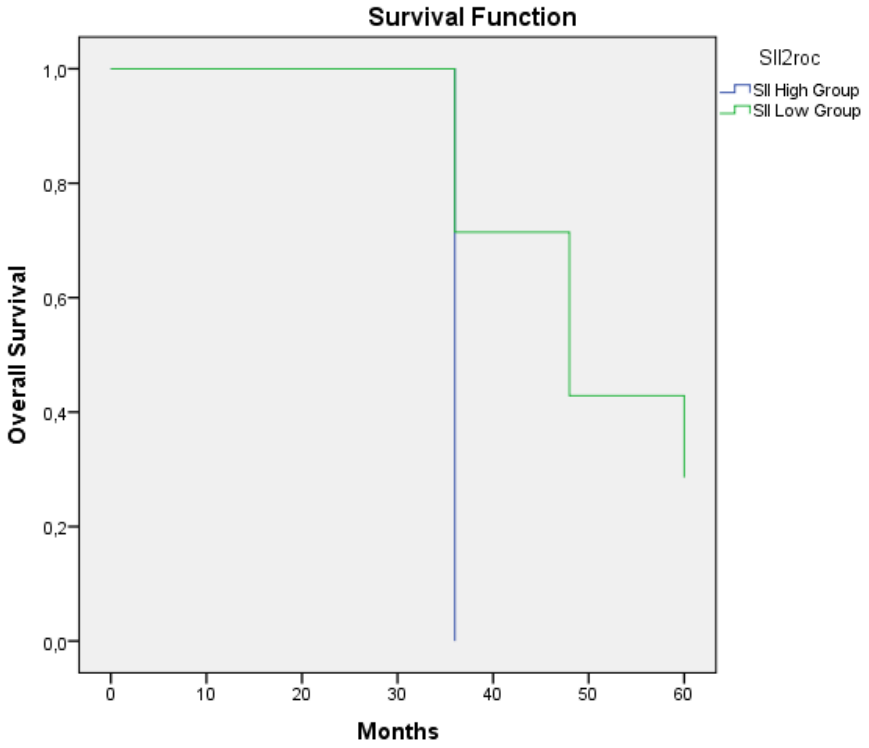


Figure 2: The 3 years and 5 years survival according to the SII groups

DISCUSSION

SII, an index based on inflammation, has previously been studied in patients with breast cancer in the adjuvant and neoadjuvant period, and according to our current knowledge, our study is the first study evaluating SII in breast cancer patients under the age of 40. In our study, we found that SII was associated with survival in breast cancer patients under the age of 40, and survival was statistically significantly worse in patients in the high SII group (P=0.051) (Figure 1).

In previous studies, it has been shown that high SII levels may be an independent prognostic factor in patients with gastric cancer, lung cancer, and hepatocellular cancer. (**11, 12, 13**)

Due to the increasing number of studies, information on the relationship between the inflammatory system and cancer is increasing. It has been confirmed in various cancer types that there was a significant relationship between pre-treatment monocyte, lymphocyte, neutrophil and platelet counts, inflammatory system and prognosis (**14, 15**). Platelets lead to tumor angiogenesis and the development of metastasis and form a shield of protection for tumor cells against antitumor immuneresponse (**16**).

Neutrophilsplay a role in the inflammatory and immuneresponse that plays a role in the proliferation and metastasis of the tumor by secreting various cytokines and inflammatory mediators (**17**). While lymphocytes have protective effects against tumor growth and metastasis, the prognosis is better in lymphocyte infiltrated tumors (**18**). Considering all of these, it is obvious that SII, a parameter determined by the use of platelets, neutrophils and lymphocytes may be prognostic in cancers.

In the study of Liu et al. on the evaluation of the prognostic effect of SII in triple-negative breast cancer patients, they have found that increased SII levels were associated with shorter disease-free survival (DFS) and overall survival (OS). They have also shown that these patients had more advanced T stages, their tumor grades had worse differentials, and Ki67 levels were statistically significantly higher (**10**).

In the study conducted by Jiang et al. with patients with Her2 (+) breast cancer who received adjuvant trastuzumab treatment, they have found that survival was significantly affected in patients with a cut off value of more than 442, which was determined according to the ROC analysis, and that these patients had shorter DFS and OS. Again, in this study, no significant relationship has been found between known prognostic factors such as ER status, tumor size, lymph node involvement and SII.

