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Formerly "Bulletin of Medical Sciences"

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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.
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CONTENT:

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Variations in Deaths due to Vascular and Unspecified Dementia in Japan

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Abstract

Background: Vascular and unspecified dementia, which is the 9th leading cause of death in Ja-pan (2018), and senility have become major public health challenges in Japan. Efforts to clarify variations in deaths due to dementia in all 47 prefectures in Japan will provide useful information for health care strategies for the elderly.

Objective: The present study was conducted to investigate variations in deaths due to vascular and unspecified dementia in all 47 prefectures in Japan.

Study design: This is an epidemiologic study.

Methods: The number of deaths due to 10 major causes, including vascular and unspecified dementia, between 1995 and 2019 in all 47 prefectures was obtained from the Statistics Bureau of Japan official website. Variations in deaths due to vascular and unspecified dementia were compared with those from other major causes. The effects of social factors on deaths due to vascular and unspecified dementia were also evaluated in an ecological study.

Results: Deaths due to vascular and unspecified dementia were the 9th major cause of death in Japan in 2018. Variations, represented by the coefficient of variation, in deaths due to vascular and unspecified dementia were the highest among the 10 major causes of death in all 47 prefectures in Japan. The number of elderly individuals (□65 years old) (%) and medical bills per elderly subject (□75 years old) (Japanese yen) were

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closely associated with deaths due to vascular and unspecified dementia in a multiple regression analysis.

Conclusion: Marked variations in deaths due to vascular and unspecified dementia were observed among all 47 prefectures in Japan.

Keywords: Dementia, Coefficient of variation, Japanese, Ecological study

INTRODUCTION

The number of elderly individuals (□65 years old) has markedly increased in Japan, and currently comprises 28.4% of the population (1). The number of deaths is also increasing with the aging of the Japanese population (2). We previously examined changes and variations in deaths due to senility, which has been the 3rd leading cause of death in Japan since 2018. The findings obtained revealed that deaths due to senility significantly increased after 1995 and variations in deaths due to senility were the highest among the 5 major causes of death (3). Therefore, the proper management of elderly individuals is urgently needed in Japan.

Vascular and unspecified dementia, which is the 9th leading cause of death in Japan (2018), and senility have become major public health challenges in Japan. A total of 4,620,000 elderly individuals (one in seven) were reported to have dementia in 2012 (4), and this number is expected to increase to 7,000,000 (one in five) by 2025. Life span was previously shown to be shorter in patients with than in those without dementia. Furthermore, life spans after the diagnosis of Alzheimer's disease and vascular dementia were 7.1 years (95%CI: 6.7-7.5) and 3.9 years (95%CI: 3.5-4.2), respectively (5). A previous study reported that the mortality rate was 2.5-fold higher in patients with than in those without dementia in developed countries (6), and dementia was identified as one of the major factors affecting this value (7-9). However, the number of deaths due to dementia is lower in Japan than in other countries (10), which suggests that deaths related to dementia are underreported (11). Therefore, efforts to clarify variations in deaths due to dementia in all 47 prefectures in Japan will provide useful information for health care strategies for the elderly.

In the present study, we examined variations in deaths due to vascular and unspecified dementia (9th leading cause of death) compared with other major causes of death in all 47 prefectures in Japan.

METHODS

Number of deaths

The 10 major causes of death in 2018 in Japan were as follows: (1) malignant neo-plasm, (2) heart diseases, (3) senility, (4) cerebrovascular diseases, (5) pneumonia, (6) accidents, (7) aspiration pneumonia, (8) renal failure, (9) vascular and unspecified dementia, and (10) suicide. Datasets on aspiration pneumonia between 1995 and 2016 were not available because it was only added as a new item in 2017. Therefore, we excluded "aspiration pneumonia" from the present analysis. The number of deaths due to the 9 major causes of death in 2018 in each of the 47 prefectures of Japan between 1995 and 2019 was obtained from the Statistics Bureau of Japan official website (12). The number of deaths due to each disease was adjusted according to the population and converted to the number of deaths per 100,000 individuals.

Social factors

The relationships between clinically important social factors and the number of deaths due to vascular and unspecified dementia between 2016 and 2019 (4 years) were examined (13). We analyzed the number of elderly individuals (□65 years old) (%), the number of single-person households (%), household income (Japanese yen), and medical bills per elderly subject (□75 years old) (Japanese yen) in the present study, as previously reported (3).

