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Review Article

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PUBLISHING HISTORY

The Albanian Journal of Medical and Health Sciences (AJMHS) is an International official journal of the University of Medicine, Tirana, (Universiteti i Mjekësisë, Tiranë - UMT) in the Republic of Albania. AJMHS is a peer-reviewed open-access scientific journal, published three times a year. It publishes articles from a variety of methodologies and approaches of high scientific standard in the full spectrum of medical and health sciences.

The journal, whose history goes back to 1961, has been previously published as "Buletini i Shkencave Mjekësore i Fakultetit të Mjekësisë, Universiteti i Tiranës" (Bulletin of Medical Sciences-Faculty of Medicine, University of Tirana) and the articles were in both Albanian and English languages. In 2012, the journal, for the first time, was only published in English Language as "Bulletin of Medical Sciences". In 2014, the journal was promoted as university journal and become the official journal of the University of Medicine, Tirana, changing its previous title to "Albanian Journal of Medical and Health Sciences".

AJMHS is established to encourage scholarly publications by national and international authors and applies a rigorous peer-review system. It offers original manuscripts that provide theoretically informed empirical analyses of issues in clinical and experimental research, as well as original theoretical or conceptual analyses, in all fields of medicine, interesting case reports and clinical images, invited reviews, editorials, letters, comments and letters to the Editor including reports on publication and research ethics. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications in the Balkans as well as in the World.

A history of the Albanian Journal of Medical and Health Sciences

The history of the Albanian Journal of Medicine and Health Sciences stretches back more than a half-century. The roots of the Journal are in publication of the Bulletin of Medical Sciences, the official Journal of the Faculty of Medicine of the State University of Tirana (Buletini i Universitetit Shteteror te Tiranes, Seria e Shkencave Mjekesore: "Bulletin of the State University of Tirana, Medical Science Series"). It is thus the first published Albanian scientific medical journal and became the most prestigious scientific tribune of medical sciences in Albania. The first issue of the Journal was published in the first quarter of 1961. The first editorial board was: Fadil Spahiu (Chief Editor), Prof. Josif Adhami (Deputy Chief Editor), Prof. Selaudin Bekteshi, Petrit Gace, Bajram Preza, Dr. Përparim Tepelena, Ylli Xhagjika (members), Aleko Rapo (secretary). In addition to the Executive Editorial Board, an Editorial Scientific Board was created that consisted mainly of the members of the Scientific Council of the Faculty of Medicine.

From 1961 to 1978, the “Bulletin of Medical Sciences” was published four times a year with an average of 150 pages for each number and provided an English summary of its papers. The main sections were: 1) Clinical studies, 2) Experimental studies, 3) Review papers, 4) Criticism and bibliography, 5) Life sciences and 6) Scientific News. From 1961 to 1962 the Bulletin was also indexed at PubMed, the US based directory of medical scientific journals.

In 1964, Fadil Spahiu left the Editorial Board and the Journal was run for one year from the Deputy Chief Editor. In the second half of 1965 Dr. Hiqmet Dibra, while acting as Dean of the Faculty of Medicine, was also appointed as Editor in Chief. At that time, the Bulletin was published with 2000 copies for each number. Starting from the number 1 of the year 1978, beside the summaries in English, abstracts in French were made available for each paper as well.
In 1969 the Bulletin was run for three consecutive years from Prof. Josif Adhami, and Prof. Selaudin Bekteshi, Petrit Gace and Bajram Preza were acting as executive editorial members. In 1972, the Rector of the University of Tirana approved the new Editorial Board, with Prof. Ulvi Vehbiu as Editor in Chief and 7 members. Since 1961, the Editorial Board reviewed and published in the Bulletin also papers of foreign authors, who had submitted for publications to the Journal. Until 1974, 35 articles from 8 different countries were published. From 1975 to 1990, due to the political conservationism and closure of the Communist Party in Albania, the Bulletin was closed to submissions from foreign authors. Almost all of the editorials of the Bulletin, till 1990, had political connotations.
In 1995, Dr. Adnan Kastrati was appointed as the new Editor in Chief of the Bulletin. At that time the Bulletin appeared with an average of 3 numbers per year including both English and French summaries for each paper. In 1998, the new Editor in Chief was appointed Dr. Myftar Barbullushi. In May 2012, a new Editorial Board was created with Prof. Bashkim Resuli as Editor in Chief. In this period, the Medical Bulletin was published for the first time entirely in English. On September 2014, the Bulletin was promoted to University Journal as the Official Journal of the University of Medicine, Tirana published with a new Journal title "Albanian Journal of Medicine and Health Sciences" and a new Executive and Scientific Board leaded by the Editor-in-Chief Prof. Dr. Genc Sulcebe.
For the first time, in 2015, the Journal set up his own website, keeping in line with the actual standards of international peer reviewed journals. AJMHS also developed a new look and logo. The ownership and management of the Journal are held by the University of Medicine, Tirana and the Journal Executive and Scientific Board maintain its initial policy of free access and free submission.
This half-century history of editorial and publishing excellence has firmly established the Albanian Journal of Medicine and Health Sciences as a scientifically credible publication that is relevant to its readers. It remains committed to the aim of the Bulletin of the Medical Sciences at its foundation in the early 1961: "to build a good and useful medical journal for the progression of medical sciences".

EDITORIAL POLICY

Scope and Mission

Albanian Journal of Medical and Health Sciences *(AJMHS)* is a published three times a year, peer-reviewed open-access international journal. The journal is the official scientific publication of the University of Medicine, Tirana, Albania. The language of the journal is English.
AJMHS was founded in 1961 as "*Buletini i Shkencave Mjekësore i Fakultetit të Mjekësisë, Universiteti i Tiranës" (Bulletin of Medical Sciences-Faculty of Medicine, University of Tirana*). In 2012 the journal was published in English for the first time as "*Bulletin of Medical Sciences*". The journal's name changed to "*Albanian Journal of Medical and Health Sciences*" in 2014.
AJMHS publishes scientific articles in basic, translational, clinical and health care research, conducted in all fields of medicine and health care, as well as interesting case reports and clinical images, invited reviews, invited medical education papers, editorials, opinions and viewpoints, comments and letters to the Editor. The structure of each edition of the publication comprises section categories determined by the Editor and reflects the views of the Editorial Board.

AJMHS encourages academicians, researchers and specialists of different medical and health care fields from all over the world to publish their valuable research in all branches of medicine and health care.

The journal's aim is to publish original articles with high scientific and ethical quality.
The Editorial Board of the *AJMHS* and the Publisher adhere to the principles of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the US National Library of Medicine (NLM), the World Medical Association (WMA), the US Office of Research Integrity (ORI), the European Association of Science Editors (EASE), and the International Society of Managing and Technical Editors (ISMTE).
AJMHS permits and encourages authors to post items approved for publication from the journal on personal websites or institutional repositories both prior to and after publication, while providing bibliographic details of the publication in *AJMHS*.

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All articles are also available in PDF format on our website http://ajmhs.umed.edu.al and can be downloaded free of charge.

The *AJMHS*'s mission is to distribute and expand worldwide good quality research, focused primarily on the medical and health care problems of the South-East European and Mediterranean countries.

AJMHS is open to publication for all the authors that comply with the scientific and ethical requirements of the journal. All manuscripts submitted for publication are strictly internally and externally peer reviewed for their originality, methodology, scientific relevance, quality, ethical nature and suitability for the journal. A similarity check is performed on all manuscripts submitted. All the articles published at *AJMHS* will be fully accessed online. No submission or publishing fee is requested.

Ethics

AJMHS is committed to the highest standards of research and publication ethics. All submitted manuscripts are screened for plagiarism in order to detect instances of overlapping and similar text. The editors will act in accordance to the relevant international rules of publication and research ethics (COPE guidelines, WAME resources, WMA policies and ORI) if any ethical misconduct is suspected.

The journal recommends an approval of the research protocols by an ethics committee in accordance with international agreements "WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (last updated: October 2013, Fortaleza, Brazil)", "Guide for the care and use of laboratory animals (8th edition, 2011)" and/or "International Guiding Principles for Biomedical Research Involving Animals (2012)". This approval is required for all experimental, clinical and drug trial studies. For articles concerning experimental research on humans, a statement should be included that informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. The journal may request a copy of the Ethics Committee Approval received from the relevant authority. Informed consent must also be obtained for case reports. More details on the ethical principles of the journal may be found at the "Ethical Guidelines" and the "Instructions to Reviewers" pages. All reference for the ethical issues must be mentioned at the method section of the article.

Conflict of interest policy

The AJMHS's editorial review process is in accordance with the Good Editorial Practice set by international editorial organizations (WAME, COPE). WAME indicates that "conflict of interest exists when an author, reviewer, or editor in the publication process (submission of manuscripts, peer review, editorial decisions and communication between authors, reviewers and editors) has a competing interest that could unduly influence his or her responsibilities (academic honesty, unbiased conduct and reporting of research and integrity of decisions or judgments) in the publication process".

The AJMHS requires that each author, reviewer, and editor must disclose to the editor-in-chief any conflict of interest related to family, personal, financial, political or religious issues as well as any competing interest outlined above at the WAME's definition. Whether or not a conflict of interest and financial support exist, they must be declared at the Conflict of Interest Statement (signed and approved from all the authors) as well as at the end of the manuscripts (Conflict of Interest Statement, before the Reference Section). If a reviewer or an editor has a conflict of interest and/or believes that it is not appropriate to be a reviewer, or an editor for a given manuscript, the reviewer or the editor should resign from the assignment.

The AJMHS editorial board members may also submit their own manuscripts to the journal. However, they cannot take part at any stage on the editorial decision of their manuscripts. They will be treated like any other author and if any, final acceptance of such manuscripts can only be made by the positive recommendation of at least two external reviewers.

Authors should not contact any of the editorial executive or scientific board members during the review process. All necessary information regarding the process of a manuscript will be regularly provided from the editorial office via the official e-mail addresses. The names of the handling editor and the reviewers are not disclosed to the author(s). Due to the AJMHS's double-blinded review principles, the names of authors and reviewers are not known to each other. Please refer to the "conflict of interest statement and copyright form" section below for the conflict of interest declaration for authors. For a conflict of interest statement for reviewers, please refer to the "Instructions to Reviewers" page.

INSTRUCTIONS FOR AUTHORS

AJMHS is based on independent and unbiased double-blind and peer-reviewing principles. Only unpublished papers that are not under review for publication elsewhere can be submitted. The authors are responsible for the scientific content and the

ethical compliance of the material to be published. *AJMHS* reserves the right to request any research materials on which the paper is based. It is highly recommended that all manuscript must be checked from a native English speaker with experience in Scientific English writing. The executive editorial board is committed to a rapid publishing process. The authors will be kept informed about all the stages of the reviewing process.

Manuscript formatting

Manuscript format must follow the guidelines described below that are in accordance with the ICMJE (Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals - updated in August 2013 - http://www.icmje.org/icmje-recommendations.pdf).
The manuscript must be submitted to the following address: ajmhs.submission@gmail.com
Papers that do not comply with the format of the Journal and submission requirements will be returned to the author for correction without further review.

General Format

The manuscript should be typed in a Microsoft Word™ file, single-column format, double-spaced with 2.5 cm margins on each side, and 11-point type in Times New Roman font.
All abbreviations must be defined the first time they are used and should be displayed in parentheses after the definition. Abbreviations should be limited to those defined in the AMA Manual of Style, current edition. Authors should avoid abbreviations in the title and abstract and limit their use in the main text.
Decimal points should be used in decimals throughout the manuscript. Measurements should be reported using the metric system according to the International System of Units (SI). Consult the SI Unit Conversion Guide (New England Journal of Medicine Books, 1992). An extensive list of conversion factors can be found at:
http://www.unc.edu/~rowlett/units.
For more details, see:
http://www.amamanualofstyle.com/oso/public/jama/si_conversion_table.html.
When a drug, product, hardware, or software is mentioned within the main text product information, it should include the name of the product, the producer of the product and the city or the country of the company .It should be provided in parenthesis in the following example format: “Examination BIO-AUTO analyzer (Beckman-Coulter, New Jersey, NJ, USA)”.

Article Type

Identification of the article type is the first step of manuscript preparation and submission. The article type dictates the rules that should be followed, including formatting and word limits of the manuscript. The main categories of article types are outlined below:
Original Article: Original contributions are manuscripts containing substantial novel research. These articles can include randomized controlled trials, observational (cohort, case-control or cross-sectional) studies, diagnostic accuracy studies, systematic reviews and meta-analyses, non-randomized behavioral and public health intervention trials, experimental animal trials, or any other clinical or experimental studies. Abstracts must begin on a separate page and should not exceed 400 words. Abstracts should be structured with the following subheadings: Background, Aims, Study Design (case control study, cross-sectional study, cohort study, randomized controlled trial, diagnostic accuracy study, meta-analysis and systemic review, animal and in vitro experimentation, non-randomized study in behavioral sciences and public health, etc.), Methods, Results and Conclusion. The main text should be structured with the following subheadings: Introduction, Material and Methods, Results, Discussion, Conclusions, Acknowledgments, Conflict of Interest statement, Authorship contribution, References, Tables, and Figure Legends. The main text should not exceed 3500 words, excluding the abstract, references, tables, and figure legends. There should be a maximum of 40 references.
Short Report: Short reports or short communications are short versions of research, applications or work in progress limited to 1500 words. These articles can include clinical or laboratory work, collected case reports of scientific significance etc. Abstracts must begin on a separate page and should not exceed 250 words. Abstracts should be structured with the following subheadings: Background, Aims, Study, Methods, Results and Conclusion. The main text should be structured with the following subheadings: Introduction, Material and Methods, Results, Discussion, Conclusions, Acknowledgments, References, Tables, and Figure Legends. The main text should not exceed 1500 words, excluding the abstract, references, tables, and figure legends. There should be a maximum of 4 tables and/or figures and 15 references.

Invited Review or Medical education articles: Invited review and Medical education articles are comprehensive analyses of specific topics in

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medicine, which are written upon invitation due to the extensive experience and publications of authors on the review subjects. They can also be articles focused on clinical teaching and guidelines. All invited review articles will also undergo peer reviewing prior to acceptance. Review articles must not exceed 5000 words for the main text (excluding references, tables, and figure legends) and 400 words for the unstructured abstract. A review article can be signed by no more than 5 authors and can have no more than 60 references.

Case Report: Interesting cases demonstrating new findings can be reported. Cases should be unique, representing a diagnostic or therapeutic challenge and having a learning point for the readers. Abstracts of case reports should mainly include information about the case and should be limited to a maximum of 250 words. The abstract must begin on a separate page and should be structured with the following subheadings: Background, Case Report and Conclusion. The main text of case reports should be structured with the following subheadings: Introduction, Case Report, Discussion, Acknowledgments and References. Case reports must not exceed 1200 words (excluding references, tables, and figure legends). Case reports can be signed by no more than 5 authors and can have no more than 10 references and 3 figures or tables. It is highly recommended that the Case reporting must follow the CARE (Case Report) guidelines.

Clinical Reasoning: Clinical reasoning represents a rational thinking through the various aspects of patient care to better define the medical strategy regarding the diagnosis and/or treatment of a clinical problem in a specific patient. Conducting a physical exam, taking a medical history, ordering complementary exams and describing safe and effective treatment are necessary steps in gathering clinical data from a patient before engaging in the process of clinical reasoning. The latter represents a critical thinking process about all the important clinical information using personal skills and abilities often achieved from the experience. This article type is intended to help clinicians think differentially and take the next step which determines the best course of action to take based on what is known or what can reasonably be hypothesized from clinical data. The authors are encouraged to present clinical cases from their experience which has generated a real diagnostic dilemma. The first section, case presentation, should include the patient's complaints as well as historical and clinical data enough to present an initial differential diagnosis. The second section, complementary exams, is dedicated to pertinent and necessary complimentary examinations according to previous topographic and clinical differential diagnosis. In the third section, the authors should present all steps (surgery, biopsy, pathological exam) needed in defining the final diagnosis. A supplementary section should include an overview of the final diagnosis. The maximum lengths of the text and the references should not exceed 2000 words and 20 references, respectively. No abstract is required.

Clinical Image: The journal publishes original, interesting, and high quality clinical images having a brief explanation (maximum 500 words excluding references but including figure legends) and of educational significance. The figure legend should contain no more than 100 words. It can be signed by no more than 5 authors and can have no more than 5 references and 1 figure or table.
Any information that might identify the patient or hospital, including the date, should be removed from the image. An abstract is not required with this type of manuscripts. The main text of clinical images should be structured with the following subheadings: Case, and References.

Letter to the Editor: Letters in reference to a journal article must not exceed 500 words (excluding references). Letters not related to a journal article must also not exceed 500 words (excluding references). An abstract is not required with this type of manuscripts. A letter can be signed by no more than 4 authors and can have no more than 5 references and 1 figure or table.
Other: Editorials, reviewer commentaries, book reviews, reports on publication and research ethics, Opinions and View-Points are requested by the Editorial Board.
A summary of the article type's characteristics is given in the table below.