Similar results have been obtained in the study of Sun Y et al. with patients with hormonereceptor-negative Her2 (+) breast cancer (**8**).

In our study, we found that higher SII levels in young breast cancer patients were worse prognostic and although the median value in terms of OS could not be reached, it was associated with short survival, but statistical significance could not be reached difference between the two groups. The 3 and 5-year survivals were significantly shorter in the high SII groups (Figure 2).

When the relationship between previously defined prognostic factors and SII was evaluated, similar to Jiang L.'s study, no significant relationship was found in our study, however, patients in the high SII group were younger, had more

advanced T stage and AJCC clinical-stage, although they did not have statistical significance.

Chen Li et al., in their study evaluating the pre-treatment SII levels in patients receiving neoadjuvant chemotherapy, have found that patients with low SII levels had better DFS and OS times, and 3, 5, and 10-year DFS and OS times were better (9). In our study, in line with these findings, 3 and 5-year survival was better in the low SII group.

Although our study is current and has not been performed in breast cancer patients under the age of 40 before, it has many restrictions. The first is that it includes a relatively small number of patients from a single center and is retrospective, secondly, it has a short follow-up period, and the third is that all patients under the age of 40 are included. We think that more significant results can be obtained in studies when more homogeneous and more specific subgroups are included. However, the use of different cut-off values for SII in the literature creates limitations in terms of comparison with other studies. Although SII is an independent predictor in many cancers, its sensitivity and specificity are not high. Prospective randomized and well-designed studies are needed for optimization of the appropriate cut-off value.

CONCLUSION

SII can be used as an easy-to-apply and easily repeatable, inexpensive, and effective marker to show the prognosis in many cancers. Our study is the first study in which the systemic immune inflammation index (SII), which is an index based on peripheral inflammation, was evaluated in patients with breast cancer diagnosed below the age of 40, and our findings show that patients with higher SII levels at the time of diagnosis have statistically significantly worse prognosis. At the time of diagnosis, more intensive treatments can be planned by considering the SII status in addition to classical prognostic indicators in young breast cancer patients. However, to clarify the SII prognostic value, it needs to be validated in larger, multi-center clinical studies.

Acknowlegment: None

Author contributions: HD, İB; Design of the study and data collection. **HD;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7–30.

2. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A, Author SO. Breast Cancer Before Age 40 Years NIH Public Access Author Manuscript. Semin Oncol. 2009;

3. Gnerlich JL, Deshpande AD, Jeffe DB, Sweet A, White N, Margenthaler JA. Elevated Breast Cancer Mortality in Women Younger than Age 40 Years Compared with Older Women Is Attributed to Poorer Survival in Early-Stage Disease. J Am Coll Surg. 2009;

4. Lal P, Tan LK, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas. Am J Clin Pathol. 2005 Apr;123(4):541–6.

5. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol. 2017 Dec;14(12):717–34.

6. Wariss BR, de Souza Abrahão K, de Aguiar SS, Bergmann A, Thuler LCS. Effectiveness of four inflammatory markers in predicting prognosis in 2374 women with breast cancer. Maturitas. 2017;

7. Cho U, Park HS, Im SY, Yoo CY, Jung JH, Suh YJ, et al. Prognostic value of systemic inflammatory markers and development of a nomogram in breast cancer. PLoS One. 2018;13(7):e0200936.

8. Sun Y, Li W, Li A-J, Su H, Yue J, Yu J. Increased systemic immune-inflammation index independently predicts poor survival for hormone receptor-negative, HER2-positive breast cancer patients. Cancer Manag Res. 2019;11:3153–62.

9. Chen L, Kong X, Wang Z, Wang X, Fang Y, Wang J. Pre-treatment systemic immune-inflammation index is a useful prognostic indicator in patients with breast cancer undergoing neoadjuvant chemotherapy. J Cell Mol Med. 2020;

10. Liu J, Shi Z, Bai Y, Liu L, Cheng K. Prognostic significance of systemic immune-inflammation index in triple-negative breast cancer. Cancer Manag Res. 2019;11:4471–80.

11. B. H, X.-R. Y, Y. X, Y.-F. S, C. S, W. G, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clinical Cancer Research. 2014.