Ethics

All data used in the present study were obtained from an official governmental website that is accessible to the public. This study was approved by the Ethics Committee of Shikoku Gakuin University, Zentsuji city, Kagawa prefecture, Japan (approval number: 2020004, approval date: 10 Feb. 2021.).

Statistical analysis

Data were expressed as means ± standard deviations (SD). The coefficient of variation (CV), which is calculated by SD/mean, was used to examine variations in deaths due to major causes. We used the Kruskal-Wallis test and Steel test to compare CV among the major causes of death (3, 14). The relationships between the number of deaths due to vascular and unspecified dementia and social factors were examined using simple and multiple regression analyses, where $p<0.05$ indicates a significant difference. Statistical analyses were performed using JMP Pro version 15 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Figure 1 shows changes in the number of deaths stratified by the major causes, except for aspiration pneumonia, between 1995 and 2019 in Japan. In 2018, the number of deaths due to malignant neoplasm was the highest, followed by heart diseases, senility, cerebrovascular diseases, pneumonia, accidents, aspiration pneumonia, renal failure, vascular and unspecified dementia, and suicide. The number of deaths due to malignant neoplasm and heart diseases has been increasing in the last 25 years, whereas that due to cerebrovascular diseases has decreased. The number of deaths due to pneumonia decreased from 2017 following the separation of "aspiration pneumonia" from this category. A marked increase has been observed in deaths due to senility since 2004 (3).

The number of deaths due to the major causes stratified by cause for 25 years is summarized in Table 1. The number of deaths due to malignant neoplasm was the highest (263.5±29.5/100,000 people), while that due to vascular and unspecified dementia was 5.7±4.7/100,000 people (Figure 1, Table 1). The increase in the rate of deaths due to vascular and unspecified dementia was 786.4% (1995 vs 2019).

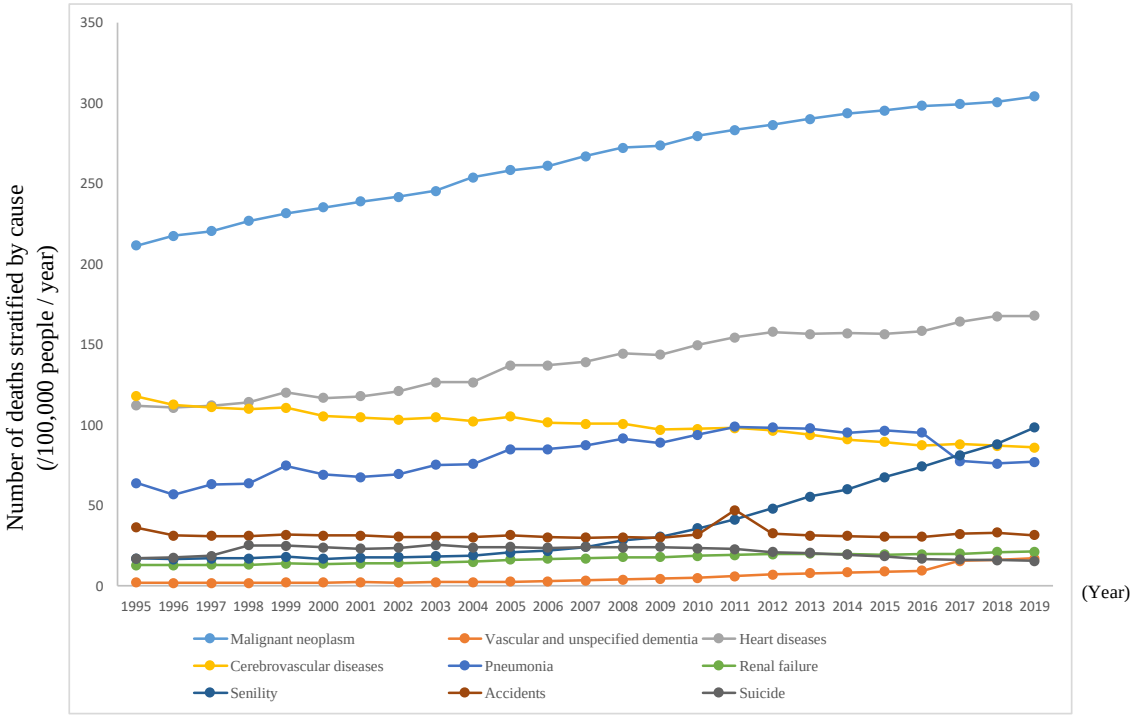

Figure 1. Changes in the number of deaths stratified by cause among all 47 prefectures in Japan (1995-2019)