Article Type	Word Limit	Abstract word Limit	Reference Limit	Author Limit	Tables/figures Limit
Original Article	3500[1]	400[4]	40	None	6
Invited Review	5000[1]	400	60	5	6
Case Report	1200[1]	250[5]	10	5	3
Clinical Image	500[2]	N/A	5	5	1
Letter to the editor	500[3]	N/A	5	4	1
Clinical reasoning	2000[3]	N/A	20	5	3
Short report	1500	250[4]	15	10	4

1. This should not include the abstract, references, tables or figure legends.
2. This should include the figure legends.
3. This should not include the references.
4. Should be structured with the following subheadings: Background, Aims, Study Design, Methods, Results, and Conclusion.
5. Should be structured with the following subheadings: Background, Case Report and Conclusion.

Preparation and submission of a manuscript

All manuscripts should be submitted via email to the following address: ajmhs.submission@gmail.com
The submission should be divided into SEPARATE files in the following order:

1. Cover Letter (separate file).
2. Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form (separate signed file).
3. Manuscript (Title page, Abstract page, main text, references, tables, and figure legends).
4. Figures (if applicable).

1 - Cover Letter

The cover letter, addressed to the Editor In Chief from the corresponding author, should include: the article title and type of article he/she is submitting (for example: original article, case report, review article or clinical image). The corresponding author should briefly summarize why their work is a valuable addition to the scientific literature. Furthermore, there should be a statement that the manuscript has not already been published, accepted or under simultaneous review for publication elsewhere. AJMHS does not accept multiple submission and duplicate submission. For manuscripts that have been presented orally or as a poster, this must be stated on the title page with the date and the place of the presentation. An example of a cover letter can be found on the journal's webpage (AJMHS Cover Letter).

2 - Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form

This is a statement of scientific contributions and responsibilities of all authors. The form is available for download at the the journal's webpage. The ICMJE recommends that authorship has to be based on the following 4 criteria:
1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. 2. Drafting the work or revising it critically for important intellectual content. 3. Final approval of the version to be published. 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
A contributor should meet all four criteria to be identified as an author. If a contributor does not meet all four criteria, he/she should be acknowledged in the acknowledgements section of the manuscript. All authors must sign the corresponding declaration.
For more details please refer to the ICMJE's definition of the role of authors and contributors at: http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html.
AJMHS recommends that the author ranking in the authorship list has to follow the importance of the contribution of the individual co-authors in the study, with the exception of the last author who is generally the author group coordinator or leader and whose contribution is comparable with the first author. The authors must state in the section dedicated to the Author Contribution Form and in the main text (before the Reference section), if they have agreed for another ranking order (for example: authors A.B and C.D. have an equal contribution to this study, etc). The specific contribution of each author must be stated at the end of the manuscript, before the references.
All contributing authors must sign the **Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form** and submit it through the submission system during submission. Please see Authorship Contributions**,** Conflict of Interest Statement and Copyright form for detailed information regarding "Acknowledgement of Authorship, Exclusive Publication Statement, Conflict of Interest Statement, and Transfer of Copyright Agreement".
Please refer to "conflict of interest policy" for more information.

3 - Manuscript must contain:

Title Page (separate page)
This should include:
a - The complete manuscript title (no more than 150 characters).
b - The running head (no more than 50 characters).
c - Word counts for the abstract and text (the text word count does not include references, tables, and figure legends).
d - The number of references and the number of figures and/or tables.

e - All authors' full names.
f - Detailed affiliations and e-mail addresses (all authors should meet the ICMJE's requirements for authorship – see details at "author contribution form").
g - The name, address, telephone and fax numbers and email address of the corresponding author.
h - Key-words: (3 to 6 key-words) from the list provided in Index Medicus under "Medical Subject Heading (MeSH)".
e - Information about where and when the study has previously been presented.

Abstract Page (separate page)
Original articles, invited review articles and case reports should include an abstract in a separate page. Abstracts for original articles and short reports should be structured with the following subheadings: Background, Aims, Study design, Methods, Results, and Conclusion. Abstracts for case reports should be structured with the following subheadings: Background, Case Report, and Conclusion. Abstracts for review articles should not be structured. Clinical images, clinical reasoning, Editorials, Letters to the Editor, and Commentaries or Opinions/Viewpoints should not contain an abstract.

Main document
The main document should include the main text, acknowledgements, conflict of interest disclosure, authorship contribution description, references, tables, and figure legends, in that order.

Main text
The main text should be structured according to the article type, as described in the Article Type section above.

Acknowledgements
All contributors who do not meet the criteria for authorship (ICMJE: authorship and contributorship: http://www.icmje.org/ethical_1author.html) should be mentioned in this subheading.
Conflict of interest disclosure: The authors should disclose any potential conflict of interest.
Statement about specific author contribution at the study (including concept, design, supervision, resource, materials, data collection and/or processing, analysis and/or interpretation, literature search, writing and critical reviewing). For example: A.B (concept, design, data collection etc); B.C. (data collection, analysis, writing, reviewing etc). They should comply with ICMJE recommendations that authorship be based on the following 4 criteria: 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. 2. Drafting the work or revising it critically for important intellectual content. 3. Final approval of the version to be published. 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
A contributor should meet all four criteria to be identified as an author. If a contributor does not meet all four criteria he/she should be acknowledged in the acknowledgements section of the manuscript.

References
Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have performed the original work. Reference listings must be in accordance with ICMJE standards and numbered consecutively at the end of the manuscript in the order in which they are mentioned in the text. While citing publications, preference should be given to the latest, most up to date publications. Full papers must be clearly differentiated from abstracts presented in scientific meetings and published as supplements in scientific journals (see below: Abstract example). If an ahead of print publication is being cited the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ Medline/Pub Med (for journal abbreviations consult the List of Journals indexed for MEDLINE, published annually by NLM). When there are 6 or less authors, all authors should be listed. If there are 7 or more authors, the first 6 authors should be listed followed by "et al". In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples:
Journal article: Korini G, Kocova O, Abani L, Vikani E, Vini T. Polymorphisms of cytochrome P464 genes in three ethnic groups from Albania. Albanian J Med Health Sci 2012;29:252-60.
Book: Benon M. Ocular manipulation. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.
Book chapter: Tos M, Stangerup SE. The relationship between secretory diarrhea and nutrition. In: Mos F, Thompton J, Peitersen E, editors. Nutrition and medical treatment. Amsterdam: Kugler & Ghedini; 1989:325-30.
Abstract: Gurakar A, Elsahwi K, Akdogan M, Wright H, Nour, B, Sebastian T, et al. Asplenia and primary sclerosing cholangitis (PSC): A mere coincidence? Hepatology 2002;36:673a (abstract).
Article in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect

Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2626828/pdf/8903148.pdf.
For other reference style, please refer to "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References".

Tables
Tables should be presented within the main document and after the reference list.
All tables should be referred to within the main text and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title should be provided for all tables and the titles should be placed above the tables. Abbreviations used in the tables should be defined below the tables (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide an easy reading.

Figures and Figure Legends
Figures, graphics and photographs should be submitted as separate files (in TIFF or JPEG format). They should not be embedded in a Word document. When there are figure subunits, the subunits should be labeled in small letters (a, b, c, etc.). Thick and thin arrows, arrowheads, stars, asterisks and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures should be blind too. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process all submitted figures should be clear in resolution and large in size (minimum dimensions 100x100 mm).
Figure legends should be listed at the end of the main document. When there are figure subunits, the figure legends should be structured in the following format.
Example: Figure 1. a-c. Primary culture of choroid plexuses on day 2 after seeding of dissociated cells (×400). Nesting staining in green (a). GFAP staining in red (b). Nuclear labeling in blue and merged images (c).

Checklist
Before submission, the corresponding author should ensure that all files mentioned below meet the journal requirements:

1. A cover letter containing
- The article title and type
- A brief statement describing the novelty and importance of the work
- A statement declaring the absence or presence of a conflict of interest
- A statement that the manuscript has not been previously published or accepted for publication and is not submitted or under simultaneous review for publication elsewhere.

2. The author contribution, Transfer of Copyright Agreement and Conflict of Interest Statement Form (all in a separate signed file) is included and signed from all the authors.

3. A title page including
- Title (less than 150 characters), running title (less than 50 characters)
- Authors' affiliations and e-mail addresses, including the name of the corresponding author
- Key words: 3 to 6 key-words
- Word count for the abstract and main text
- A statement of the date and place of the meeting where the manuscript was presented orally or as a poster, if occurred.

4. Structured Abstract (on a separate page-see above)

5. Structured Main text (see above)
- Ethical approval and/or informed consent has to be mentioned in the text (Methods)
- References are in the correct format and cited sequentially in the text
- All Tables and Figures have been included and appear correctly

6. Permission for reprinted figures, tables, materials or photographs has been obtained (if available)

REVIEWING PROCESS

Revisions
When submitting a revised version of a paper, the author must submit a detailed "Response to reviewers" reporting in great detail how each issue raised by the reviewers was covered.
Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option will be automatically cancelled. If the submitting author(s) believe that additional time is required, they should request a 2-week-extension before the initial 30 day period is over.

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CONTENT:

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Knowledge, Attitude and Practices of Health Professionals in Albania Regarding Infection Prevention and Control in Healthcare Settings

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Abstract

Background: Infection Prevention and Control (IPC) in healthcare facilities constitutes an important public health issue, especially in light of the ongoing COVID-19 pandemic.

Aim: The aim of this study was to assess the level of knowledge, attitude and practices (KAP) of health professionals in Albania regarding IPC aspects in healthcare settings.

Study design: Before-and-after surveys (cross-sectional studies).

Methods: The surveys were carried out in March 2021 (before IPC training) and next in April 2021 (after IPC training) including a nationwide representative sample of 505 physicians and nurses (84 men and 421 women) working in primary health care centres and maternity services in Albania. A structured questionnaire developed by the World Health Organization was administered (in March 2021) and re-administered (in April 2021) online to all participants assessing the KAP level regarding the IPC approach employed at health facility level. Wilcoxon's signed rank test (for two related samples) was used to compare the median differences in the KAP level before and after the IPC training received by survey participants.

Results: The KAP level exhibited a significant increase after the training of health personnel compared with the KAP level before the IPC training course, including the following dimensions: the importance of the number of personnel at health facility level; fulfilment of the

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standard of one patient per bed; the importance of adequate spacing between patient beds; availability of water services at health facility; the importance of the number of toilets at health facility level; the importance of functional hand hygiene and sanitation facilities; the importance of power supply, the importance of materials for cleaning; the importance of personal protective equipment; and the importance of medical waste management, including adequate labelling (all $P<0.001$).

Conclusion: This study provides useful evidence on the KAP level of physicians and nurses in Albania regarding IPC aspects related to healthcare settings. This evidence helps in the identification of the remaining gaps and needs for further training and support of professionals in Albania at different levels of health care provision.

Keywords: Albania, before-and-after survey; epidemiology, healthcare related infections, infection prevention and control, knowledge, attitude, practices (KAP)

INTRODUCTION

After the collapse of the communist regime in 1990, Albania has undergone a substantial demographic change and epidemiologic transition (1,2). The official sources of information mainly consisting of the national Institute of Statistics (http://www.instat.gov.al/) report that the share of the older population (individuals aged 65 years and above) in Albania was 15% in January 2021 (3), a proportion which was only about 4% in 1990. This remarkable aging of the Albanian population is due to an increase in life expectancy, a significant decrease in fertility rate (1,4), as well as due to massive emigration especially of the younger population (1). As expected, this fast demographic change is reflected in a sharp increase in the share of non-communicable diseases (NCDs) (1,5). Hence, in 2018, the mortality rate (number of deaths per 100,000 people for all ages) from all NCDs combined was about 752 (793 in males vs. 711 in females). For the same year, the mortality rate from all injuries was about 28 deaths per 100,000 population (6). On the other hand, based on the information provided by the Institute for Health Metrics and Evaluation, the mortality rate from infectious diseases, maternal, neonatal and nutritional diseases in Albania in 2019 was estimated at 27 per 100,000 population. This infectious disease burden constitutes only 3% of the overall mortality, whereas in 1990 it accounted for more than 20% of the all-cause mortality in Albania (6). Nevertheless, there is no specific information neither from national sources nor from international sources about the healthcare-related mortality or burden of infectious diseases in Albania.

The World Health Organization (WHO) has recently developed a self-assessment monitoring tool about "Infection prevention and control health-care facility response for COVID-19" (7). The objective of this operational tool is to assess Infection Prevention and Control (IPC) capacities to respond to COVID-19, but also to other infections in health facilities at different levels of care such as primary, secondary, or tertiary level, including also long-term care institutions (7,8). Of note, the WHO tool has also benefited from other useful instruments suggested by the Centre for Disease Prevention and Control (CDC) in USA (9) and the European Centre for Disease Control and Prevention (ECDC) (10).

The instrument developed by WHO supports health facilities to identify, prioritize and address the gaps in IPC capacities, structures and resources in order to respond adequately to COVID-19 and other infectious diseases (7). Following the WHO guidelines and recent developments, this instrument was recently translated and adapted into the Albanian context. In March-April 2021, in line with the translation and adoption into the Albanian context of the WHO self-assessment instrument regarding IPC aspects at health facility level (11), many health professionals (both physicians and nurses) were trained on monitoring procedures that should be applied for ensuring an adequate implementation of safety measures related to IPC. The trainings

were carried out online with technical support from the University of Medicine in Tirana and technical and financial support from UNICEF, Office in Albania.

In this context, the objective of this study was to assess the level of knowledge, attitude and practices (KAP) regarding IPC aspects among health professionals working at different levels in Albania, before and after a specific training course on a wide array of IPC aspects. We hypothesized an increase in the KAP level after the IPC training among at least 80% of health professionals included in this study.

MATERIAL AND METHODS

Two cross-sectional studies were conducted in Albania during the period March-April 2021. The first survey was administered in March 2021 including a nationwide representative sample of health professionals in Albania who were subsequently trained online about different aspects regarding healthcare-related IPC. A second cross-sectional study was carried out in April 2021 in the same sample of health professionals following the IPC training course.

Of note, during the period March-April 2021, there were trained online 1593 health professionals (585 physicians and 1008 nurses) from all regions of Albania working in primary health care centres (n=1411, of whom 550 physicians and 861 nurses), or in maternity services (n=182, of whom 35 physicians and 147 nurses).

On the whole, there are 413 primary health centres in Albania in which provide services a number of 1538 family physicians, 287 specialized doctors, as well as 6864 nurses and laboratory technicians.

The surveys conducted during March-April 2021 included a representative sample of 505 health professionals (84 men and 421 women) working in primary health care centres (n=453, or 32% of the overall trained personnel), or maternity services (n=52, or 29% of the overall trained personnel) in different districts of Albania. The survey forms were sent twice (before and after the IPC training) to one-third of training participants (n=531). Of these, only 505 survey forms were completed and returned by study participants. Hence, the response rate was: 505/531=95%.

Data collection consisted of an adopted version of the Infection Prevention and Control Assessment Framework (IPCAF) developed by the WHO (11). The IPCAF consists of a structured questionnaire, which was administered online twice (through the platform JotForm: https://www.jotform.com/) to all study participants (before and after the IPC training).

Besides demographic characteristics (age, gender), job profile (position, working experience) and general characteristics of the health facilities (district, residence, type of facility), all participants were asked (before and after the IPC training) to rank in a scale ranging from 1 (little) to 10 (a lot) their opinions (indicating their KAP level) regarding the importance of different components related to an

effective IPC approach, including the following dimensions: the number of personnel at health facility level; fulfilment of the standard of one patient per bed; the importance of adequate spacing between patient beds; availability of water services at health facility; the importance of the number of toilets at health facility level; the importance of functional hand hygiene and sanitation facilities; the importance of power supply, the importance of materials for cleaning; the importance of personal protective equipment; the importance of medical waste management, including adequate labelling (11). A full version of the questionnaire administered to all study participants is presented in Appendix 1.

The survey was approved by the Scientific Committee of the national Institute of Public Health, Tirana, Albania.

Measures of central tendency (mean and median values) and dispersion (standard deviations and interquartile ranges) were calculated (before and after the IPC training) for the KAP dimensions, which were expressed as numerical terms (variables) in a scale from 1 to 10. On the other hand, frequency distributions (absolute numbers and their respective proportions) were reported for categorical variables including the availability of information materials at health facility level (Table 1), or monitoring procedures applied at health facility level for an adequate IPC approach (Table 2).

Wilcoxon's signed rank test (for two related samples) was used to compare the median differences in the KAP level before and after the IPC training received by survey participants.

A p-value of ≤ 0.05 was considered as statistically significant in all cases.

Statistical Package for Social Sciences (SPSS, version 22) was used for all the statistical analyses.