12. Tong YS, Tan J, Zhou XL, Song YQ, Song YJ. Systemic immune-inflammation index predicting chemoradiation resistance and poor outcome in patients with stage III non-small cell lung cancer. J Transl Med. 2017;

13. Chen L, Yan Y, Zhu L, Cong X, Li S, Song S, et al. Systemic immune–inflammation index as a useful prognostic indicator predicts survival in patients with advanced gastric cancer treated with neoadjuvant chemotherapy. Cancer Manag Res. 2017;

14. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. Journal of the National Cancer Institute. 2014.

15. Mei Z, Shi L, Wang B, Yang J, Xiao Z, Du P, et al. Prognostic role of pretreatment blood neutrophil-to-lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. Cancer Treatment Reviews. 2017.

16. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. Blood. 2015 Jul;126(5):582–8.

17. Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, et al. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med. 2010 Feb;16(2):219–23.

18. Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12 439 patients. Ann Oncol. 2014;

MSD

Medical Science and Discovery
ISSN: 2148-6832

Reflections of Covid-19 pandemic: Turkey's results during the first month of the pandemic

Huseyin Aygun[1], Suna Eraybar[1]*

1 University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa TR
* **Corresponding Author:** Suna Eraybar **E-mail:** sunaeraybar@gmail.com

ABSTRACT

Objective: This study aims to evaluate the clinical and epidemiological findings of patients with suspected COVID-19 admitted to the emergency service of a 3rd step training and research hospital.

Material and Methods: Patients older than 18 years of age, suspected COVID-19 disease and received diagnostic combined nasal and oropharyngeal swab between April 1, 2020 and April 30, 2020 were evaluated retrospectively. Demographic, laboratory, radiological findings and PCR results of the patients were recorded. In addition, the patients' home isolation, hospitalization, intensive care follow-up requirements and 28-day mortality were analyzed.

Results: Total 3020 patients were included in the study, and the mean age of the patients was found to be 41 ± 16.22. 55.4% (n = 1673) of the patients were female and 83.0% (n = 2508) of them were found to have negative PCR results. Mortality occurred in 3.5% of the patients (n = 107) within 28 days. The relationship between the PCR results, pneumonia status and type of hospitalization and 28-day mortality results were compared, and a statistically significant relationship was found. [($p<0.001$), ($p<0.001$), ($p<0.001$)].

Conclusion: In line with these data obtained at the beginning of the pandemic, positive PCR results, presence of pneumonia and the history of intensive care unit hospitalization are risk factors for mortality.

Keywords: Covid-19, coronavirus infection, pandemic, emergency

INTRODUCTION

In December 2019, the acute respiratory disease, now known as the novel coronavirus infection, emerged in Wuhan, Hubei district, China. The disease quickly spread around Wuhan (**1, 2**). On January 30, 2020, the World Health Organization recognized this rapidly spreading infectious disease as an international public health emergency, currently known as coronavirus disease 2019 (COVID-19), and then defined it as a COVID-19 pandemic on March 11, 2020 (**3**).

Coronavirus disease was firstly diagnosed on March 11, 2020 in Turkey. From this period on, the guidelines covering the viral characteristics of COVID-19 disease, patient diagnosis and treatment have been published and updated by Republic of Turkey Ministry of Health General Directorate of Public Health under the title of COVID-19 Scientific Committee Study. Follow-up and treatment schemes in our country were created in line with these guidelines.

In the period of about 1 year spent with the virus, many publications including the characteristics of the virus and the disease, as well as many studies including the differences between countries and geographical distribution have contributed to the literature. According to the data of the World Health Organization, the Covid-19 pandemic continues its current spread today with 79 million definite diagnoses and 1.7 million deaths (**4**). Clinical involvement, symptomatology, treatment protocols, and patient perspective about the disease are evaluated with the help of shared data from different continents all over the world.

Received 09-01-2021

Accepted 27-01-2021

Available Online: 28-01-2021

Published 30-01-2021

http://dx.doi.org/10.36472/msd.v8i1.461

Today, it is known that Sars-Cov2 is transmitted through droplets and causes lower respiratory tract infection that develops as a result of the increase in cytokine-mediated immune response characterized by fever, cough, and shortness of breath. Hematological examinations and Real-time PCR performed in the presence of contact with infected persons and symptoms associated with Covid provide guidance during the diagnosis phase. Despite standard supportive and antiviral treatment, according to Chinese data mortality rates in critically ill patients are still 62% in critically patients and 81% in patients who require mechanical ventilation (5).