Table 1. Number of deaths per 100,000 stratified by cause among all 47 prefectures in Japan (1995-2019)

	Mean ± SD	Minimum	Maximum	Rate of increase (%: 1995 vs 2019)
Number of years		25		
Malignant neoplasm	263.5 ± 29.5	211.6	304.2	143.8
Heart diseases	138.8 ± 19.5	110.8	167.9	149.9
Cerebrovascular diseases	100.2 ± 8.8	86.1	117.9	73.0
Pneumonia	81.0 ± 13.0	56.9	98.9	120.4
Senility	38.2 ± 26.0	16.7	98.5	569.4
Accidents	32.1 ± 3.4	30.0	47.1	86.8
Suicide	21.5 ± 3.4	15.7	25.5	91.3
Renal failure	17.0 ± 2.9	13.0	21.5	165.4
Vascular and unspecified dementia	5.7 ± 4.7	1.8	17.3	786.4

Per 100,000 in 47 prefectures in every year for 25 years. SD: standard deviation.

Table 2. Comparison of the coefficient of variation among deaths stratified by cause in all 47 prefectures in Japan (1995-2019)

	The value of the coefficient of variation Mean ± SD	95% CI	*p*
Malignant neoplasm	0.119 ± 0.003	0.084 - 0.155	<0.001
Heart diseases	0.162 ± 0.012	0.127 - 0.197	<0.001
Cerebrovascular diseases	0.220 ± 0.012	0.184 - 0.255	<0.001
Pneumonia	0.195 ± 0.017	0.159 - 0.230	<0.001
Senility	0.309 ± 0.039	0.274 - 0.344	<0.001
Accidents	0.274 ± 0.276	0.238 - 0.309	<0.001
Suicide	0.153 ± 0.031	0.118 - 0.188	<0.001
Renal failure	0.223 ± 0.010	0.187 - 0.258	<0.001
Vascular and unspecified dementia	0.372 ± 0.031	0.337 - 0.407	

vs Vascular and unspecified dementia as the control group by the Steel test.
SD: standard deviation.
95% CI: 95% Confidence Interval

We then compared CV among deaths stratified by the major causes in all 47 prefectures in Japan (Table 2). The CV of vascular and unspecified dementia was the highest among the major causes ($p<0.001$).

To assess the effects of social factors on the number of deaths due to vascular and unspecified dementia, we investigated the relationships between the number of deaths due to vascular and unspecified dementia and social factors between 2016 and 2019 using a simple correlation analysis (Table 3). The number of deaths due to vascular and unspecified dementia correlated with the number of elderly individuals (≥65 years old) (%), the number of single-person households (%), and medical bills per elderly subject (≥75 years old) (Japanese yen).

We also performed a multiple regression analysis with the number of deaths due to vascular and unspecified dementia between 2016 and 2019 as the dependent variable and the number of elderly individuals (≥65 years old) (%), the number of single-person households (%), and medical bills per elderly subject (≥75 years old) (Japanese yen) as independent variables (Table 4). The number of elderly individuals (≥65 years old) (%) and medical bills per elderly subject (≥75 years old) (Japanese yen) were important factors contributing to the number of deaths due to vascular and unspecified dementia between 2016 and 2019.

Table 3. Relationships between the number of deaths due to vascular and unspecified dementia and socioeconomic factors

	2016		2017		2018		2019	
	r	*p*	r	*p*	r	*p*	r	*p*
The number of elderly individuals (≥65 years old) (%)	0.483	**<0.001**	0.544	**<0.001**	0.540	**<0.001**	0.534	**<0.001**
The number of single-person households (%)	-0.449	**0.001**	-0.409	**0.001**	-0.363	**0.012**	-0.395	**0.006**
Household income (Japanese yen)	-0.040	0.788	-0.018	0.905	-0.025	0.870	0.021	0.891
Medical bills per elderly subject (≥75 years old) (Japanese yen)	-0.454	**0.001**	-0.400	**0.005**	-0.367	**0.011**	-0.387	**0.007**