RESULTS

Mean age in the study sample was 40±11 years. About 83% of survey participants were women; around 70% worked in urban health care facilities; almost 90% worked in primary health care centres, whereas the rest worked in maternities (paediatric services); two-thirds were nurses, and one-third were physicians; mean working experience was about 15 years; almost one in four participants was the manager/director of the health facility (data not shown in the tables).

Table 1 presents the distribution of information materials available at health facilities according to survey participants. About 93% of survey participants reported availability of informational materials for an adequate hand hygiene; about 42% reported availability of informational materials about antibiotic-resistance; 72% about disinfection and sterilization; 83% about protection and safety of health personnel; about 74% reported availability of materials on proper waste management; and 64% about safe injections.

Table 1. Informational materials available at health facilities included in the survey

INFORMATIONAL MATERIALS	FREQUENCY	PERCENT
Hand hygiene	468	92.7
Antibiotic-resistance	213	42.2
Disinfection and sterilization	363	71.9
Protection and safety of health staff	417	82.6
Waste management	371	73.5
Safe injections	322	63.8
None	3	0.6
TOTAL	**505**	**100.0**

Conversely, the distribution of the monitoring procedures available at health facilities according to participants' reports was as follows (Table 2): about 58% reported hand hygiene procedures; 56% waste management procedures; 54% cleaning of rooms or other spaces at health facilities; 50% consumption of soap or alcohol solutions; 35% disinfection and sterilization of instruments, or procedures related to the change of wounds. Slightly more than half of participants (52%) reported the availability of all the aforementioned monitoring procedures, whereas further 4% of participants reported none of these procedures.

Table 2. Monitoring procedures available at health facilities included in the survey

PROCEDURE	FREQUENCY	PERCENT
Disinfection and sterilization of instruments	176	34.9
Hand hygiene	292	57.8
Consumption of soap or alcohol solution	253	50.1
Change of wounds	176	34.9
Cleaning of rooms/spaces	271	53.7
Waste management	284	56.2
All	264	52.3
None	19	3.8
TOTAL	**505**	**100.0**

Table 3 presents the distribution of KAP level before and after the IPC training received. The KAP level regarding all the items exhibited a significant increase after the training of health personnel compared with the knowledge level before the IPC training course (all p-values <0.001 according to Wilcoxon's signed rank test for comparison of two related samples). Hence, after the IPC training, participants had a better knowledge about the importance of the number of staff in the health facilities (mean scores before and after the training were respectively: 6.4±2.0 vs. 8.3±2.0, where higher scores indicating a higher level of knowledge). Similarly, after the IPC training, participants had a better knowledge about the importance of fulfilling the standard of one patient per bed in the health facilities (mean scores before and after the training were respectively: 5.4±2.0 vs. 8.3±2.4) [Table 3].

Furthermore, after the IPC training, health professionals had a better knowledge about the importance of adequate spacing between patient beds in the health facilities (mean scores before and after the training were respectively: 7.7±2.0 vs. 8.7±2.0). Also, after the IPC training, interviewees had a better knowledge about the importance of water services available at all times and of sufficient quantity in the health facilities (mean scores before and after the training were respectively: 6.5±1.0 vs. 9.2±1.2). Likewise, after the IPC training, participants had a better knowledge about the importance of the number of toilets in the health facilities (mean scores before and after the training were respectively: 5.9±1.8 vs. 8.8±2.0). In addition, after the IPC training, health staff had a better knowledge about the importance of hand hygiene stations available in the health facilities (mean scores before and after the training were respectively: 7.1±1.7 vs. 9.0±1.8) [Table 3].

Through the same lines, there was a significant improvement in the level of knowledge of the health personnel about the following additional dimensions: the importance of the energy/power supply (7.4±1.3 vs. 9.3±1.3, respectively); the importance of materials for cleaning (7.3±1.2 vs. 9.4±1.2, respectively); the importance of personal protective equipment (7.5±1.1 vs. 9.5±1.2, respectively); the importance of the number of waste collection containers (7.6±1.3 vs. 9.7±1.3, respectively); and the importance of correct labelling of waste containers in the health facilities (7.7±1.2 vs. 9.6±1.2, respectively) [Table 3].

Table 3. KAP level before and after the IPC training in a nationwide sample of health professionals in Albania in 2021 (N=505)

KAP QUESTION*	Before IPC training		After IPC training		P†
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
How important do you consider the **number of personnel** in your health facility?	6.4±2.0	7 (5-8)	8.3±2.0	9 (7-10)	<0.001
How important do you consider the standard of **one patient per bed** fulfilled in your facility?	5.4±2.0	7 (5-7)	8.3±2.4	9 (7-10)	<0.001
How important do you consider **adequate spacing** (of >1 meter) between patient beds in your facility?	7.7±2.0	9 (7-9)	8.7±2.0	10 (8-10)	<0.001
How important do you consider **water services** at all times and of sufficient quantity in your facility?	6.5±1.0	7 (7-8)	9.2±1.2	10 (8-10)	<0.001
How important do you consider the **number of toilets** at your health facility?	5.9±1.8	7 (5-7)	8.8±2.0	9(8-10)	<0.001
How important do you consider the functioning **hand hygiene stations** at your health facility?	7.1±1.7	8 (6-8)	9.0±1.8	10 (8-10)	<0.001
How important do you consider the **energy/power supply** at your health facility?	7.4±1.3	8 (7-8)	9.3±1.3	9 (8-10)	<0.001
How important do you consider the **materials for cleaning** at your health facility?	7.3±1.2	8 (7-9)	9.4±1.2	10 (9-10)	<0.001
How important do you consider the **personal protective equipment** at your health facility?	7.5±1.1	8 (7-8)	9.5±1.2	10 (9-10)	<0.001
How important do you consider the functional **waste collection containers** at your health facility?	7.6±1.3	8 (6-8)	9.7 ±1.3	10 (8-10)	<0.001
How important do you consider the waste collection containers **labelled** according to their content?	7.7±1.2	8 (7-8)	9.6±1.2	9 (9-10)	<0.001

* For all questions presented in the table, participants were asked to rank their opinions/perceptions in a scale ranging from 1 (little) to 10 (a lot).

†P-values for comparison of median differences by use of Wilcoxon's signed rank test (for two related samples). SD = standard deviation; IQR = interquartile range.

DISCUSSION

This KAP study (before-and-after survey) was carried out in a national sample of physicians and nurses in Albania working in primary health care services and maternity services. Main findings of this before-and-after survey consist of a significant increase in the KAP level in more than 80% of the health personnel trained about IPC issues. In particular, participants' KAP level indicated a considerable increase after the training in terms of the following IPC dimensions: the importance of the number of personnel at health facility level; fulfilment of the standard of one patient per bed; the importance of adequate spacing between patient beds; availability of water services at health facility; the importance of the number of toilets at health facility level; the importance of functional hand hygiene and sanitation facilities; the importance of power supply, the importance of materials for cleaning; the importance of personal protective equipment; and the importance of medical waste management, including adequate labelling.

These findings highlight the importance of the training courses on IPC in order to enable the Albanian health professionals for employing adequate and effective safety measures. Of note, as of 2018, there has been a new reform regarding the restructuring and reorganization of health care services in the Republic of Albania, following the territorial and administrative reform undertaken in 2015-16 (1,5). Thus, in the past few years, administrative and managerial tasks regarding provision of health services (public health, primary health care services, as well as hospital services) have been transferred to a new institution that is the "Operator of Health Care Services" (which has four regional branches, each of which covering several local health care units) (1,5).

The information generated from this study carried out in Albania regarding the KAP level of health professionals about IPC issues helps to identify and single out training deficits, knowledge gaps and thereby inform about the needs for future training. Based on the needs identified, priorities should be set and incorporated into future planning of health care institutions at different levels of care and in all regions of Albania.

Yet, this study may have several limitations. Firstly, generalization of the findings may be confined by the sample representativeness, notwithstanding the seemingly big and nationwide representative sample of health professionals included in this study. In addition, the issue of potential information biases cannot be excluded, regardless of the fact that the instrument of data collection consisted of a standardized international questionnaire developed by WHO (11). More importantly, associations observed in cross-sectional designs are not assumed to be causal.

CONCLUSIONS

Regardless of potential limitations, this study provides useful evidence on the KAP level of physicians and nurses in Albania regarding IPC aspects related to healthcare settings. This

evidence helps in the identification of the remaining gaps and needs for further training and support of professionals in Albania at different levels of health care provision.

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Conflicts of interest: None declared.

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Appendix 1. Questionnaire administered to health professionals

- Socio-demographic characteristics: gender; age.
- Position and job profile: position (physician vs. nurse); years of working experience; director/manager of health facility (yes vs. no).
- Characteristics of health facility: district and municipality; area (urban vs. rural areas); type (primary health care centre vs. maternity services).
- What informational materials are available at your facility for monitoring of infection control and prevention programs/measures? (circle all options that apply: materials on hand hygiene; antibiotic-resistance; disinfection and sterilization; protection and safety of health staff; waste management; safe injections; none).
- What monitoring procedures are actually available at your health facility? (circle all options that apply: disinfection and sterilization of medical instruments; hand hygiene; consumption of soap or alcohol solution; change of wounds; cleaning of rooms and other spaces; waste management; all; none).
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the number of personnel in your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the standard of one patient per bed fulfilled in your facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider adequate spacing (of >1 meter) between patient beds in your facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider water services at all times and of sufficient quantity in your facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the number of toilets at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the functioning hand hygiene stations at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the energy/power supply at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the materials for cleaning at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the personal protective equipment at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the functional waste collection containers at your health facility?
- In a scale ranging from 1 (little) to 10 (a lot): How important do you consider the waste collection containers labelled according to their content?

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The Role of Complete Blood Count Derived Inflammatory Markers in Gestational Trophoblastic Diseases

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Abstract

Background: Gestational trophoblastic disease (GTD) originates from the placenta, which can show local invasion and metastasis.

Aims: To investigate the value of the parameters of the complete blood count in our GTD patients and their usage as an inflammatory marker.

Study Design: Between January 1, 2016 and December 31, 2019, the records of patients who were followed up with the diagnosis of Gestational Trophoblastic Disease and underwent curettages at the Okmeydani Training and Research Hospital Obstetrics and Gynecology Department, were analyzed retrospectively.

Methods: A total of 52 cases were included, including 27 partial and 25 complete mole cases. (Group 1). 62 pregnant women under 12 weeks of age (Group2) and 66 non-pregnant gynecology patients (Group3) were determined as the control group. All values in the complete blood count and Neutrophil / Lymphocyte Ratio (NLR), Platelet / Lymphocyte Ratio (PLR) values were recorded.

Results: Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) values were statistically different between the 3 groups (p.0.02 and p <0.001 respectively). RDW-CV values were statistically lower in the group 2 compared to the group 3 (p: 0.039). When RDW-SD values were compared, both group 1 and group 2 were found statistically significantly lower than the group 3 (p.0.019 and p: 0.028 respectively). There was a significant difference between the 3 groups in

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NLR values. (P = 0.006).

Conclusions: The neutrophil / lymphocyte Ratio can be evaluated as an important parameter in gestational trophoblastic patients. However, multicentered and prospective studies with a large number of patients are needed to be conducted for the routine use of these parameters.

Keywords: Blood count parameters ,Gestational trophoblastic disease, Neutrophil / Lymphocyte Ratio (NLR), Platelet / Lymphocyte Ratio (PLR)

INTRODUCTION

Gestational trophoblastic disease (GTD) forms the gynecological malignant group that originates from the placenta, which can show local invasion and metastasis, but can be provided the most cure. Within this disease group, complete hydatiform mole (CHM), partial hydatiform mole (PHM), exaggerated placental site (EPS) and placental-site nodule (PSN) are defined as premalignant disorders. Invasive mole, choriocarcinoma (CC), placenta -site trophoblastic tumor (PSTT) and epitheloid trophoblastic tumor (ETT) are malignant disorders and are also referred to as gestational trophoblastic neoplasia (GTN). The incidence of GTD differs in various parts of the world. While CHM and PHM appear 1-3 in 1000 pregnant women in North America and Europe, this rate increases in Asia and Latin America. CC, PSTT and ETT are very rare tumors and are seen in 1 in 50000 pregnant women (1,2).

Maternal age, menarche age, previous history of molar pregnancy, genetic factors, parity, socioeconomic status, malnutrition, infections, and oral contraceptive usage are all defined as possible risk factors (3,4).

In recent years, MPV (Mean Platelet Volume), Platelet Distribution Width (PDW), Platelet Count (PC) and Platelet Crit (PCT) Neutrophil / Lymphocyte Ratio (NLR), Platelet / Lymphocyte Ratio (PLR), Red Cell Distribution Width (RDW) have been used as diagnostic markers in many inflammatory diseases (5,6).

The aim of our study is to investigate the value of the accessible parameters of the complete blood count in our GTD patients and their usage as inflammatory markers.

MATERIAL AND METHODS

Between January 1, 2016 and December 31, 2019, the records of patients who were followed up with the diagnosis of Gestational Trophoblastic Disease and underwent curettages at the Okmeydani Training and Research Hospital Obstetrics and Gynecology Department, were analyzed retrospectively. During this 4-year period, 30 partial moles, 26 complete moles and 1 choriocarcinoma cases were followed up and treated in our clinic. However, a total of 52 cases, including 27 partial and 25 complete mole cases of whose records can all be accessed completely, were included in our study (Group 1). In addition, the choriocarcinoma case was excluded because it may affect the results since it was a malignant case. Age, gravidity, parity and abortus numbers, gestational weeks, demographic information and complete blood count at the time of diagnosis were recorded. 62 pregnant women under 12 weeks of age (Group 2) and 66 non-pregnant gynecology patients (Group 3) who applied to our outpatient clinic on the same dates were determined as the control group and complete blood count values of these patients were also examined.

Patients with any systemic disorder, acute or chronic inflammatory disease, any previous history of hematopoietic system disease, history of malignancy or drug use that may affect the blood count were excluded from the study.

The diagnosis of GTD was confirmed with pathological results. The blood test was taken into EDTA (potassium ethylenediaminetetraacetic acid) tubes and examined within two hours. In GTD patients, blood was collected before any therapeutic intervention was made after hospitalization.

Neutrophil / Lymphocyte Ratio (NLR) was calculated by dividing absolute neutrophil count to absolute lymphocyte count, and Platelet / Lymphocyte Ratio (PLR) was calculated by dividing absolute platelet count to absolute lymphocyte count.

All information obtained was entered into a statistical package for the social sciences, version 25.0, SPSS Inc, Chicago, Illinois, USA (SPSS). Descriptive statistics were used to calculate the frequency (n), percentage (%), central tendency (mean, median&mode) and dispersion (range, variance, SD, maximum & minimum) for each variable when appropriate. Continuous data was evaluated by the Kolmogorov-Smirnov test for normal distribution. According to the result of the Kolmogorov Smirnov test, the difference between the groups were investigated by Student t test or Mann Whitney U test. In the triple comparison we used the Kruskal Wallis test with pairwise comparisons after Bonferroni corrections if statistically significant. A p-value<0.05 has been considered statistically significant.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Health Sciences, Okmeydanı Training and Research Hospital Prof.Dr. Cemil Tascıoglu (Date 23/06/2020/No 48670771-514.10-243).

RESULTS

Between January 1, 2016 and December 31, 2019, 11873 deliveries took place in our clinic and the incidence of molar pregnancy was found to be 4.37 / 1000. Of the total 52 molar pregnancy cases, 27 were partial (51.9%) and 25 (48.1%) were complete hydatiform moles.

When the age distribution of the group 1 (the mole group) included in the study was examined; mean age was found as 29.52 ± 8.603. The parity of 11.5% of the patients was 4 and above (Table 1).

In the study, the mean age of the patients who were diagnosed with partial moles was 29.59 ± 7.732, and the mean age of the patients who were diagnosed with complete moles was 29.44 ± 9.618 (P = 0.595 There was no significant difference in the number of gravidity, parity and abortus (Table 2).