The aim of our study is to evaluate the characteristics of patients affected by COVID-19 disease and determine the follow-up rates of patients with asymptomatic, or mild, moderate, and severe pneumonia, and to obtain epidemiological data of our country in the light of the data of a tertiary education research hospital.

MATERIAL AND METHODS

Patients older than 18 years of age who were evaluated for suspected COVID-19 disease in the emergency department of our hospital between April 1, 2020 and April 30, 2020 and who received diagnostic combined nasal and throat swab were screened retrospectively.

PCR results obtained in the emergency department at the time of first admission were evaluated and test positivity depends on this first test result.. On-control swabs of outpatients at 14th day or 3rd day follow-up swabs from hospitalized patients were not included in the study. Patients under the age of 18 and pregnant women were excluded from the study. During the planning stage, permissions were obtained from the ethics committee of our hospital (2011-KAEK-25 2020 / 05-15).

Patients' age, gender, chronic disease history, and admission complaints were recorded. Complete blood count, biochemistry, CRP, d-Dimer and ferritin values, and thorax computed tomography findings of the patients, which were made at the first admission in the emergency department, were evaluated through the patient records in the hospital automation system. Patients were divided into two groups as positive and negative according to real-time COVID PCR test results.

Also, the patients' home isolation or hospitalization status, length of stay and intensive care follow-up requirements and 28-day mortality were analyzed.

Statistical analysis: The data of the study were analyzed using the SPSS 21.0for Windows (SPSS Inc., Chicago, IL, USA) computer program. Descriptive statistics; categorical variables were shown as numbers and (%), while continuous numerical variables were expressed as mean ± standard deviation. Kolmogorov-Smirnov test was used for normality distribution of the data. Chi-square and Fisher's exact test were used to analyze whether there was a relationship between categorical variables. P <0.05 was considered statistically significant.

RESULTS

A total of 3020 patients were included in the study, and the mean age of the patients was found to be 41 ± 16.22 years. 55.4% (n = 1673) of the patients were female and 83.0% (n = 2508) of them were found to be negative for PCR results. 3.5% (n = 107) of the patients developed mortality within 28 days. According to the computerized thoracic CT findings, no typical finding was found in 75.6% of them. Typical findings such as ground glass opacity and patchy infiltration were found at only 5.6%.

While 81.5% of the patients were follow with home isolation, only 2.1% of them needed intensive care hospitalization. The need for mechanical ventilation developed in 39 (1,29 %) among 3020 patients.Clinical and demographic data of the patients are given in Table 1.

No statistically significant correlation was found in the Chi-square test analysis conducted to determine the relationship between the gender of the patients and the 28-day mortality results (p> 0.05).

The relationship between the PCR results, pneumonia status and hospitalization type and 28-day mortality results were compared and a statistically significant relationship was found respectively. [(p <0.001), (p <0.001), (p <0.001)]. It was determined that this difference was caused by those who had positive PCR results, those who had negative pneumonia and those admitted to intensive care. (Table: 2)

When the laboratory results of the patients were evaluated, the mean lymphocyte count was 2.67 in the PCR-group and 2.04 in the PCR + group. CRP, D-dimer, Ferritin and Troponin values were relatively high in PCR + group. Laboratory testing results are given in Table: 3.

Table 1: Clinical and demographic characteristics of Covid-19 patients

		Frequency (n)	Percent (%)
Sex	Female	1673	55.4
	Male	1347	44.6
Symptoms	More than 2 symptoms	1898	62.8
	Cough	377	12.5
	Weakness	54	1.8
	Shortness of breath	149	4.9
	Fever	372	12.3
	Sore throat	20	0.7
	Nausea Vomiting	39	1.3
	Fever + Cough	100	3.3
	Loss of Taste	11	0.4
PCR	Positive	512	17.0
	Negative	2508	83.0
Pneumonia	typical	170	5.6
	İntermediate	335	11.1
	Atypical	233	7.7
	Negative	2282	75.6
Final Status	Home isolation	2460	81.5
	Hospitalization	496	16.4
	İntensive care unit admissions	64	2.1
28-Day Mortality	Alive	2913	96.5
	Exitus	107	3.5
Total		3020	100.0

http://dx.doi.org/10.36472/msd.v8i1.461

Table 2: Comparison of the current status of the patients with the PCR results.