Bold values: $p<0.05$ by a simple correlation analysis

Table 4. Relationships between the number of deaths due to vascular and unspecified dementia and socioeconomic factors by a multiple regression analysis

	B	95%CI		Standardized β	*p*	VIF
2016						
Constant	9.817	-5.414	25.048	0.000	0.201	.
The number of elderly individuals (≥65 years old) (%)	0.675	0.307	1.042	0.453	**0.001**	1.169
The number of single-person households (%)	-0.107	-0.393	0.180	-0.105	0.456	1.515
Medical bills per elderly subject (≥75 years old) (Japanese yen)	0.000	0.000	0.000	-0.408	**0.003**	1.352
R^2=0.450 p<0.001						
2017						
Constant	8.510	-10.266	27.286	0.000	0.366	.
The number of elderly individuals (≥65 years old) (%)	0.985	0.532	1.438	0.531	**<0.001**	1.169
The number of single-person households (%)	-0.067	-0.421	0.286	-0.053	0.702	1.515
Medical bills per elderly subject (≥75 years old) (Japanese yen)	0.000	0.000	0.000	-0.380	**0.006**	1.352
R^2=0.462 p<0.001						
2018						
Constant	5.548	-15.058	26.154	0.000	0.590	.
The number of elderly individuals (≥65 years old) (%)	1.071	0.574	1.568	0.541	**<0.001**	1.169
The number of single-person households (%)	-0.012	-0.400	0.376	-0.009	0.950	1.515
Medical bills per elderly subject (≥75 years old) (Japanese yen)	0.000	0.000	0.000	-0.368	**0.009**	1.352
R^2=0.430 p<0.001						
2019						
Constant	8.613	-13.266	30.492	0.000	0.432	.
The number of elderly individuals (≥65 years old) (%)	1.110	0.583	1.638	0.523	**<0.001**	1.169
The number of single-person households (%)	-0.066	-0.478	0.345	-0.046	0.747	1.515
Medical bills per elderly subject (≥75 years old) (Japanese yen)	0.000	0.000	0.000	-0.371	**0.008**	1.352
R^2=0.440 p<0.001						

95% CI: 95% Confidence Interval
VIF: Variance Inflation Factor

DISCUSSION

In the present study, we compared variations, expressed as CV, in vascular and unspecified dementia with those in the other major death causes in Japan. The CV of vascular and unspecified dementia was the highest among the major death causes.

The number of deaths due to vascular and unspecified dementia has been in-creasing. However, the number of deaths due to dementia in Japan is markedly lower than that in other countries (10, 12). The low mortality rate of dementia in Japan has been attributed to the following reasons: (1) the number of deaths due to dementia was very low, (2) deaths due to dementia were diagnosed as another cause of death, or (3) doctors did not indicate dementia in the death certificate due to family reluctance (10). An underestimation of deaths due to dementia has been reported in other countries (15, 16). In USA, only 25% of deaths due to dementia have actually been attributed to dementia (15). Previous studies that examined death certificates indicated that more direct causes of death, such as pneumonia, sepsis, and cardiovascular disease, were applied to cases of dementia (17). Patients with dementia often have multiple comorbidities, which increases the difficulties associated with the identification of the cause of death (18, 19). Although 28.8% of dementia-related deaths, including Alzheimer's disease, were diagnosed as deaths due to dementia in 2003 in Japan, and this rate rap-idly increased to 47.3% by 2016 (12), this is still lower than those in France and Italy (60%) (20). In many cases, a diagnosis other than dementia was listed as the cause of death.

Stigma towards dementia may also contribute to variations in its diagnosis (21). A lack of awareness and understanding of dementia has led to some prejudice or barriers to its diagnosis and care in many countries (22-24). In Japan, the Ministry of Health, Labour and Welfare changed the Japanese term "Chihou", a negative expression, to "Ninchisyo", a more positive word, in 2004 to reduce prejudice against dementia and deepen our understanding of the disease (25).