Table 1: Demographic characteristics of gestational trophoblastic disease

		n	%
Age (Year)	15-19	6	11,5
	20-24	9	17,3
	25-29	16	30,8
	30-34	9	17,3
	35-39	5	9,6
	40-44	2	3,8
	45-49	5	9,6
Gravida	1	16	30,8
	2	11	21,2
	3	13	25
	4 and above	12	23
Parity	0	18	34,6
	1	16	30,8
	2	11	21,2
	3	1	1,9
	4 and above	6	11,5
Abortion	0	40	76,9
	1	6	11,5
	2	1	1,9
	3	3	5,8
	4 and above	2	3,9
O Rh	+	15	28,8
	-	0	0
A Rh	+	17	32,7
	-	6	11,5
B Rh	+	7	13,5
	-	1	1,9
AB Rh	+	6	11,5
	-	0	0
Pathology Report	Complete	25	48,1
	Partial	27	51,9

Table 2: Comparisons of partial and complete molar pregnancies

	Partial	Complete	*p*
Age	29,59 ± 7,732	29,44 ± 7,618	0,595
Gravida	2 (1-14)	2 (1-10)	0,706
Parity	1 (0-5)	1 (0-8)	0,142
Abortion	0 (0-12)	0 (0-6)	0,264
Week of pregnancy	8,83 ± 2,335	8,7 ± 2,259	0,57

The mean age was 28.73 ± 5.926 in the healthy pregnant group (group 2), and the mean age was 35.41 ± 9.79 in the gynecology patient group (group 3). When the demographic characteristics of molar pregnancy (group 1) and healthy pregnancy under 12 weeks (group 2) were compared, there was no statistically significant difference in the mean age (p: 0.882). Gravidity mean was 2 (1-6) (min-max), parity mean was 1 (0-3) (min-max), abortus mean was 0 (0-3) (min-max), pregnancy week mean was 8.44 ± 2.199 in healthy pregnant group. There was no significant difference between group 1 and group 2 on gravidity, parity, abortus and gestational weeks. (p.0.179, p: 0.209, p.0.751, p: 0.694 respectively)

Complete blood count results of 180 patients with inclusion criteria are shown in table-3 as "mean ± SD" values.

Pairwise comparisons of the variables after the Bonferroni correction in statistically significant triplet comparisons are shown in table 4.

There was no statistically significant difference between the 3 groups in White Blood Cell (WBC), Red Blood Cell (RBC), Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Crit (PCT), Platelet Distribution Width (PDW) values. In contrast, Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) values were statistically different (p.0.02 and p <0.001 respectively). While P.0.034 was detected between Group 1 and Group 3 in MCH; the values were found as p <0.001 between Group 1 and Group 3, and p <0.001 between Group 2 and Group 3 in MCHC.

There were statistically significant differences between 3 groups in Red Cell Distribution Width Coefficient of Variation (RDW-CV) and Standard Deviation (RDW-SD) values. (P: 0.028, p: 0.008 respectively). When the subgroups were examined, RDW-CV values were statistically lower ingroup 2 patients compared to the group 3 (p: 0.039). When RDW-SD values were compared, both group 1 and group 2 were found to be statistically significantly lower than the group 3 (p.0:019 and p: 0.028 respectively).

Table 3: Comparisons of blood count parameters among mole hydatidiform, non-mole pregnancy and gynecology groups

	Group 1	Group 2	Group 3	*p*
	n=52	*n=62*	*n=66*	
WBC (10^3/µl)	9,09 ± 3,15	8,49 ± 2,32	7,94 ± 2,29	0,051
RBC (10^6/µl)	4,36 ± 0,47	4,36 ± 0,33	4,45 ± 0,33	0,343
HGB	12,45 ± 1,307	12,25 ± 1,098	11,97 ± 1,56	0,243
HCT (%)	36,93 ± 3,61	41,47 ± 38,89	36,62 ± 3,95	0,852
MCV	84,805 ± 5,23	83,73 ± 5,96	82,55 ± 7,81	0,553
MCH (pg)	28,58 ± 2,01	36,89 ± 48,42	27,139 ± 3,56	0,02*
MCHC (g/dL)	33,69 ± 1,008	33,57 ± 1,05	32,68 ± 1,69	<0,00
PLT (10^3/µl)	244,15 ± 53,72	257,26 ± 57,79	273,12 ± 77,806	0,138
MPV	11,46 ± 10,74	9,6 ± 2,004	10,075 ± 1,02	0,357
PCT	0,244 ± 0,506	0,39 ± 1,16	0,2715 ± 0,706	0,111
PDW	15,22 ± 1,801	17,99 ± 19,01	15,06 ± 1,92	0,44
RDW-CV (%)	13,68 ± 1,08	13,79 ± 1,6	14,49 ± 1,87	0,028
RDW-SD	40,703 ± 3,46	41,08 ± 4,02	42,85 ± 4,17	0,008
NEU (%)	67,13 ± 8,38	65,92 ± 8,07	60,77 ± 11,68	0,001
NEU (10^3/µl)	7,0019 ± 5,62	5,78 ± 1,87	5,03 ± 2,09	0,001
LYM (%)	25,18 ± 7,24	26,38 ± 7,33	29,47 ± 9,34	0,014
LYM (10^3/µl)	2,18 ± 0,63	2,19 ± 0,59	2,25 ± 0,76	0,964
NLR	3,0637 ± 1,64	2,8115 ± 1,204	2,52 ± 1,601	0,006
PLR	122,32 ± 42,29	122,96 ± 36,82	133,44 ± 55,38	0,696

**: statistically significant*

Table 4: Pairwise comparisons of the variable after Bonferroni correction in statistically significant triplet comparisons

					Group 3				
		MCH(pg)	MCHC(g/dL)	RDW-CV(%)	RDW-SD	NEU(%)	NEU(10^3/µl)	LYM(%)	NLR
Group 1	MCH(pg)	0,034							
	MCHC(g/dL)		<0,001						
	RDW-CV(%)								
	RDW-SD				0,019				
	NEU(%)					0,002			
	NEU(10^3/µl)						0,001		
	LYM(%)							0,015	
	NLR								0,007
Group 2	MCH(pg)								
	MCHC(g/dL)		<0,001						
	RDW-CV(%)			0,039					
	RDW-SD				0,028				
	NEU(%)					0,017			
	NEU(10^3/µl)						0,03		
	LYM(%)								
	NLR								

A statistically significant difference was found between neutrophil numbers and percentages among the 3 groups (p <0.001). When group 1 and group 3 were compared, the number and percentage of the neutrophils were significantly higher in group 1 than group 3 (p.0.001 and p: 0.002 respectively). Likewise, the number and percentage values were higher in group 2 compared to group 3. (p: 0.03 and p: 0.017 respectively). When we look at the lymphocyte percentages, there was a significant difference between the 3 groups. (P = 0.014). Group 1 lymphocyte percentage values were statistically significantly lower than group 3. (P = 0.015). While there was no statistically significant difference in platelet / lymphocyte ratio (PLR) values, there was a significant difference between the 3 groups in Neutrophil / lymphocyte ratio (NLR) values. (P = 0.006). NLR values in group 1 were significantly higher than the group 3. (P = 0.007).

DISCUSSION

The incidence of molar pregnancy differs between countries. In a comprehensive study by Matsui et al., they reported that the incidence decreased in Japan over the years and was 1.65 per 1000 live births in 2000 (7).

In our study, this rate was found to be 4.37 / 1000. Although complete moles are seen more frequently in many studies, the ratio of CHM in a British study was found to be 1 / 1423 and the

ratio of PHM was found to be 1 / 1058 (8). In our cases, the PHM rate was 2.27 per 1000 live births, and the CHM rate was 2.1 per 1000 live births.

When the average age range of the patients in the mole group in our study was examined; it was determined that the highest rate was 30.8% with the age range of 25-29, 11.5% were below 19 years old and 13.4% were above 40 years old. Compared to the other age groups, gestational trophoblastic diseases were more common in the reproductive age group. In the study of Lurain et al., it has been reported that the risk of gestational trophoblastic diseases increases 1.5 times under the age of 20 and 5.2 times over the age of 40 (9).

The incidence of hydatiform moles increased in early and late fertile pregnancies. The number of parities has no place among the risk factors of molar pregnancy (2,10). In the study of Bagshwe et al., they reported that blood typeA was more common in molar pregnancy (11).

In our study, in accordance with the literature, blood type A was found to be significantly higher, at 44.2%.

A complete mole may present with vaginal bleeding, larger uterus for the gestational age, hypertension and hyperemesis. In our cases, vaginal bleeding and larger than expected uterus were the most important findings. In partial moles, as in our cases, missed or incomplete abortus findings are more common and it can be diagnosed with the curettage material. In all cases, considering the risk of recurrence, weekly follow-ups were performed until serum ß-HCG values decreased to normal, and three times weekly follow-up after normalization, and then serum ß-HCG levels were followed for one year by monthly checks. Patients were offered contraception for approximately a year.

Leukocytosis is an expected finding in a healthy pregnancy. In our study, the number of leukocytes was significantly higher in molar and healthy pregnancies compared to the gynecological patient group. But there was no statistically significant difference. (P: 0.051). Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) in our study were also significantly higher in molar and healthy pregnancies than in the gynecological patient group. However, this increase may be secondary to the changes in pregnancy-related blood parameters and may not be clinically relevant.

Another indicator of platelet activation is the Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Crit (PCT) in the complete blood count. The increase in these values is associated with ongoing inflammation (12,13,14).

Compared to healthy pregnancies, an increase in leukocyte, MPV and PDV values is observed in cases of preeclampsia with increased inflammatory response and abnormal placental invasion. Preeclampsia and hyperemesis gravidarum are also expected findings in GTD. Dilutional thrombocytopenia due to increased intravascular volume is observed in

healthy pregnant women, and a compensatory increase in MPV can be observed (15).

In our study, MPV values were found to be higher in molar pregnancy compared to healthy pregnancy and gynecology patient group, but no statistically significant difference was found. In the study of Eskicioglu et al., Leukocyte count and PDW values were found to be significantly lower in molarpregnancy than in the healthy control pregnancy group. There was no difference in PLT and MPV values (16).

Especially due to the lack of cytotrophoblast invasion which is usually seen in complete molar pregnancy; the relationship between leukocyte values and GTD can be observed. There are very few studies on this subject, and most of the studies have examined platelet counts more than CBC (complete blood count) parameters (17,18). RDW (Red Cell Distribution Width), another marker used in the clinic, is a parameter that shows the distribution of erythrocyte volume in hemogram examinations. There are many conditions in which the width of the distribution of erythrocytes is clinically related, apart from anemia. RDW values increase as a result of defective erythropoiesis, increased inflammation or hemolysis. In pregnant women, RDW does not change much between 16-34 weeks and remains stable (19,20).

In our study, the gestational week was the first trimester in both molar and healthy pregnancy groups. The RDW values were found to be statistically low in these two groups compared to the gynecological patient group.

Recently, studies with markers that show systemic inflammation in the peripheral blood such as Neutrophil / lymphocyte ratio (NLR), Platelet / lymphocyte ratio (PLR) and are easily obtained by simple complete blood count (hemogram) tests attract attention. The high value of Neutrophil / lymphocyte ratio (NLR) is related to the increased neutrophil count in value because of increased inflammation; on the other hand, the low lymphocyte count is related to a defect in a patient's general health condition, increased cortisol levels because of physiological stress and increased apoptosis (21).

NLR and PLR values, which are thought to reflect systemic inflammation, are studied in many diseases. It has been reported that high NLR and PLR values show increased inflammation; and are associated with worsening renal function in diabetic patients, increased mortality in malignancy patients, and poor prognosis in cardiovascular disease patients (22). In our study when molar pregnancy, healthy pregnancy and gynecological patients were compared, NLR values were statistically significantly higher in molar pregnancies. This increase is also present in healthy pregnancies. However, especially when comparing molar pregnancy and gynecological patient groups, NLR value was found much higher (p: 0.007). There was no statistical difference in PLR values. In a study of Guzel et al., invasive and non-invasive GTDs were compared and it was found that the rate of NLR increased significantly in the invasive group (23).

Increased neutrophil levels inhibit lymphocyte activity and stimulate lymphopenia by increasing lymphocyte apoptosis (24).

In our study, although the neutrophil count and percentage were significantly higher in both the molar and healthy pregnancy groups, the percentage of lymphocytes was significantly lower compared to the gynecological patient group in the molar pregnancy patient group. (P: 0.015). In cases such as inflammation and malignancy, the immune response of circulating leukocytes causes an increase in the number of neutrophils and a decrease in the number of lymphocytes (25). In addition, the growth factor and interleukins cause neutrophil accumulation, and an increase in the number of neutrophils is also observed in solid tumors (26,27).

CONCLUSIONS

There are a small number of studies examining inflammatory markers in complete blood count parameters in relation to gestational trophoblastic patients. Although a significant difference was observed between the molar pregnancy group and the gynecological patient group in some parameters in our work, no statistical difference was found between the molar and the healthy pregnancy groups.

Easily accessible inflammation markers in the complete blood count are also important in Gestational Trophoblastic Diseases. Specifically, the neutrophil / lymphocyte ratio can be considered as an important parameter for diseases in which systemic inflammation is evident. However, multicentered and prospective studies with a large number of patients must be conducted to routinely use these parameters.

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High-Performance Liquid Chromatography Method Appropriate for the Determination of Mycophenolic Acid in Renal Transplantation

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Abstract

Background: Mycophenolate mofetil and mycophenolate sodium, both produgs of the active metabolite mycophenolic acid, are immunosuppressive agents used in transplantation for the prevention of acute rejection. The inter-patient variability in mycophenolic acid exposure is wide compared with the therapeutic window. Therapeutic drug monitoring for mycophenolic acid levels in renal transplantation has been suggested to optimize outcomes by reducing rejection or drug related toxicities.

Aim: The aim of this study is to validate a simple, rapid and sensitive high-performance liquid chromatography method combined with protein precipitation for the determination of the concentration of mycophenolic acid in human plasma.

Method: HPLC analysis was carried out using the chromatographic system Agilent Technologies 1200 DAD. Precipitation of plasma proteins was performed by the addition of acetonitrile. Samples were injected manually and the compounds were separated on a Lichrosphere select B C18 analytical column (particle size 5μm). The mobile phase consisted of 5:55 (v/v) acetonitrile-buffer phosphate adjusted at pH 2.5, flow rate was 1.0mL/min and column temperature was kept at 30°C. Detection was performed at 215nm. Naproxen was used as

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internal standard. Inter-day and intra-day precision and accuracy were evaluated from the analysis of control samples (low QC of 1 μg/ml, medium QC of 5 μg/ml and high QC of 10 μg/ml) measured on five different days. The precision and accuracy of this HPLC assay were estimated.

Results: The proposed method showed appropriate linearity for mycophenolic acid (MPA) with correlation coefficient greater than ($r2 > 0.999$). The precision and accuracy of intra-day and inter-day of this HPLC assay is suitable for routine therapeutic drug monitoring applications. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be respectively 0.1 μg/ml and 0.4 μg/ml.

Conclusion: This HPLC-UV method for the determination of MPA concentration in human plasma is simple and suitable to be used for therapeutic monitoring. The method was intended to be applied in the analysis of human plasma from renal transplanted patients followed at the University Hospital Center "Mother Theresa" in Tirana, Albania.

Keywords: mycophenolic acid, HPLC method, renal transplantation

INTRODUCTION

Mycophenolate mofetil (MMF) is an immunosuppressive drug used to prevent rejection following solid organ transplantation. MMF is used at fixed-dose regimen of 1 g per os twice daily in renal allograft recipients in association with cyclosporine, tacrolimus and steroids (1). The prodrug mycophenolate mofetil (Figure 1) is rapidly and completely absorbed and hydrolysed to the active compound mycophenolic acid (MPA). The free fraction of the highly protein bound MPA (97%) is thought to be responsible for the immunosuppresive effect (2). To prevent gastrointestinal adverse events, which are frequently seen during MMF treatment, enteric-coated mycophenolate sodium (EC-MPS, Myfortic ®) was developed. EC-MPS and MMF are both prodrugs of MPA that showed similar efficacy and safety profiles and are alternatively used as immunosuppressive agents in de novo and stable kidney transplantation recipients (3). MPA potently, selectively, and reversibly inhibits inosine monophosphate dehydrogenase (IMPDH) and therefore inhibits the de novo pathway of purine synthesis in T and B cells (T and B lymphocytes) (4,5). MPA is primarily metabolized by glucuronidation of the phenolic hydroxy group by uridine diphosphate - glucuronosyltransferases (UGTs) to an inactive mycophenolic acid glucuronide (MPAG), which is the major urinary excretion product of MPA (6).

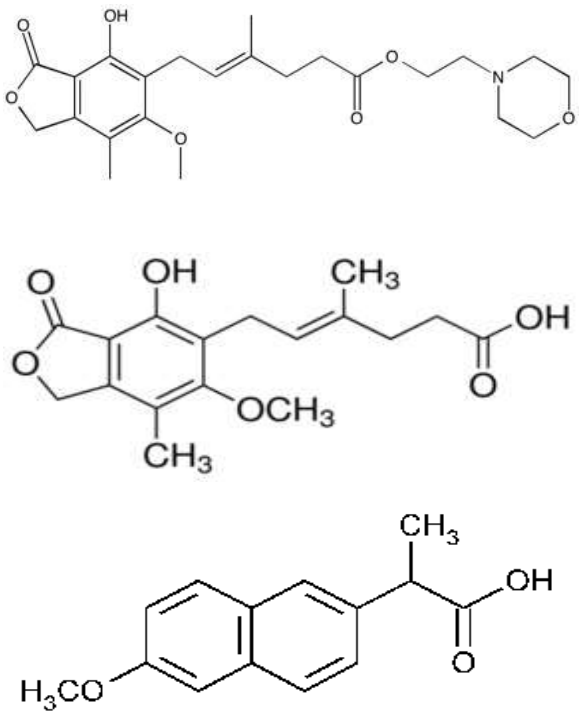


Figure 1. Structures of mycophenolate mofetil, MMF (a), mycophenolic acid, MPA (b), and naproxen internal standard, IS (c)

The inter-patient variability in MPA exposure is wide compared with the therapeutic window (7) and is influenced by many factors like coadministration of cyclosporine, low plasma albumin levels and impaired renal function (8). Furthermore, MMF can produce hematologic and/or gastro-intestinal toxicity. Therapeutic drug monitoring (TDM) for mycophenolic acid (MPA) levels in renal transplantation by using different analytical methods has been suggested to optimize outcomes by reducing rejection or drug related toxicities (9).