			28 day mortality		Total	Chi square test
			Alive	Exitus		
Sex	Female	n	1617	56	1673	x2=0.42, p>0.05
		%	96.7%	3.3%	100.0%	
	Male	n	1296	51	1347	
		%	96.2%	3.,8%	100.0%	
PCR	Positive	n	442	70	512	x2=185.08, p<0.001
		%	86.3%	13.7%	100,0%	
	Negative	n	2471	37	2508	
		%	98.5%	1.5%	100.0%	
Pneumonia	Typical	n	156	14	170	x2=51.30, p<0.001
		%	91.8%	8.2%	100.,0%	
	Intermediate	n	312	23	335	
		%	93.1%	6.9%	100.0%	
	Atypical	n	213	20	233	
		%	91.,4%	8.6%	100.0%	
	Negative	n	2232	50	2282	
		%	97.,8%	2.,2%	100.0%	
Admission	Home isolation	n	2435	25	2460	x2=1519.28, p<0.001
		%	99.0%	1.0%	100.,0%	
	Hospitalization	n	473	23	496	
		%	95.4%	4.6%	100.0%	
	Intensive care unit admissions	n	5	59	64	
		%	7.8%	92.2%	100.0%	
Total		n	2913	107	3020	
		%	96.5%	3.5%	100.0%	

PCR: Polymerase chain reaction

Table 3: Laboratory values of Covid-19 patients at the time of admission

	Total			**PCR(+)**			**PCR(-)**		
Variables	n	mean	Std.Dev.	n	mean	Std.Dev.	n	mean	Std.Dev.
WBC	2968	9.36	4.20	512	8.05	5.60	2456	9.63	3.66
Lymphocyte	2957	2.56	5.03	511	2.04	2.97	2446	2.67	5.36
Platelet	2969	248.38	70.06	512	221.68	75.61	2457	253.94	67.54
CRP	2870	19.21	42.45	487	29.63	54.53	2383	17.08	39.20
TROPONIN	1598	75.38	448.46	163	91.08	506.50	1435	73.59	441.55
D-DİMER	2148	0.80	3.56	187	2.06	7.32	1961	0.68	2.94
FERRİTİN	2004	123.14	203.13	137	233.15	382.32	1867	115.07	180.78

PCR: Polymerase chain reaction WBC: White blood cell, CRP: C-reactive protein

DISCUSSION

Covid-19 maintains its importance as a national public health problem despite the active struggle we carry out with the whole world today. Our study reveals our patient profile and results in the first month of the struggle against the Covid pandemic in our hospital.

Studies have shown that the most important symptoms associated with covid-19 are fever, cough and fatigue, and this was frequently followed by headache, hemoptysis and dyspnea (**6-8**). In our study, especially when the patients presented with multiple symptoms, the most common symptoms were cough (15.8%) and fever (**7-9**).

In addition to the laboratory tests that support the diagnosis in a patient presenting with Covid-19 symptoms, nasopharyngeal and oropharyngeal swab tests are the standard approach for diagnosis.

With the Real Time-PCR test, it is aimed to identify RNA-dependent RNA polymerase nucleocapsid genes belonging to Sars COV2.

However, the positivity rates of the RT-PCR test vary between 21.4% and 38% (**10, 11**). In our study, the positivity rate was found to be 17% in our patients who had typical symptoms associated with Covid, had contact with a Covid + person, or had a history of traveling abroad, and who were treated with a possible case definition with laboratory tests. This difference may have developed as a result of quantifying the probable case definition by us on a wide margin.

Typical CT findings such as peripheral and subpleural ground-glass opacities, often in the lower lobes were detected in approximately 5.6% of our patients who were evaluated for Covid-19 and scheduled for treatment, while intermediate findings were found in 11.1%. In our PCR positive patients, this rate was 91.8%, and typical pneumonic involvement was correlated with PCR results. The association of PCR positivity and the presence of typical findings in CT is observed with a rate that reaches 98% in the literature (**12**).

Fang et al. Reported that the sensitivity of thorax CT for COVID-19 was 98%, while PCR test sensitivity was 71%. In addition, due to the false negative results of RT-PCR, it was recommended to isolate the patient and repeat the PCR test result in the presence of typical BT findings (13).