In the present study, the number of elderly individuals (≥65 years old) (%) and medical bills per elderly subject (≥75 years old) (Japanese yen) were identified as important factors contributing to the number of deaths due to vascular and unspecified dementia. In our previous study, the same factors affected the number of deaths due to senility (3). The prevalence of dementia is higher in older age groups and the proportion of dementia-related deaths to total deaths in Japan has increased 2.3-fold from 1.91% in 2003 to 4.45% in 2016 (10). Therefore, the number of elderly individuals (□65 years old) (%) may affect variations in the number of deaths due to vascular and un-specified dementia. Furthermore, patients with dementia often have multiple comorbidities (18, 19). A detailed examination and treatment of the more immediate cause of death, which is reflected by medical bills per elderly subject (□75 years old) (Japanese yen),

may lead to the adoption of a cause of death other than dementia.

Collectively, these factors, i.e., a suspected low rate of the diagnosis of dementia as the cause of death, stigma towards dementia, aging in Japan, and a detailed examination, may have contributed to the larger variations observed in the number of deaths due to vascular and unspecified dementia than in other major causes of death in all 47 prefectures in Japan in the present study. Therefore, the accurate definition of death due to vascular and unspecified dementia is important.

There were a number of limitations that need to be addressed. This was an eco-logical study and, thus, individual data were not used. Furthermore, the social factors examined in the present study were not sufficient for assessing the number of deaths due to vascular and unspecified dementia. Therefore, more detailed studies using individual data are urgently needed in the future.

CONCLUSION

Marked variations in deaths due to vascular and unspecified dementia were observed among all 47 prefectures in Japan.

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Conflicts of Interest statement:

The authors declare no conflict of interest.

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Introducing Speech Audiometry in Albanian Language

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Abstract

Background: In Albania, there are no validated speech audiometry lists of words.

Aims and Methods: The aim of this study was to create and to evaluate lists of words for children and young adults with normal hearing. In total, 20 lists of 10 spondaic dissyllabic words were created (10 for adults and 10 for children), taking into account the idiosyncrasies of the Albanian language. Eighty-three young adults and eighty-six children were included in this study from November 2018 to November 2019. For each patient's ear the difference between the Speech Recognition Threshold (SRT) and the Pure Tone Average threshold (PTA) was calculated to analyse if the value was less or equal to 7 dB in order to validate the 20 wordlists. The psychometric function slope of the resulted speech audiometry graph was calculated to compare it with the ones of other Indo-European languages.

Results: The difference between the SRT and PTA was 3.2 ± 2.1 dB in adults and 3.3 ± 2.4 dB in children. A value ≤ 7 dB was observed in 93 % of adults and 92% of children. The psychometric function slope was 7.3%/ dB in adults, and 7.9%/ dB in children, similar to those found in other Indo-European languages.

Speech audiometry in the Albanian language can now be performed using the lists defined in the present study.

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Keywords: Albania; Audiology; Pediatrics; Hearing loss, Auditory performance.

INTRODUCTION

Listening is a function of the perception of an acoustic signal and at the same time it realizes its identification.

Vocal audiometry plays a crucial role in assessing hearing capacity and assessing the possibility of speech recognition by the human hearing apparatus. Vocal audiometry is an examination, a test, used to diagnose hearing problems. It requires the implication not only of the neurosensory peripheral apparatus of hearing, but also, the knowledge of the spoken language, and the culture associated with a particular language as it is based on a list of specific words. (1) (2) (3)

The first studies on vocal audiometry were done by a monk (L'Abbé ROUSSELOT, 1886-1924). In the middle of the 20th century, first wordlists were created in English (Hudgins & Hawkins, 1947), in French (Fournier, 1951; Lafon, 1964), in Italian (Azzi Bocca Pellegrini, 1950), in Russian (Aleksandrowski, 1998), in Polish (Skarzynski, 2004), in Saudi Arabic speech. (Ashoor AA 1985) (4) (5) (6) (7) (12)

The purpose of speech audiometry is to assess the intelligibility, which is the ability to perceive and process speech, by understanding and repeating correctly a specific list of words. This test, associated with pure tone audiometry, helps to better qualify hearing impairment.

In the clinical auditory practice, there are two forms of vocal audiometry: one for adults and one for children, due to the different spoken vocabulary used by these two age groups.

In adults, vocal audiometry is a more sensitive and accurate indicator than tonal audiometry in various auditory pathologies, such as "immediate deafness" or neurological pathology. Vocal audiometry is an irreplaceable tool for the auditory examination of a patient who wants to use a hearing prosthesis.