Several gas chromatographic and HPLC methods have been developed for the determination of MPA. According to the current literature, HPLC-

UV (10-17) and HPLC-MC (18-23) methods in combination with protein precipitation were described for the determination of MPA and its metabolites in human plasma, serum, urine, saliva and microsomal incubations. HPLC-MS methods offer better sensitivity in comparison with HPLC-UV methods. However, HPLC-MS equipment is costly and HPLC-UV methods are thus more commonly used in clinical practice.

The proposed HPLC-UV method combined with protein precipitation is rapid and suitable to be used for the determination of MPA in human plasma. The developed method is based on simple sample preparation that can be performed in every laboratory.

The aim of the present study was to validate the proposed HPLC method in order to be applicable for therapeutic monitoring of MPA in human plasma.

MATERIALS AND METHODS

Reagents and Chemicals MPA (6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phtalanyl) -4-methyl-4-hexenoic acid) was kindly provided by the representative office of the pharmaceutical company Novartis (Tirana, Albania). Solid Naproxen was used as an internal standard.

Acetonitrile HPLC grade was purchased from Sigma-Aldrich.

Ortho-phosphoric acid analytical reagent to reach a certain pH suitable for HPLC grade.

HPLC system HPLC analysis was carried out using the chromatographic system Agilent Technologies 1200 DAD equipped with on-line degasser, binary pump, column oven and photo diode array detector. The HPLC column used was Lichrosphere select B C18 analytical column (particle size 5μm) eqquiped with a precolumn guard. The water used for chromatography was previously purified. Before use, the mobile phase was degassed and purified by vacuum filtration through 0.45μm Millipore filters. 20 μL of the sample was injected manually into the chromatographic system Agilent Technologies 1200 DAD. Data were collected from the Agilent's ChemStation software. Statystical analysis was carried out by using the Microsoft Excel software.

Chromatographic conditions The mobile phase was 45:55 (v/v) acetonitrile-buffer phosphate. Buffer phospate (pH equal to 2.5 adjusted with 1 M ortho-phosphoric acid). The flow rate was 1.0 mL/min and column temperature was set at 30°C. Detection was performed at 215 nm.

Sample preparation A 200 μL aliquot of blank plasma was transferred in a tube and was spiked with working standard solutions of MPA, followed by addition of 10 μL of naproxen (from a solution of 0.5mg/ml in acetonitrile) as internal standard and 400 μL of acetonitrile (v/v) as a protein precipitating agent. Each tube was vortex mixed for 30 sec and then centrifuged for 10 min at 10000 rpm. 20 μL of the supernatant was injected into the HPLC system for analysis.

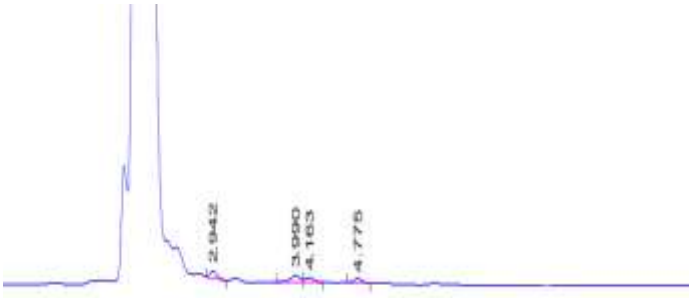
Calibration curve Stock solution for the construction of the standard curve of MPA was prepared by dissolving the MPA in methanol to yield concentration of 1.0 mg/ml. Working standard solutions of MPA (0.5, 1.0, 2.0, 4.0 and 8 μg/ml) were prepared by serial dilutions with water. Stock solutions were stored at +4°C. Blank plasma samples were treated as described above, spiked with the working standard solutions and 10 μL of naproxen as internal standard. The calibration curve was constructed from the peak-height ratio of the MPA to the naproxen internal standard from the HPLC chromatograms and then plotted against the nominal MPA concentration.

Assay validation Inter-day and intra-day precision and accuracy were evaluated from the analysis of control samples (low QC of 1 μg/ml, medium QC of 5 μg/ml and high QC of 10 μg/ml) measured on five different days. Inter-day precision and accuracy were evaluated by analyzing spiked plasma samples five times over the course of one day in random order. Precision of the HPLC method at each concentration was determined by comparing the coefficient of variation (CV) with the accuracy estimated for each spiked control. Relative recovery was estimated by the measured ratio of control samples at low QC of 1 μg/ml, medium QC of 5 μg/ml and high QC of 10 μg/ml to the aqueous solutions at the same concentrations.

RESULTS

Chromatograms This HPLC-UV method combined with protein precipitation for the determination of MPA concentration in human plasma is simple and suitable to be used in any laboratory for therapeutic drug monitoring. Typical chromatograms obtained from blank plasma and plasma spiked with MPA concentration of 1μg/ml and 10μL of naproxen as internal standard are shown in Figure 2 (a) and (b), respectively. Retention times for MPA was about 7 min and for the internal standard about 8 min. This method requires 200μl of plasma. Each chromatographic run lasts 10 min.

A



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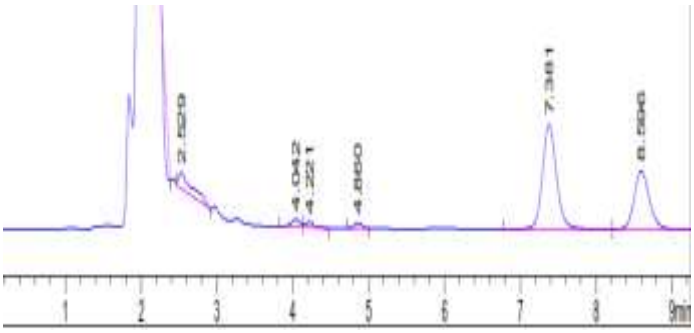

Figure 2. (a) Blank plasma (b) Plasma spiked with MPA and Naproxen as internal standard

Calibration Curve The calibration curve for MPA plasma was found to be linear over the concentration range of 0.5 – 8 μg/ml. The typical calibration curve was obtained :

$y= 0.0806x + 0.0151$; $r^2 = 0.999$, where y is the peak area ratio, x is the concentration of the compound and r is the correlation coefficient.

The Limit of Detection (LoD) and Limit of Quantification (LoQ) were found to be respectively 0.1 μg/ml and 0.4 μg/ml.

Precision and Accuracy The coefficients of variation (CV) and accuracy for intra- and inter-day assays were determined at Quality control concentrations of 1 – 10 μg/ml for MPA. Precision and accuracies values for intra- and inter-day assays are shown in Table 1. The precision and accuracy of this HPLC assay is suitable for routine therapeutic drug monitoring applications.

Relative recovey was estimated by the measured ratio of control samples at low QC of 1 μg/ml, medium QC of 5 μg/ml and high QC of 10 μg/ml three times to the aqueous solutions at the same concentrations shown in Table 2. Recovery ranged from 90% to 93%.

Table 2. Relative recovery of MPA by the proposed HPLC method (n=3)

Added μg/ml	Recovery %	Mean (%)
1	86 92 87	91
5	110 87 76	90
10	88 96 96	93

Table 1. Accuracy and Precision of HPLC assay for the determination of MPA in Human Plasma (n=5)

Added μg/ml	Intra-day			Inter-day		
	Found mean ±SD	Precision (%)	Accuracy (%)	Found mean ± SD	Precision (%)	Accuracy (%)
1	1 ± 0.04	4.3%	4.5%	0.9 ± 0.1	12.1%	12.1%
5	5.1 ± 0.1	1.5%	1.5%	5.2 ± 0.3	5.7%	4.7%
10	10 ± 0.4	4.2%	3.7%	9.9 ± 4.6%	4.6%	4.1%

DISCUSSION

This HPLC-UV method combined with protein precipitation has been validated for the analysis of MPA in human plasma from renal transplanted recipients. The method showed appropriate linearity for MPA with correlation coefficient greater than 0.999. The proposed method is suitable for routine MPA analysis as well as pharmacokinetic studies. The determination of mycophenolic acid could help the physician assess if concentrations are within the therapeutic range, limiting toxicity and preventing rejection due to low drug concentration.

CONCLUSION

The proposed HPLC method combined with protein precipitation is suitable for MPA analysis in plasma obtained from renal transplant recipients. Therapeutic monitoring of MPA might contribute to a better management of renal transplant recipient with the goal of optimizing therapeutic regimens in order to reduce the risk of MPA-related toxicity and prevent rejection.

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Conflict of Interest Disclosure: The authors declare that they have no conflict of interest.

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The Effect of Ramadan on Glycaemic Control in Type 2 Diabetic Patients

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Abstract

Background: People with type 2 diabetes fasting during Ramadan have significant increases in glycaemic excursions. Therefore, diabetes management in Ramadan should target glycaemic variability to empower people with diabetes to fast safely for prolonged periods. For this purpose, we planned to evaluate the glucose variability that might occur during the month of Ramadan by monitoring the treatments and blood glucose levels in our patients.

Aim: The current study aimed to evaluate the glucose variability that might occur during Ramadan.

Study Design: Methodological study analyzing the glucose variability during Ramadan.

Methods: One hundred patients, diagnosed with Type 2 diabetes and wanted to fast during Ramadan, were recruited. Patients were divided into three groups: the metformin group, the multiple oral anti-diabetic (OAD) group, and the insulin group. During Ramadan, the patient's capillary blood glucose was monitored and recorded five times per day (before sahur, 11 am, 5 pm, before iftar and two hours after iftar). Biochemical data, pre- and post-Ramadan fasting glycated haemoglobin (HbA1c) levels, and glucose measurements were compared between the three groups.

Results: There was no significant difference between the three group in terms of age, gender, body mass index (BMI), or diabetic complications. Diabetic nephropathy frequency,

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fasting plasma glucose, and HbA1c levels were significantly lower in the metformin group. There was no significant association between HbA1c changes among the three groups. There was also a significant association between before sahur, 11 am, 5 pm, and iftar blood glucose levels among the three groups. A higher risk of post-iftar excursions was observed in insulin-treated patients.

Conclusion: Patients on insulin treatment were less tolerant of fasting and had an increased risk for hypoglycaemia. The insulin group required more frequent warnings regarding the acute and chronic complications that may develop due to fasting.

Keywords: blood glucose measurements, Ramadan fasting, type 2 diabetes mellitus,

INTRODUCTION

The month of Ramadan is a holy month for Muslims. Fasting during this month is one of the five basic conditions of Islam imposed on all Muslims. During fasting, Muslims avoid any oral intake, including medication from sunrise (Sahur) to sunset (Iftar). According to a demographic study conducted in 2009, 23% of the world's 6.8 billion people, i.e., 1.57 billion people, have an Islamic faith and this rate increases by 3% each year (1). In the EPIDIAR study conducted in 13 Muslim countries, it was observed that 78% of patients with type 2 diabetes preferred to fast for at least 15 days during Ramadan (2).

People with type 2 diabetes fasting during Ramadan have significant increases in glycaemic excursions (2). Glycaemic excursions are disruptive to the individual and measures of variability are associated with subsequent hypoglycaemia (3). Therefore, diabetes management in Ramadan should target glycaemic variability to empower people with diabetes to fast safely for prolonged periods (4). For this purpose, we planned to evaluate the glucose variability that might occur during the month of Ramadan by monitoring the treatments and blood glucose levels in our patients.

MATERIALS AND METHODS

This study included one hundred patients diagnosed with type 2 Diabetes Mellitus who presented to the Sakarya University, Education and Research Hospital Outpatient Diabetes Clinic. At three months follow-up, laboratory studies were conducted at least 15 days before Ramadan started. Patients with type 1 diabetes, gestational diabetes, chronic kidney and liver failure, thyroid dysfunction, severe anemia, and oncologic and hematological malignancies were excluded.

Demographic data, anthropometric characteristics, additional diseases, and diabetic complications of patients were evaluated. Patient's biochemical parameters and three-month glycated hemoglobin (HbA1c) values were scanned and recorded at pre-Ramadan (days before Ramadan start) and post-Ramadan (within o week after Ramadan fasting was completed).

The treatment they had received before Ramadan was reassessed to reduce the risk of hypoglycemia (defined as glucose level below 70 mg/dl or 3,9 mmol/L) that may occur during fasting. Blood glucose levels were evaluated at the follow-ups and a dose reduction was performed when necessary. According to their treatment, patients were divided into three groups: the metformin group, the multiple oral anti-diabetic group (OAD), and the insulin group. Group 1 consisted of patients who received metformin only.

Group 2 consisted of patients who received DPP-4 Inhibitors + Metformin, Sulphonylurea + Metformin, or meglitinide + metformin therapy. Patients were instructed to take Sulphonylurea before iftar (the breaking of fast with a meal at sunset).

Group 3 consisted of patients who received intensive insulin or premixed insulin therapy.

All patients were interviewed by their physicians, diabetes educators and nutritionists before fasting. All patients were instructed to check and keep records of their blood glucose reading meters. Capillary blood glucose was monitored five times per day (before sahur, at 11 am, 5 pm, before iftar, and two hours after iftar) and was performed once a week during Ramadan (four weeks). Patients were also screened for the presence of hypoglycemia symptoms.

Demographic data, anthropometric data, biochemical data, and pre- and post-Ramadan fasting HbA1c levels were compared between the three groups. The lowest and highest blood glucose measurements, taken over the four weeks, five times a day, were labelled as minimum/maximum values and treatment groups were compared accordingly. We also selected the minimum and maximum blood glucose measurements in each group and documented them as "total". The differences between the minimum/maximum values for each period and the totals were calculated and labelled as "delta". Groups were compared based on the "delta" values. Also, the changes in HbA1c levels before and after Ramadan were compared.

Statistical Analysis

Data analysis was performed using the SPSS 10.0 statistical program [SPSS Inc. Chicago, IL]. Normally distributed continuous data were reported as the mean and standard deviation (SD), whereas non-normally continuous data were reported as the median and interquartile range (IR). ANOVA was used for the comparison of normally distributed data, and the Kruskal-Wallis test was used for the comparison non-normally distributed data. The Chi-square test was used to determine the association between the categorical data. $P < 0.05$ was considered significant

RESULTS

A total of one hundred patients (age, mean (SD) 57 years; Female/Male (F/M) 48/52) were included in the study. There were 38 patients in the metformin group (age, mean (SD) 56 years; F/M 19/19), 44 patients in the multiple OAD group (age, mean (SD) 55 years, F/M 24/20), and 18 patients in the insulin group (age mean (SD) 60 years; F/M 9/9). Demographic data of the patients are summarized in Table 1.

There was no significant difference between the metformin group, multiple OAD group and insulin group in terms of age, gender, BMI (basal metabolic index), additional diseases, diabetic complications (except diabetic nephropathy) or hypoglycemia symptoms ($p> 0.05$). Diabetic nephropathy and duration of diabetes mellitus were statistically different between the three groups ($p < 0.05$) (Table 1). Fasting plasma glucose (FPG) levels and HbA1c levels at baseline and after fasting were also significantly different between the three groups ($p < 0.05$) (Table 2).