Lymphopenia is accepted as a cardinal finding in Coronavirus disease, which plays an active role in the hematopoietic system. (9, 14). In the studies conducted, lymphopenia in Covid -19 patients confirmed by RT-PCR accompanies the current diagnose in approximately 80% -90% cases (9, 15). In our study, especially in the PCR positive group, the tendency for lymphopenia draws attention similar to the literature.

In addition to hematological parameters, acute phase reactants such as ferritin, CRP and Troponin, d-dimer markers indicating thrombosis are high in Covid-19 patients whose diagnosis was confirmed by RT-PCR. Ferritin is an important poor prognostic marker followed especially in patients with Covid-19. A significant relationship has been found between ferritin level and the development of Covid-associated ARDS (16, 17). Similarly, in our study, ferritin level was found to be high in the PCR positive patient population.

81.5% of our patients were isolated and treated at home with outpatient follow-up. Our intensive care hospitalization rate is 2.1%. In studies conducted in China, outpatient follow-up rates are at the level of 92.3%, and these rates indicate the need for a healthy regulation of social isolation and quarantine prevention (18).

In a study of 222 patients conducted in our country in March 2020, mortality rates were found to be 5.4%, and it was stated that this rate was lower than 6.9% worldwide (19). in our study, our 28-day mortality rate is 3.5%, and it can guide us that we can provide effective follow-up and treatment considering our number of patients and pneumonia rates.

Limitations: Our study includes the first days of the Covid 19 pandemic and includes the diagnosis, treatment and hospitalization indications and patient management used in that period. In the course of time, in line with the changes in literature, both treatment and home follow-up or hospitalization practices have changed.

CONCLUSION

In our study, we examined a period that included the first days of the Covid-19 pandemic with the data of our hospital. Thanks to the nationwide implementation of the diagnosis, treatment and isolation measures determined by the central administration of our country's healthcare system and the guidance of Covid-19, we found lower mortality and intensive care needs

The key point in the pandemic is the early diagnosis of patients with current symptomatology, contact with the patient or a history of traveling abroad, and early isolation with appropriate tests and laboratory tests. Our fight against the pandemic continues and our study can form a basis for multi-center studies that reflect our country's data.

Acknowlegments: Thanks to Melih Yuksel, MD, Assoc.Prof. For his supervision.

Author contributions: HA, SE; Design of the study and data collection. **SE;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

REFERENCES

1. Xia XY, Wu J, Liu HL, Xia H, Jia B, Huang WX. Epidemiological and initial clinical characteristics of patients with family aggregation of COVID-19. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2020;127:104360.

2. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. Jama. 2020;323(11):1061-9.

3. WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020. World Health Organization; 2020.

4. Organisation WH. Coronovirus disease (COVİD_2019) situation report. 29 December 2020.

5. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020.

6. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497-506.

7. Carlos WG, Dela Cruz CS, Cao B, Pasnick S, Jamil S. Novel Wuhan (2019-nCoV) Coronavirus. Am J Respir Crit Care Med. 2020:P7-P8.

8. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020:102433.

9. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang Y-Q, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal transduction and targeted therapy. 2020;5(1):1-3.

10. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clinica Chimica Acta. 2020.

11. Xiao AT, Tong YX, Zhang S. False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: rather than recurrence. Journal of medical virology. 2020.

12. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020:200642.

13. Long C, Xu H, Shen Q, Zhang X, Fan B, Wang C, et al. Diagnosis of the Coronavirus disease (COVID-19): rRT-PCR or CT? European journal of radiology. 2020:108961.

14. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. New England journal of medicine. 2020;382(18):1708-20.

15. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical characteristics of Covid-19 in New York city. New England Journal of Medicine. 2020.

16. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The lancet. 2020.

17. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine. 2020.

18. Qiu C, Deng Z, Xiao Q, Shu Y, Deng Y, Wang H, et al. Transmission and clinical characteristics of coronavirus disease 2019 in 104 outside-Wuhan patients, China. Journal of medical virology. 2020.

19. GÜNER HR, Hasanoğlu İ, Kayaaslan B, Aypak A, Kalem AK, Eser F, et al. COVID-19 experience of the major pandemic response center in the capital: Results of the pandemic's first month in Turkey. Turkish journal of medical sciences. 2020;50(8):1801-9.

MSD

Medical Science & Discovery

International Journal of
Medical Science and Discovery
Open Access Scientific Journal
ISSN: 2148-6832
Lycia Press LONDON U.K.
www.medscidiscovery.com

lycians

www.lycians.com