In children, vocal audiometry is used to:

- assess the child's ability to repeat words and his/ her communication development;
- help in deciding if an "auditory rehabilitation" or an auditory prosthesis is necessary (in case we are dealing with a child with significant hearing loss);
- make it possible in a concrete and visual way for the child's parents to understand the lack of the child's ability to hear and understand a word or sentence;
- help in a concrete way in choosing the type of hearing prosthesis (conventional device or cochlear implant).

The patient is presented with different lists of ten words (disyllabic) at different sound intensities, for each ear. The results of the vocal audiometry are then represented on a graph for each ear with the sound intensity in decibels (dB) on the abscissa, and the percentage of words understood on the ordinate. The curve takes on a sigmoid shape in healthy subjects. One of the most fundamental measures in speech audiometry is the Speech Recognition Threshold (SRT). It is

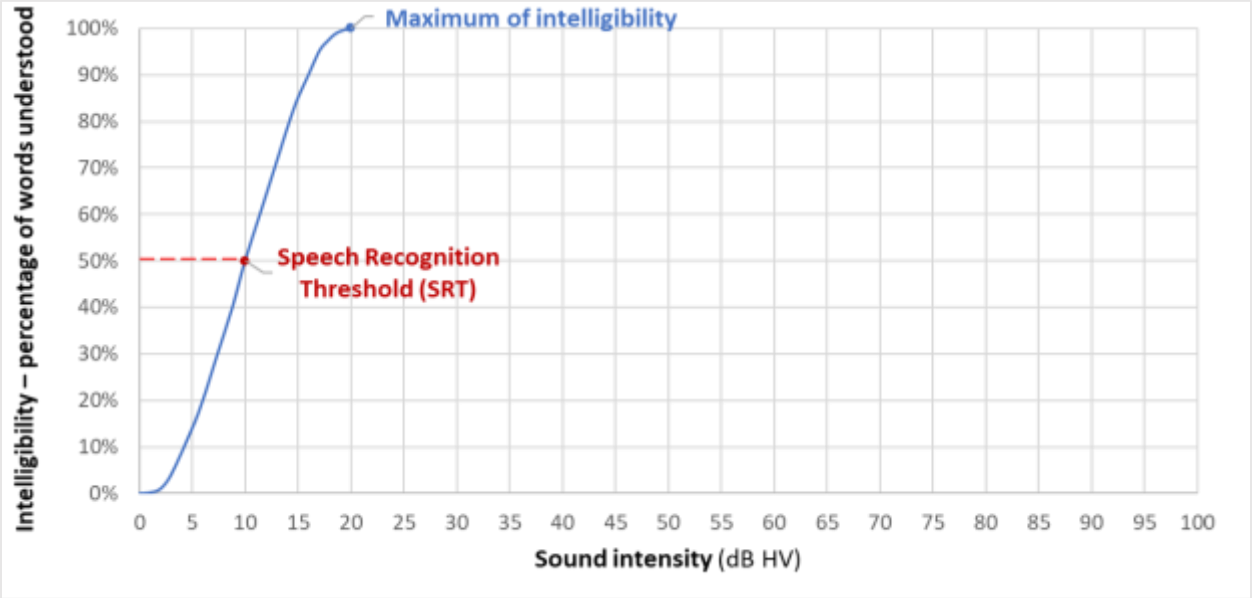

Figure 1. Theoretical normal speech audiometry graphic

defined as the lowest level in dB HL at which an individual can correctly identify 50% of the words (« Guidelines for Determining Threshold Level for Speech », 1988). As for the maximum of intelligibility, it is the highest point on the curve. (**Fig. 1**) (1) (3) (8) (9) (11)

Familiarity, homogeneity and standardisation are critical elements to consider when creating lists for speech audiometry evaluations. Because the purpose of SRT testing is to measure the auditory threshold for speech, the words selected as stimuli should be as familiar as possible (Hudgins & Hawkins, 1947, Jahner et al, 1994; Young et al, 1982). (10)

Objectives

The first goal of this study was to create 20 lists of 10 words each, dealing with the following criteria:

- dissyllabic words with 4 or 5 phonemes;
- familiar to each age group (adults or children);
- auditory and grammatically homogenous;
- phonetically different in spectral analysis;
- almost all the phonemes of the language must be represented in a list of 10 words, taking into consideration the occurrence of these phonemes in the Albanian language;
- each syllable must be pronounced at the same intensity (spondaic words).