When blood glucose measurements and their variability were examined, Sahur maximum, 11 am minimum and maximum, 5 pm minimum and maximum, and iftar minimum and maximum

Table 1. Demographic Data

	METFORMİN	OAD	INSULIN	P
Number of person (N)	38	44	18	
Age (Y)	55,9 ± 10,1	55,2 ± 9,7	59,5 ± 8,2	0,274
Gender (Female/Male)	19 / 19	24 / 20	9 / 9	0,903
*BMI (KG/M^2)	30.1 ± 5.65	30.2 ± 7,3	33,3 ± 10,3	0,813
*DM duration (year)	6 ± 6	6 ± 7	11 ± 8	0,003
Hypertension (N/%)	20 (52,6)	25 (56,8)	15 (83,3)	0.077
Hyperlipidaemia (N/%)	22 (57,9)	34 (77,3)	14(77,8)	0.118
Coronary artery disease (N/%)	2 (5.3)	4 (9,1)	2 (11.1)	0.707
Peripheral artery disease (N/%)	0	0	0	
Cerebrovascular disease (N/%)	1 (2,6)	1 (2,3)	1 (5,6)	0.778
Diabetic neuropathy (N/%)	2 (5.3)	1 (2,3)	1 (5,6)	0.736
Diabetic retinopathy (N/%)	1 (2,6)	1 (2,3)	0	0.794
Diabetic nephropathy (N/%)	1 (2.6)	3 (6,8)	4 (22.2)	0.038
Hypoglycaemia (N/%)	1 (2,6)	1 (2,3)	0	0.794

*Abnormal distribution; BMI, body mass index; DM, diabetes mellitus; F, female; M, male

Table 2. Laboratory Data

	METFORMIN	OAD	INSULIN	P
*FPG1 (mg/dl) median (IR)	138 (34,5)	150 (48)	200 (113)	0.000
HBA1C (%) mean (SD)	6.6 (0.75)	7.4 (1.1)	8.4 (1.8)	0.000
LDL 1 (mg/dl) mean (SD)	138 (39.9)	146 (33.8)	143 (45.8)	0.645
*TG 1 (mg/dl) median (IR)	137 (86.5)	146 (124)	170 (187)	0.137
Cr (mg/dl) mean (SD)	0.74 (0.16)	0.76 (0.21)	0.80 (0.24)	0.55
ALT 1 (mg/dl) mean (SD)	22 (15.5)	20 (9)	22 (12)	0.319
*FPG 2 (mg/dl) median (IR)	134 (37)	146 (47)	164 (77)	0.042
HBA1C2 (%) mean (SD)	6.5 (0.9)	6.9 (0.9)	7.9 (1.4)	0.000
*LDL2 (mg/dl) median (IR)	125 (52)	131 (34)	144 (51)	0.624
*TG2 (mg/dl) median (IR)	162 (84)	151 (116)	155 (195)	0.699
Cr 2 (mg/dl) mean (SD)	0.72 (0.16)	0.72 (0.17)	0.79 (0.2)	0.38
*ALT 2 (mg/dl) median (IR)	20 (8.5)	21 (9)	22 (15)	0.740

1: Pre-Ramadan; 2: Post-Ramadan; FPG, fasting plasma glucose; LDL, low density lipoprotein; TG, triglyceride; HBA1C, glycated hemoglobin; ALT, alanine transaminase; AST, aspartate transaminase

blood glucose levels were significantly different ($p < 0.05$) (Table 3).

Table 4. Blood Glucose Measurement Data of the Events

	METFORMIN	OAD	INSULIN	P
*SAHUR MIN median (IR)	110 ± 19	112 ± 46	117 ± 76	0.394
SAHUR MAX mean (SD)	177 ± 51	186 ± 46	246 ± 76	0.000
11 MIN mean (SD)	129 ± 25	132 ± 27	162 ± 36	0.000
11 MAX mean (SD)	180 ± 44	193 ± 47	243 ± 62	0.000
*17 MIN median (IR)	107 ± 21	106 ± 24	135 ± 57	0.003
*17 MAX median (IR)	135 ± 49	137 ± 41	198 ± 79	0.000
*IFTAR MIN median (IR)	106 ± 26	100 ± 29	129 ± 67	0.000
*IFTAR MAX median (IR)	135 ± 50	132 ± 55	195 ± 107	0.001
*IFTAR 2 MIN median (IR)	135 ± 70	136 ± 55	178 ± 59	0.110
*IFTAR 2 MAX median (IR)	206 ± 109	215 ± 81	288 ± 147	0.128

2: 2 hours after iftar; MIN, minimum ; MAX, maximum

Table 3. Blood Glucose Variability Factors in Patients

	METFORMIN	OAD	INSULIN	P
*SAHUR MIN median (IR)	110 ± 19	112 ± 46	117 ± 76	0.394
SAHUR MAX mean (SD)	177 ± 51	186 ± 46	246 ± 76	0.000
11 MIN mean (SD)	129 ± 25	132 ± 27	162 ± 36	0.000
11 MAX mean (SD)	180 ± 44	193 ± 47	243 ± 62	0.000
*17 MIN median (IR)	107 ± 21	106 ± 24	135 ± 57	0.003
*17 MAX median (IR)	135 ± 49	137 ± 41	198 ± 79	0.000
*IFTAR MIN median (IR)	106 ± 26	100 ± 29	129 ± 67	0.000
*IFTAR MAX median (IR)	135 ± 50	132 ± 55	195 ± 107	0.001
*IFTAR 2 MIN median (IR)	135 ± 70	136 ± 55	178 ± 59	0.110
*IFTAR 2 MAX median (IR)	206 ± 109	215 ± 81	288 ± 147	0.128

2: 2 hours after iftar

There was also a significant association between before sahur, 11 am, 5 pm, and iftar blood glucose levels among the three groups ($p < 0.05$). There was no significant association between HbA1c changes ($p > 0.05$) (Table 4).

Blood glucose levels and HbA1c variability are summarized in Figure 1.

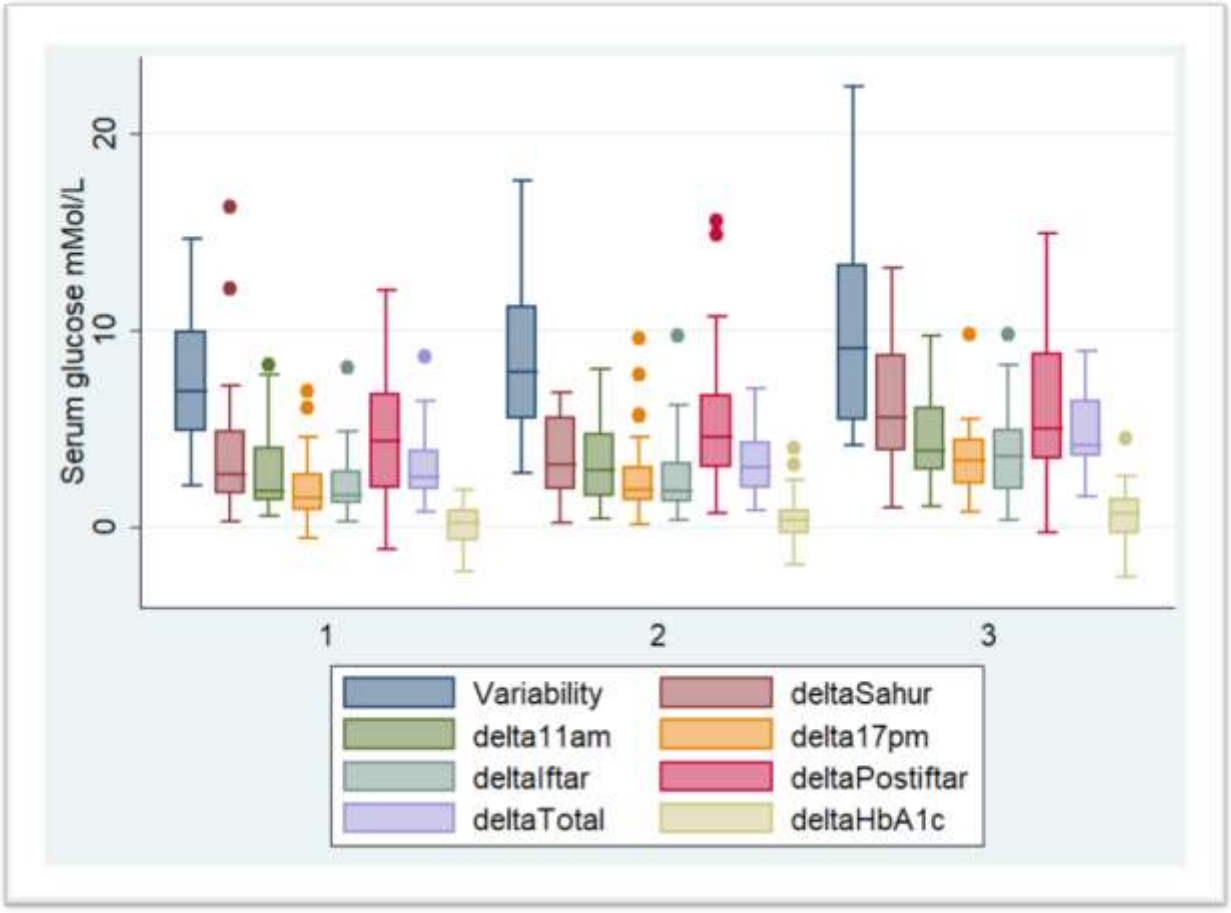

Figure 1. Blood glucose levels and HbA1c variability

DISCUSSION

It is known that there is an increasing prevalence of diabetes in Muslim countries, and people with diabetes who want to fast due to their religious beliefs frequently attend diabetes centres in our country and other Muslim countries. Numerous studies have been conducted on diabetes regulations, hypoglycaemia, and hyperglycaemia. On the contrary, in many other studies, it was found that fasting did not impair blood glucose regulations and did not increase the risk of hypoglycaemia. The studies are very different and varied in terms of design, the number of patients, and the study objectives. In our study, we aimed to investigate the effect of fasting on glycaemic regulation and variability in patients with type 2 diabetes who visited our clinic.

One of the most important complications that may occur in the summer months, due to the prolonged fasting period is hypoglycaemia (5,6). In the EPIDIAR study, severe hypoglycaemia rates were found to be high especially in the patients who used insulin (2). Expert opinions suggested that reducing the incidence of hypoglycaemia can be achieved by reducing the

total dose of insulin by 20–50% (2,7). In another study, continuous glucose monitoring recordings, during, before, and after Ramadan showed wide intra- and interindividual variability. When the mean glucose measurements were evaluated, there was a slow decline before the fasting period and a rapid increase after iftar. The results indicated a higher risk of after iftar excursions, poorer glucose control in insulin-treated patients as well as in patients taking sulphonylurea. This difference was more significant in the group receiving insulin treatment compared to the group receiving OAD (8)

In our study, there was no increase in glycaemic variability in the patient group using only metformin and using more than one OAD, whereas in the patients using mixed and basal-bolus insulin, there was a significant increase in the risk of glycaemic fluctuations and hypoglycaemia. Most patients with baseline blood glucose levels regulated by OAD and without comorbidities are able to tolerate long periods of fasting. When these results are evaluated, it should be kept in mind that hypoglycaemia and associated comorbidities may be seen more in patients receiving insulin therapy. In particular, the insulin group required more frequent warning of the acute and chronic complications of fasting.

In our study, control HbA1c values decreased in all groups, including the insulin group. Compared with BMI, LDL, and triglyceride levels, there was no significant difference between the groups using OAD and insulin. In many studies, significant decreases have been observed when comparing other periods of the year with the month of Ramadan in the lipid profile, HbA1c levels, arterial blood pressure, and uric acid levels (9). In a study involving 23 patients, a significant decrease in the body fat mass was found when there were no differences in BMI measurements, and it was thought that the decrease in HbA1c could be due to a decrease in body fat (10). Again, a study conducted in Turkish non-fasting diabetic showed that patients had higher plasma glucose levels during Ramadan and after Ramadan compared to fasting diabetic patients (11). These results suggest that fasting may be useful for some of the diabetic patients.

As a result of the EPIDIAR study, there was an increase in the risk of hypoglycaemia, hyperglycaemia, dehydration, ketoacidosis, and thrombosis in patients with type 2 diabetes (2) However, in many studies, there was no increase in risk (2,12-16). Of course, different factors such as patient group, fasting period and percentage of patients with complications may affect the results. In our study, none of the patients had acute complications such as ketoacidosis, hyperosmolar hyperglycaemic coma, or severe hypoglycaemia requiring hospitalisation. Proactive and focused nutritional advice, together with appropriate dose adjustments of anti-diabetic medications, should help keep blood glucose levels better controlled and more stable during Ramadan fasting (8,17-21).

CONCLUSION

There is no clear consensus on how to treat patients with diabetes who want to fast. In our study, patients using single or several OADs were more tolerant of fasting, whereas patients who were on insulin were found to be less tolerant. As a result, the increasing number of diabetic patients with the growing prevalence of diabetes is still a problem for physicians. The most appropriate approach is for the treatment decision to be based on the patients' characteristics and aim to provide the least harm to the patients.

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Hypertension and Hyperuricemia

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Abstract

Essential hypertension affects about 25% of the world population and is considered as a major causal factor of myocardial infarct, congestive heart failure, stroke, end-stage renal disease and also risk factor for type 2 diabetes. Hypertension is implicated in 13% of deaths globally. The essential hypertension is a multifactorial and complex disease and the number of contributory factors in its etiology are increasing each year as being appreciated in recent decades. Recent experimental and clinical studies in animals and humans have implicated uric acid in the early onset mechanism of essential hypertension in children and adolescents. An association exists also between uric acid, cardiovascular diseases and mortality, metabolic syndrome, subclinical atherosclerosis, stroke, kidney diseases, type 2 diabetes and endothelial dysfunction. Asymptomatic hyperuricemia was also a strong risk factor for resistant hypertension in the elderly. Epidemiological data suggest that hyperuricemia and gout is becoming more prevalent worldwide, probably as the consequence of Westernization of diet and life style, obesity and the increased availability of certain medications. These data suggest that the prevalence of hyperuricemia in the adult population is 20%-40% and this continues to increase over time.

The aim of this article was to review the results of the most recent studies about the possible relation between elevated uric acid levels and the

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onset or worsening of hypertension and other cardiovascular, renal and metabolic diseases.

All these studies support a role for high serum uric acid levels (>6mg/dl or 60mg/l) in hypertension–associated morbidities and should bring attention to physicians in regard to their patients. The relationship between serum uric acid and hypertension is lost with increasing age and with duration of hypertension.

The regular physical exercise, Mediterranean diet and decreased consumption of beverages with high fructose corn syrup may lower the risk for hypertension. It is postulated that xanthine oxidase inhibitors would be of greater benefit than uricosuric agents in reducing the cardiovascular, renal and cerebral risk as they both lower serum uric acid and block the production of proinflammatory reactive oxygen species (ROS). Urate lowering therapy is associated with normalization of both serum uric acid and blood pressure in younger population. In contrast, in older hypertensive population, urate lowering therapy have minimal effects on blood pressure but appear to improve cardiorenal endpoints.

Keywords: Hypertension, uric acid, hyperuricemia, risk.

INTRODUCTION

Uric acid (UA) is a weak and final production of purine mononucleotide catabolism which means that cannot be further metabolized. In other mammals UA is degraded by uricase into allantoin, which is a more soluble substance. Unfortunately, uricase during Miocene was lost in humans. This loss of function provided supposedly some evolutionary advantages.

Firstly, UA acts as an antioxidant of the plasma. It can scavenge single oxygen, peroxyl and hydroxyl radicals, reacts with peroxynitrite and stabilizes endothelial nitric oxide synthase (eNOS) activity. Its antioxidant effects need the presence of ascorbate.

Secondly, UA increases salt sensitivity and may have maintained BP in the poor salt environment of these early times.

Thirdly, UA increases fat storage and lipogenesis. Fruits contain more fructose at the end of the summer, resulting in an enhanced lipid deposition to better cope with the nearby winter. Fructose corn syrup is the only sugar which can raise UA level. It is metabolized in the liver by the phosphofructokinase in fructose-1- phosphate (F1P). F1P will be converted into glucose, glycogen, lactate and lipids which step consumes a large amount of adenosine triphosphate (ATP), resulting in substrates for the purine metabolism (1).

UA is the last product of purine metabolism. The last two steps of the pathway are catalyzed by the xanthine oxidoreductase (XOR) which exists under two interconvertible isoforms: the xanthine dehydrogenase (XDH), which uses nicotine adenine dinucleotide (NAD+) as an electron acceptor, and xanthine oxidase (XO), which uses oxygen as an electron acceptor (2).

Only the XO is able to create reactive oxygen species (ROS) during the generation of UA. This latter form is more activated during ischemia, extensive surgery and stress, and is mainly present in the liver, the intestine and the vascular wall (3). UA acts as a strong antioxidant compound in the extracellular environment but has pro-inflammatory effects within the intracellular area. XO-related oxidative stress may decrease nitric oxide (NO), increase oxidative stress in the macula densa, induce endothelial and mitochondrial dysfunction, renal vasoconstriction, activation of rennin-angiotensin system (RAS), vascular smooth muscle cell proliferation and in chronic phase of hyperuricemia lead in interstitial renal fibrosis and arteriosclerosis. In early stage hyperuricemia increases blood pressure (BP) by high reabsorption of Na+ in renal tubes and it is urate lower therapy (ULT) depended but in late stage the micro renal injury causes salt sensitive kidney – hypertension depended. These studies revealed that hypertension (HT) develops in two steps (4). At the physiological pH of 7.40 in the extracellular compartment, 99% of uric acid is in ionized form as urate (as monosodium urate in blood and calcium, potassium and ammonium in urine). Urate is more soluble in plasma than in urine. The lower pH in urine can favorize the crystals formation. (2)

The physiological daily amount of endogenous and exogenous uric acid is about 700mg, which is balanced by an equal output via urine and feces. About 25-30% of uric acid is broken down by intestinal flora and excreted in the stool, while 65-75% is excreted through the kidneys. The majority of circulating uric acid is free in plasma and is readily filtered by the glomeruli, but up to 90% may be reabsorbed. UA homeostasis is governed by the balance between the rate of UA generation (determined by purine catabolism), renal excretion, and intestinal secretion. Multiple urate transporters have been identified as playing a role in renal tubular reabsorption and secretion of urate and intestinal secretion, thus helping regulate homeostasis (URATE 1, GLUT9, ABCG2 and recent one NPTI). These transporters are encoded respectively by the genes (SLC22A12, SLC2A9, ABCG2, SLC17A1) (5).

Purines can be synthesized endogenously or can derive from dietary sources. Hypoxanthine and Xanthine are the intermediate products of this catabolism. UA is derived mainly from the breakdown of purines in the liver and bowel, as well as the kidneys, muscle and vascular endothelium. The exogenous supply of UA is derived from dietary sources of purines including fatty meat, seafood and alcohol. (4)

Normal serum UA levels range between 2.4 to 7 mg/dl (180 to 415 μmol/l) in temperature 37˚and neutral pH, but age- and sex-related normative ranges for serum UA exist, too. Hyperuricemia is typically defined as SUA concentration > 6.0 mg/dL (> 360μmol/L) in women, >7.0mg/dL (>415μmol/L) in men and >5.5mg/dL (> 330 μmol/L) in children and adolescents. (2)

The uricosuric effect of estrogens leads to lower SUA levels in premenopausal women. Following menopause, however, urate levels tend to be more comparable to those of males of similar age.

A serum UA concentration of 7.0 mg/dL (415 μmol/L) is defined as the upper limits of normal, as it approaches the limits of UA water solubility. The gold standard is to maintain UA < 6.0mg /dl in hyperuricemic patients (1).

Reduced UA excretion is a common cause of hyperuricemia and found in renal failure and in the presence of insulin resistance, in part due to an increased renal reabsorption under the effect of high insulin levels. Loop and thiazide diuretics reduce renal UA excretion. Other clinical disorders may reduce the UA excretion such as: obesity, small bowl disease, hyperparathyroidism, hypothyroidism, preeclampsia, sarcoidosis, volume depletion, etc. Genetic polymorphism in urate transporter one (URAT-1) and Glut 9 transporter can also result in hyperuricemia. A small number of individuals with hyperuricemia have inherited defects resulting in primary overproduction of UA (5).

The history of link between hypertension and hyperuricemia

The strong association between hyperuricemia and hypertension has been recognized as far back as 1879, where a relationship between “gouty

families" and elevated blood pressure was noted (6).

Gout is one of the oldest recognized disease in humans, described by Hippocrates as ″arthritis of the rich″ due to its association with animal food and alcohol, but a documented history dates back to the Egyptians in 2640 BC.

Haig in 1890 made the link between of (UA) and multiple comorbidities including HT (7). Due to the lack of data to suggest a direct association, it was believed that UA acts as a marker for comorbidities such as diabetes, kidney disease and obesity.

Studies conducted between 1950 and 1960 evidenced that 25%–47% of adults with untreated hypertension were hyperuricemic (8, 9, 10). This prevalence rose to 58% among those receiving diuretics and to 75% in those with malignant hypertension (9).

In 1972, the Israeli Heart Trial demonstrated that for young males (aged 17 to 25 years) within the highest tertile for the plasma UA measurement, there was a two-fold increase in risk of hypertension after 5 years of follow up (11). This association has been described worldwide across different ethnicities including African American, Asian American and Japanese populations (12, 13, 14, 15, 16).

Klein et al. one year later demonstrated a linear relationship between serum uric acid (SUA) level and systolic blood pressure (SBP) in both white and black people (17). Since then, many epidemiological studies showed a strong association between UA and HT and particularly the risk of developing HT.

In 2011, Grayson and colleagues published a systematic review and meta-analysis of 18 prospective cohort studies of 55,607 patients (18). They revealed that a 1 mg/dl increase in UA level was associated with an increased risk of incident HT by 13% (pooled RR = 1.13). These effects were significantly larger in women and in younger population studies especially in those with metabolic syndrome (14). Therefore, UA is often considered as an independent factor for HT, especially earlier in the life course than at a later stage (18, 19, 20). With regards to an association with gender and age, hyperuricemia (> 6.8 mg/dL [410 μmol/L]) has been found to predict refractory hypertension in females ≥ 65 years (odds ratio 3.11, 95% CI 1.06–9.1) independent of chronic kidney disease (CKD), although this association was not found in males (21).

In a cohort of 45,908 Korean adults who had never been on either ULT or antihypertensive therapies, in the men < 60 years, hyperuricemia increased the relative risk of hypertension by approximately 30%, and in women < 40 years, this risk was elevated 2.6 fold (22). More recent data from the same group showed a positive association between SUA and incident hypertension {specifically increase in diastolic blood pressure, (DBP) } over a mean follow up of 3.3 years in those < 55 years (relative risk 1.74 per 1.0 mg/dL [60 μmol/L] of SUA) compared with those ≥ 55 years (23). The conclusion from one study is that SUA production may have a

causal role in raising DBP, an indicator of increased systemic vascular resistance and a risk factor for cardiovascular disease in younger individuals (24). The relationship between SUA levels and both SBP and DBP was continuous, and SBP relationship was stronger in adults (25). Multiple studies support a stronger association between SUA and hypertension in those of younger age. The Moscow Children's Hypertension Study and the Hungarian Children's Health Study were the first to describe this association in detail, despite much lower incidence of hyperuricemia in this younger cohort (26, 27). These trials, found that hyperuricemia was strongly correlated with hypertension. Hyperuricemia (> 8.0 mg/dL [> 480 μmol/L]) was present in 9.5% of normotensive adolescents, 49% of those with borderline hypertension, and 73% of those with moderate to severe hypertension. Essential hypertension has been reported in 89% of children and adolescents with UA levels > 5.5 mg/dL (> 330 μmol/L), compared with 30% in those with secondary hypertension and 0% in healthy controls or those diagnosed with white-coat hypertension (28).

In a short-term crossover study involving adolescents with newly diagnosed hypertension, treatment with 200 mg allopurinol twice a day resulted in reduction of SUA levels, which was associated with a suppression of HT in 20 out 30 patients treated (29). This supports the concept that an early intervention aiming to prevent hypeuricemia may be beneficial regarding HT and its comorbidities. In a prospective trial, 113 patients with estimated eGFR<60ml/min/1.73m² and hyperuricemia were randomized to receive allopurinol 100 mg/day or to continue their usual therapy. After 23 months, SUA level was reduced in 6mg/dl in Xanthine oxidase (XO) inhibitor group but was unchanged in control group. The e GFR value was not significantly changed in allopurinol group but it was worsened in the control group, suggesting that lowering SUA by XO inhibitors is expected to slow the progression of renal disease (30). Other two studies achieved positive impact of XO inhibitors (febuxostat) versus placebo in hypertensive and/or hyperuricemic patients. It was estimated that after four years, for every 1mg/dl reduction of SUA level, there would be a preservation of 1.15ml/min/1.73m² of eGFR (31), and after six months with SUA in 6mg/dl, was showed a reduction of plasma renin and aldosterone concentrations with a significant increase eGFR (+5.5%, p=0.001) (32).

Importantly, nowadays, according to a recent meta-analysis, there is still a lack of evidence to recommend the use of allopurinol or other uric acid lowering therapy as a treatment of HT (33). Xanthine oxidase not only generates UA but also produces superoxide. The increase of SUA may be due to the activation of XO and any benefit observed with blocking XO is more likely due to the XO associated oxidants rather from lowering UA. Allopurinol can also reduce fructose-induced proinflammatory mechanisms (monocyte chemoattractant protein-1 synthesis). This explain why XO inhibitors have been found

to improve endothelial function in contrast to probenecid, but more data are needed (34). A protective effect of UA on coronary arteries has been shown in isolated perfused hearts in the 1980s 2. Conversely, early UA infusion after an acute ischemic stroke did not improve functional outcomes at 90 days (35).

Choi and colleagues analyzed a cohort of 24.768 people with newly diagnosed gout and 50.000 matched control and found that use of calcium channel blockers and losartan was associated with a moderately lower risk of incident gout among patients with HT (36). LIFE study confirmed the same for uricosouric capacity of losartan. In contrast, the use of diuretics, β-blockers, ACE inhibitors and non-losartan angiotensin II receptors blockers was associated with an increased risk of incident gout among patients with HT (37).

These results contrast with a Mendelian randomization studies, which investigate genetic polymorphisms in the SLC2A9 gene (responsible for urate reabsorption via GLUT9), the primary genetic determinant of SUA levels, where no evidence for causal associations between UA and ischemic heart disease or blood pressure (BP) was found. Body mass index (BMI) was implicated as a potential confounder (5, 38).

Genome wide association studies (GWAS) analyzed the results of 28,283 Caucasian individuals and identified single nucleotide polymorphisms (SNPs) at 8 genetic loci demonstrating statistically significant genome-wide association with SUA levels, and 2 of these loci (ABCG1 and SLC2A9) achieved significant association with gout risk, estimated 6.0–7.7% of SUA variability. There was found to be no association with BP, fasting glucose, eGFR, chronic kidney disease (defined as eGFR < 60 mL/min/1.73m2) or coronary heart disease (CHD) in this cohort (39, 40).

However, it is important to note that Mendelian studies involve gene-dependent association, and even though hyperuricemia has an important genetic component (40% to 73%) (41), it is primarily caused by life habit and diet, and alternative biochemical pathways, such as fructose metabolism, affect UA levels.

Prolonged fructose consumption may therefore contribute to the development of metabolic syndrome by increasing circulating concentration of UA, Gama Glutamyl Transferase (GGT) activity and productions of adipokine retinol binding protein-4(RBP-4) (41). Increased circulating concentration of RBP-4 have been linked with increased visceral adiposity and have been shown to directly contribute to hepatic insulin resistance via induction of hepatic glucose production and impairment of insulin signaling in muscle. (16)

The inability to establish direct causality between hyperuricemia secondary to genetic polymorphisms and the risk of hypertension does, however, need to be interpreted with caution. The hypothesis regarding the antagonistic pro- and antioxidant effects of UA described in the literature as the “oxidant-antioxidant paradox”

may help explain the mixed and contradictory results of studies. What remains clear is that UA demonstrates different physiological properties within different biological systems and biochemical environments. On the basis of the aforementioned experimental studies, it is postulated that subjects with hyperuricemia who have not had hypertension long enough to develop secondary arteriolar injury could benefit most from ULT but the side effect profile of ULT agents limits their widespread use. In particular, allopurinol is associated with dose-related adverse effects that range from a relatively common rash to rarer effects such as aplastic anemia and severe hypersensitivity reactions. (38).

CONCLUSION

The relationship between SUA and HT is lost with increasing age and with duration of HT. The regular physical exercise, Mediterranean diet and decreased consumption of beverages with high fructose corn syrup may lower the risk for HT. It is postulated that XO inhibitors would be of greater benefit than uricosuric agents in reducing the cardiovascular, renal and cerebral risk as they both lower SUA and block the production of proinflammatory ROS. Urate lowering therapy is associated with normalization of both SUA and BP in younger population. In contrast, in older hypertensive population, ULT have minimal effects on BP but appear to improve cardiorenal endpoints.

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Rhabdomyolysis Due to Carnitine Palmitoyltransferase II Deficiency – a Common but Underrecognized Condition

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Abstract

A young 33-year-old male goes to the emergency room with weakness, nausea, anuria, which started three days before admission. The symptoms appeared after a prolonged exercise. An acute kidney injury developed, and Hemodialysis treatment was needed. At the clinical presentation, he had a high plasma Creatine Kinase (CK) level and CK-MB level. The genetic testing confirmed the diagnosis of Inherited Rhabdomyolysis, a metabolic disorder of Carnitine Palmitoyl transferase II Deficiency.

Keywords: Carnitine Palmitoyltransferase II deficiency, Rhabdomyolisis, metabolic disorder, acute renal failure, genetic disorder

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INTRODUCTION

Carnitine palmitoyltransferase II deficiency is an autosomal, recessively inherited genetic metabolic disorder that causes an amino acid change from Ser to Leu at position 113 (1). Carnitine palmitoyltransferase (CPT) catalyzes the transfer of long-fatty acids from the cytoplasm into mitochondria, where β-oxidation happens. The two forms of CPT are CPT I localized at the outer membrane of mitochondria and CPT II localized inside the mitochondria. If there is a defect of the CPT2 enzyme, long-chain fatty acid can not be transported into the mitochondria and can not be used as an energy source (1,2).

The three clinical presentations of CPT 2 deficiency described in the literature are:

- the *lethal neonatal* form that is lethal during the first months of life.
- the *infantile hepatic-cardiac-muscular* form is presented as severe attacks of hypoketotic hypoglycemia, associated with cardiac damage, which causes sudden death before the first year.
- the *myopathic* form characterizes by episodes of rhabdomyolysis triggered by intense exercise, drugs, or febrile episodes (3).

The most frequent pattern in clinical practice is the Myopathic form, and the symptomatology consists of recurrent attacks of rhabdomyolysis, presenting as myalgias, cramps, weakness, and myoglobinuria. Rhabdomyolysis may result in complications such as Acute Renal Failure and Respiratory Insufficiency (4).

CASE REPORT

A 33-year-old male patient was admitted to our hospital with muscle weakness, nausea, and anuria. The patient mentioned he had performed excessive physical activity three days before hospitalization and felt low back pain, muscle pain, and lightly brown-colored urine. He then experienced anuria for about two days before arriving at the emergency unit.

Six years ago, his older brother went through the same medical condition after a strenuous exercise.

Laboratory testing revealed high serum levels of CK64 180 U/L, CK-MB 1057 U/L, LDH 2890 U/L, AST 4017 U/L, ALT 1000 U/L, Creatinine 6.6 mg/dl, and Urea 169 mg/dl (Table 1). Kidney ultrasound showed normal kidneys in terms of size and structure. Other lab tests were within the normal range.

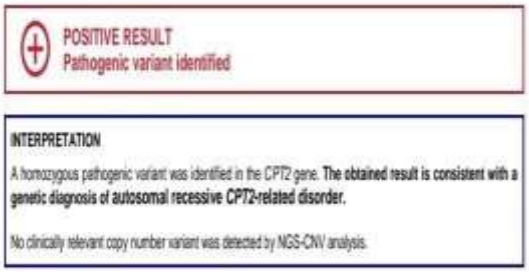
Table 1. Presentation of the laboratory findings during the patient hospitalization.

	First day	Last day	
CK	64180	62	U/L
CK MB	1057	35	U/L
LDH	2809	216	U/L
AST	407	97	U/L
ALT	1088	145	U/L

CREATININE	6.6	1.4	mg/dl
UREA	169	166	mg/dl
K	4.7	4.1	mmol/l
Ca	8	9	mg/dl
P	6.1	4	mg/l
WBC	10000	86000	/mm3
PLT	16300	25800	/mm3
TOTAL PROTEINE	7	8.5	g/dl
ALBUMINE	3.9	4.5	g/dl

The persistence of anuria and progressive decline of renal function were strong indicators to initiate dialysis treatment. The patient was treated with eight hemodialysis sessions until the improvement of the renal function.

Genetic testing (molecular analyses) for our patient and his brother confirmed Carnitine palmitoyl transferase II deficiency (Figure 1).


POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the CPT2 gene. **The obtained result is consistent with a genetic diagnosis of autosomal recessive CPT2-related disorder.**

No clinically relevant copy number variant was detected by NGS-CNV analysis.


CENTOGENE

RESULT SUMMARY

Figure 1. Results of genetic testing

The CPT2 variant c.338C>T p. =(Ser113Leu) causes an amino acid change from Ser to Leu at position 113. The substitution is in close proximity to the highly conserved donor splice site of intron None.

Our patient was discharged from the hospital in good condition, with proper recommendations regarding his lifestyle to prevent similar episodes in the future.

DISCUSSION

Carnitine palmitoyltransferase II deficiency is the most common inherited disorder of mitochondrial long-chain fatty acid oxidation, characterized by attacks of myalgia and myoglobinuria.

The most common "classic" Myopathic form occurs in young adults and is characterized by recurrent episodes of rhabdomyolysis. The CPT2 defect prevents the transportation of long-fatty acids into mitochondria, as a result preventing the use as an energy source. The patient usually has muscle pain and weakness associated with the breakdown of muscle tissue. When the muscle

tissue breaks down, myoglobin is released. It is processed by the kidneys and released in the urine as myoglobinuria. This protein causes the redness of the urine. It can also damage the kidneys, in some cases leading to kidney failure (3,5).

In literature are described 300 cases. About 80% of the patients are males. It is linked to the X-chromosomal genes or hormonal factors (estrogen) that might regulate the CPT enzyme (6). The first description of this condition was made in 1973 by the brother's Di Mauro, and the mutation that is mainly found was p. (Ser113Leu) that causes an amino acid change from Ser to Leu at position 113. This variant has previously been described as disease-causing for Carnitine palmitoyl transferase II (CPT2) deficiency by Taroni (7).

For the diagnosis of CPT II deficiency is essential the muscle biopsy (to find reduced CPT enzyme activity in muscle) or molecular genetic testing to identify pathogenic variants of CPT2 (3).

It is characterized by recurrent episodes of rhabdomyolysis triggered by prolonged and intense exercise, drugs, fasting, or febrile illness. The treatment consists of a carbohydrate-rich diet and lifestyle changing.

We presented a patient with myalgia, fatigue, and dark urine (brown-colored), with Acute Liver Injury and Acute Kidney Injury requiring hemodialysis. Laboratory data suggested that it was due to rhabdomyolysis triggered by an intensive exercise. Six years ago, at University Hospital Center "Mother Theresa," his brother was admitted with the same medical history presentation. He also developed acute hepatic lesions, acute kidney injury and rhabdomyolysis. He was treated with supportive therapy and hemodialysis sessions. Genetic testing (molecular analyses) for our patient and his brother confirmed Carnitine palmitoyltransferase II deficiency. Our patient was discharged in good condition, without need for hemodialysis. We recommended a diet rich in carbohydrates, a low amount of fat and protein, eating often, and changing his lifestyle. He ought to stay away from habits that may trigger symptoms such as fasting, exposure to cold, stress, and intense exercise.

The same medical situation with the acute hepatic lesion, rhabdomyolysis, acute kidney injury requiring hemodialysis and then discharged in good condition was described by Nikola Gjorgjievski (8). Another case was described by M Valvukis (9), where a patient with CPT2 deficiency was presented with cardiomyopathy and rhabdomyolysis triggered by febrile episodes and intense physical work.

The patient was treated with supportive therapy and hemodialysis, and further was discharged in good condition with proper advice for a lifestyle modification.

In conclusion, we could say that Carnitine palmitoyltransferase II deficiency is an underdiagnosed cause of rhabdomyolysis. We should be vigilant to this defect if we have a patient presented with rhabdomyolysis and acute kidney injury. Recognition of CPT II deficiency

can prevent further episodes of acute kidney injury and other life-threatening conditions.

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Conflict of interest: None declared.

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Recurrent Primary Hyperparathyroidism – A Case Report
The Importance of Examination Before and During Surgery

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Abstract

Background: Primary hyperparathyroidism, in 85% of the cases generates from solitary parathyroid adenoma. The selected treatment is surgery on the gland after imaging evaluation (scintigraphy with Sestamibi). But in about 11% of cases hyperstimulation is noticed by more than one adenoma and imaging examination which reaches a sensitivity of 75% and specificity of 78% in the case of solitary adenomas, fails to assess the full involvement of all glands noticing only the dominant nodule.

Case report: The case we present is that of a man, 51 years old who presents with complaints of muscle pain and physical weakness. During laboratory tests results hypercalcemia and PTH 197 pg/ml (8 – 76). On additional examinations hyperfixation is observed on the right upper parathyroid on scintigraphy with Sestamibi. Based on these data, the patient underwent right parathyroidectomy. Postoperative biopsy confirms the diagnosis of parathyroid adenoma. The patient continues treatment with Calcium to avoid transient postoperative hypoparathyroidism. Three months after the surgery, during the follow-up, there is an increase of the level of PTH (71.54 pg / ml for a normal range of 15 - 65 pg / ml) after an initial post-op decrease. The patient continues to be in follow up to assess the origin of recurrent hyperparathyroidism leaving open the possibility of multiple adenomas.

Conclusion: Preoperative evaluation for the initial localization of the lesion is a necessary

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diagnostic step but not the final one in identifying the entire disease. For a successful and long-term treatment, is advised an intraoperative evaluation and regular postoperative follow-ups of the patient.

Keywords: primary hyperparathyroidism, recurrence, PTH, scintigraphy, parathyroidectomy

INTRODUCTION

Primary hyperparathyroidism is a slow-growing but high-risk endocrine pathology resulting in multiple organ damage, mainly the kidneys and bones. It is mostly characterized by increased levels of Parathormone (PTH) and hypercalcemia, but there are non-classical forms of the disease where biochemical parameters vary and as a result its diagnosis becomes more difficult and the risk of complications increases (1).

Over 80% of primary hyperparathyroidism comes from parathyroid gland adenomas. The incidence is highest in the fifth and sixth decade of life. Women are three times more at risk than men. There are two rare forms such as glandular hyperplasia, parathyroid carcinoma or ectopic PTH which make up a smaller percentage.

The treatment of choice in parathyroid nodular pathologies is surgical removal of the gland after imaging evaluation (ultrasound, scanner or magnetic resonance and 99Tc-Sestamibi scintigraphy). But in about 11% of cases the hyperstimulation is noticed by more than one adenoma and the imaging fails to assess the full involvement of all glands noticing only the dominant nodule (2,3).

Therefore, intraoperative assessment of PTH levels is recommended to localize the in-situ lesion and depending on this, the most appropriate surgical method is then selected. In the case of solitary or multiple adenomas, minimally invasive parathyroidectomy (MIP) is preferred under local anaesthesia with intraoperative monitoring of PTH before and after the removal of the parathyroid gland. If within 2-10 min of the removal of the gland, the PTH level falls more than 50% of the normal range, the removal of adenoma is considered safe. If the PTH level does not decrease more than 50% and / or remains above normal, the surgery is continued and if necessary, a full neck examination is performed to look for other over productive glands (4).

Post-operative recurrences can occur in up to 5% of patients over a 10-year period (5).

Through intraoperative assessment methods, the probability of a successful intervention increases and the risk for pathological residual tissue and recurrent hyperparathyroidism decreases. The case that we present is that of a patient intervened for parathyroid adenoma and for a short postoperative period, the biological levels of the parathyroid turn out to be altered, suggesting for recurrence of the pathology.

CASE REPORT

The patient, male 51 years old, presented to the physician with complaints: muscle pain and physical weakness. Objectively the patient's condition was good. No pathological data. The neck was free, without pathology of thyroid and parathyroid glands. The patient underwent laboratory examinations. Everything resulted within normal range, except by high level of total calcaemic blood level (over 10mg/dl). Based on hypercalcemia, the patient was checked for levels of vitamin D3 and PTH. The hormone of the

parathyroid glands was 197 pg/ml, for a normal range of 8 - 76 pg / ml. Neck ultrasound revealed a suspicious formation in the upper pole of the right thyroid lobe. For this reason, the patient performed scintigraphy with Sestamibi. The examination revealed hyperfixation in the right upper parathyroid, suggestive for parathyroid adenoma (Figure 1).

Osteoporosis was observed in bone densitometry (Figure 2).

Meanwhile, the patient also performed Magnetic Resonance Imaging of the neck with intravenous contrast where a hyperintense nodule is described in T2 near the thyroid isthmus, measuring 12x11 mm. Based on these data, the patient underwent right parathyroidectomy. Postoperative biopsy confirmed the diagnosis of parathyroid adenoma. The patient was treated with Calcium and vitamin D3 to avoid transient postoperative hypoparathyroidism. The last inpatient PTH was

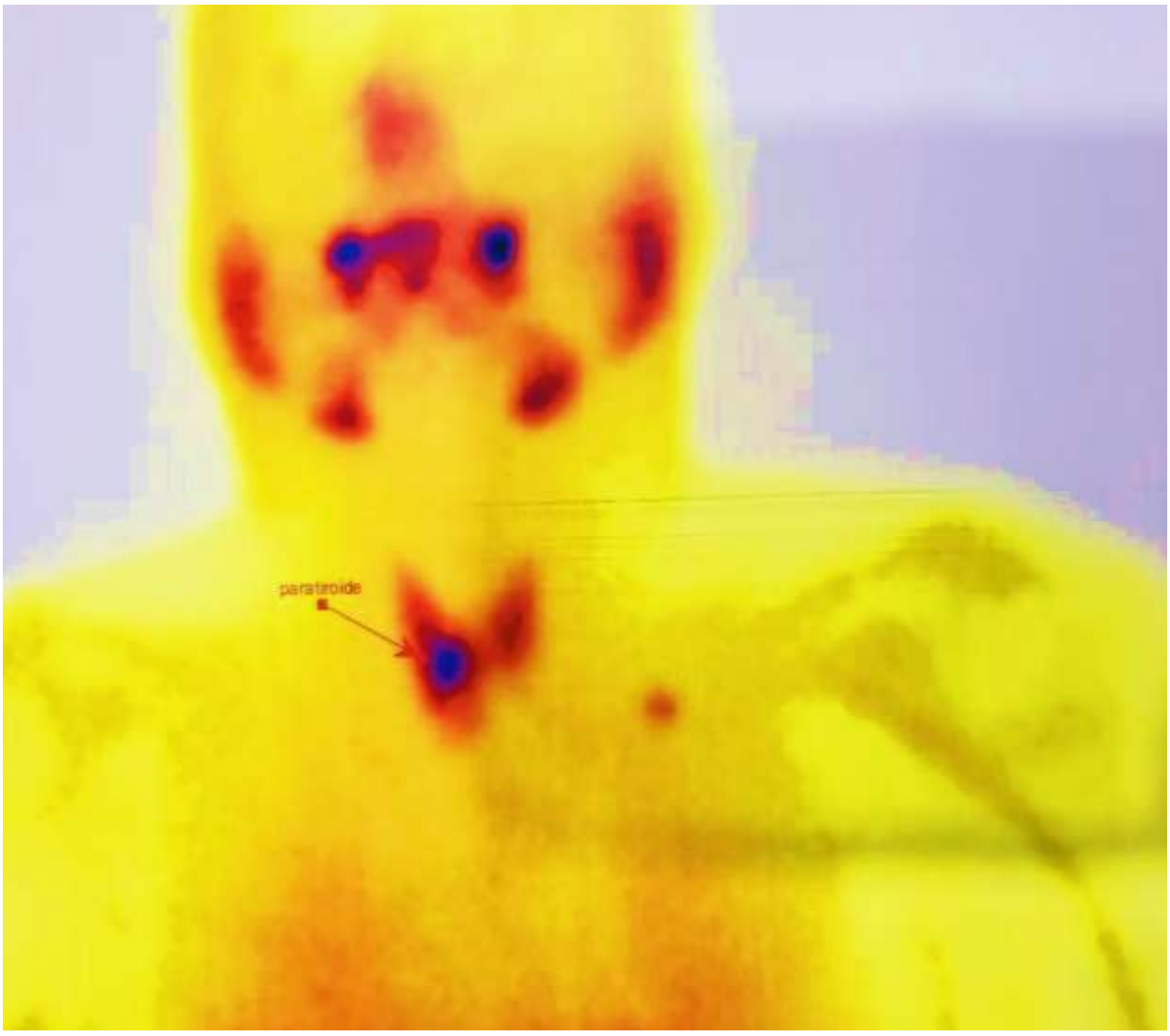

Figure 1. Scintigraphy with Sestamib

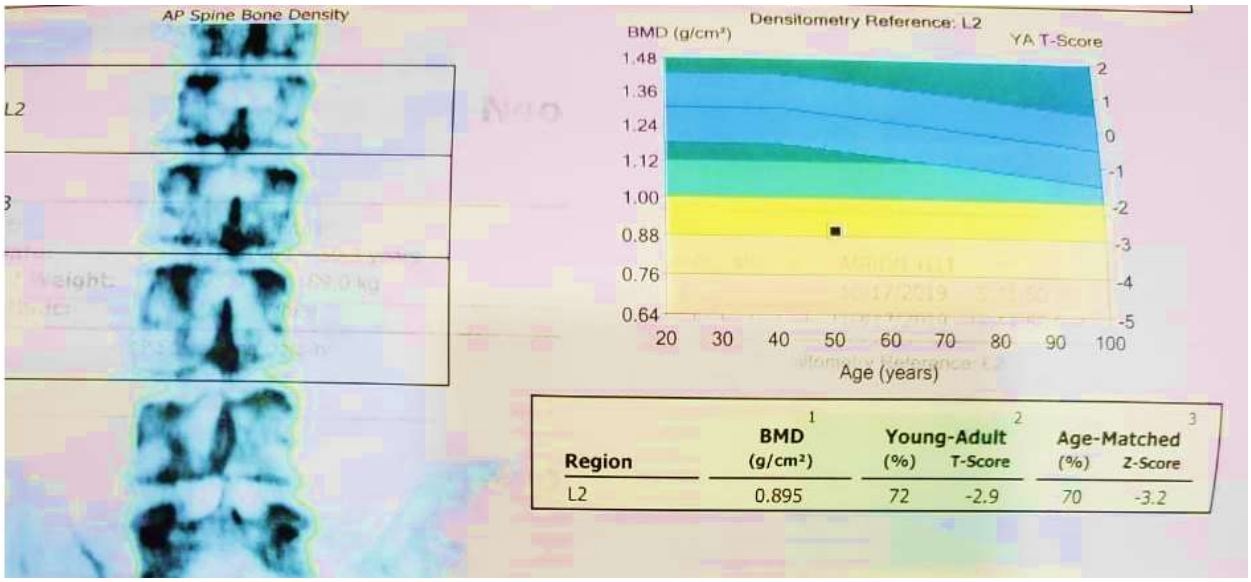

Figure 2. Bone densitometry

3.62 pg / ml. Three months after the intervention, in the next follow up, there was an increase of PTH (71.54 pg / ml for a normal range of 15 - 65 pg / ml) after an initial decrease post-op, calcemia (total and ionized) at the upper limit of range and phosphorus to the minimum range, as presented in the table below.

Table 1. Laboratory findings 3 months after intervention

	Values	**Normal Range**
Total Calcium	9.96 mg/dl	8.6-10 mg/dl
Ionised Calcium	1.25 mg/dl	1.13-1.32 mg/dl
Phosphorus	3.1 mg/dl	2.6-4.5 mg/dl
Vitamin D3	19.9 ng/ml	30-70 ng/ml
PTH	71.54 pg/ml	15-65 pg/ml

Clinically the patient was calm, with no subjective concerns. He continues to be in follow up to assess the origin of recurrent hyperparathyroidism leaving open the possibility of multiple adenomas.

DISCUSSION

Parathyroid adenoma is one of the most common pathologies of the gland. It constitutes the main cause of hyperparathyroidism. Meanwhile multiple adenomas cover only 4-5% of cases (6). The average age of diagnosis is 50-60 years. In most cases, patients are asymptomatic or with non-specific symptoms and often hypercalcemia is accidentally detected in routine blood tests. Even in our case, the patient, 51 years old, had no specific clinic. In the initial laboratory blood check, the high level of calcium suggested further biochemical and imaging exploration. Ultrasound

evaluation of the neck has a sensitivity of up to 80% in detecting parathyroid adenomas. They are visualized, mostly, as hypoechoic nodules at the upper or lower poles of the thyroid lobes. The image is amplified by CT or MRI of the neck which have a sensitivity of 75 and 85% respectively for the parathyroid glands (7). Sestamibi scintigraphy, combined with SPECT (Single photon emission computed tomography), is the 'gold standard' imaging examination in the diagnosis of parathyroid pathologies. Scintigraphy with 99Tc-Sestamibi utilizes the selective affinity of adenoma cells and has a sensitivity of up to 90%. Large solitary adenomas (over 1.8 cm) are more easily identified during examination (8). In our case, the ultrasound showed a suspicious formation (1.2 cm), which was also confirmed by magnetic resonance. But the diagnosis was not clarified until the lesion was localized by scintigraphy. Thanks to the complete imaging evaluation, a specific surgical intervention was performed: selective parathyroidectomy, with the condition that the function of other glands was preserved. But the sensitivity of all imaging methods fails on multiple parathyroid pathologies, compared to solitary adenomas. In such cases, ultrasound has a sensitivity of up to 16% and scintigraphy up to 78% (9). For this reason, preoperative evaluation in the case of multiple pathologies is more difficult and not always successful. Identifying a dominant nodule in imaging methods reduces the chance of finding a secondary lesion with less affinity for radioactive material. For this reason, today intraoperative evaluation of parathyroid glands is proposed by measuring PTH in situ (10). In the presented case, selective surgery was performed based on preoperative evaluation. In such cases, the risk for primary hyperparathyroidism is high. Our patient, in a period of less than 6 months after surgery came with high PTH, while in the postoperative period PTH levels were reduced below normal. This suggests there is still presence of pathological tissue in the parathyroid. Since preoperative scintigraphy with Sestamibi did not identify another focus, a second examination is recommended to correlate with the post-intervention PTH hormone level and the best therapeutic solution for the patient.

In conclusion, cases of involvement of more than one gland in primary hyperparathyroidism and low specificity of scintigraphy with Sestamibi, make intraoperative evaluation of PTH and close follow-up of post-parathyroidectomy patients important in order to avoid the risk of recurrence or the presence of hyper functional residual tissue.

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