The second goal of the study was to evaluate these lists of words in the Albanian language in a sample of children and adults with normal hearing, by comparing SRT and PTA. Indeed, to validate these lists, the difference between SRT and PTA thresholds (average 500, 1000, 2000, 4000 Hz) must be less than or equal to 7 or 10 dB. (11) (13) (14) (15) (16) (17)

Some characteristics of the Albanian language

The Albanian language contains 36 letters: 7 vowels and 29 consonants, of which 9 are digraphs. A letter represents a phoneme. Most of

the syllables are “open syllables CV” (starting with a consonant - C - followed by a vowel - V). 92% of words start with a consonant. 67% of words end with a vowel.

The phoneme « ë » is used in about 29% at the end of words, but it is a deaf phoneme and as such it is not used (only spondaic words are chosen).

The average length of a word in Albanian is 1.8 syllables / 4 phonemes.

Vowels are rare at the beginning of a word; only 8% of words start with them, while 92% of words start with consonants (digraph or not).

The selected words are always used in the nominative form (Prominent form).

The phonetic principle is the basic principle of orthography of the Albanian language not only because in the Albanian language words are written as they are pronounced, but also for because the phonetic principle corresponds to the morphological principle of the word. (18) (19) (20)

MATERIALS AND METHODS

Twenty lists of ten words were created (10 with adult vocabulary, 10 with children one), meeting the criteria mentioned in the “OBJECTIVES” paragraph and the idiosyncrasies of the Albanian language (**Table 1**). Thus, all words started with a consonant, and no word ending with the phoneme « ë » was selected since it is a deaf phoneme.

Table 1. The 20 wordlists created

	List 1	List 2	List 3	List 4	List 5	List 6	List 7	List 8	List 9	List 10
Adults	Pinca Vëndi Lëngu Kafsha Bari Pyka Dhëmbi Fuçia Demi Kocka	Pragu Tulla Gishti Peshku Busti Krimbi Porta Plaga Trungu Vesa	Njolla Dëngu Truri Forma Klubi Tregu Lakra Brazda Kushti Bloza	Lista Peshku Plaku Muri Gjesti Qëngji Shkëmbi Gjoksi Shuli Veshka	Kashta Shkalla Kllapa Pisha Vepra Mushka Varka Birra Plumbi Pushka	Grimca Lënda Kisha Gjuha Cungu Gozhda Zemra Brumi Pirgu Basti	Tanku Djathi Bota Motra Kocka Predha Trari Bluza Vajza Dushi	Gjalpi Supi Gjëza Brryli Vrima Shansi Salla Posta Shega Lisi	Fshati Shtegu Filmi Shtypi Delja Ngjyra Gripi Sherri Kafja Kyçi	Koka Derri Rrushi Bregu Deti Sheshi Banka Viçi Sofra Xhami
Children	Balli Syri Gjuri Lufta Goma Kali Babi Puna Libri Darka	Veza Mami Dora Buka Lecka Boja Kosi Luga Furça Topi	Lapsi Shoku Thesi Vula Goja Trimi Lulja Pasta Tymi Rrota	Çanta Treni Kënga Dera Gjumi Noti Pleshti Rrufa Ferra Pema	Pula Buza Mali Xhaja Gjeli Burri Gota Vapa Kolla Dielli	Gjyshi Leshi Macja Zogu Bora Rrypi Luani Pjata Dreka Gruri	Kripa Gjaku Shala Xhaxhi Thika Krahu Toka Drita Guri Dimri	Veshi Triko Lumi Qeni Nata Gryka Forca Shteti Lodra Fshesa	Shkolla Çifti Pulla Fusha Pika Kati Princi Nëna Miku Koha	Llampa Torta Shkopi Fiku Supa Barku Sharra Xhepi Këmba Pylli

Another criteria taken into consideration is that each wordlist contains all frequencies of the Albanian language. A special software program (Sound spectral analysis; AUDACITY programs) allowed for acoustic analysis of spondaic words and made it possible to find the words with equal phonetic value in Albanian language. (**Fig. 2**) (21)

(Tympanometry type A). They all had normal tonal hearing, which corresponds to an average loss of less 26 dB HL from 250 to 8000 Hz (Silman & Silverman's classification system) or less than 20 dB SPL.

The following equipment were used: a type 1 of Audiometer (IEC 60645-1; IEC 60645. Type A) INTER ACOUSTICS AD229b calibrated in

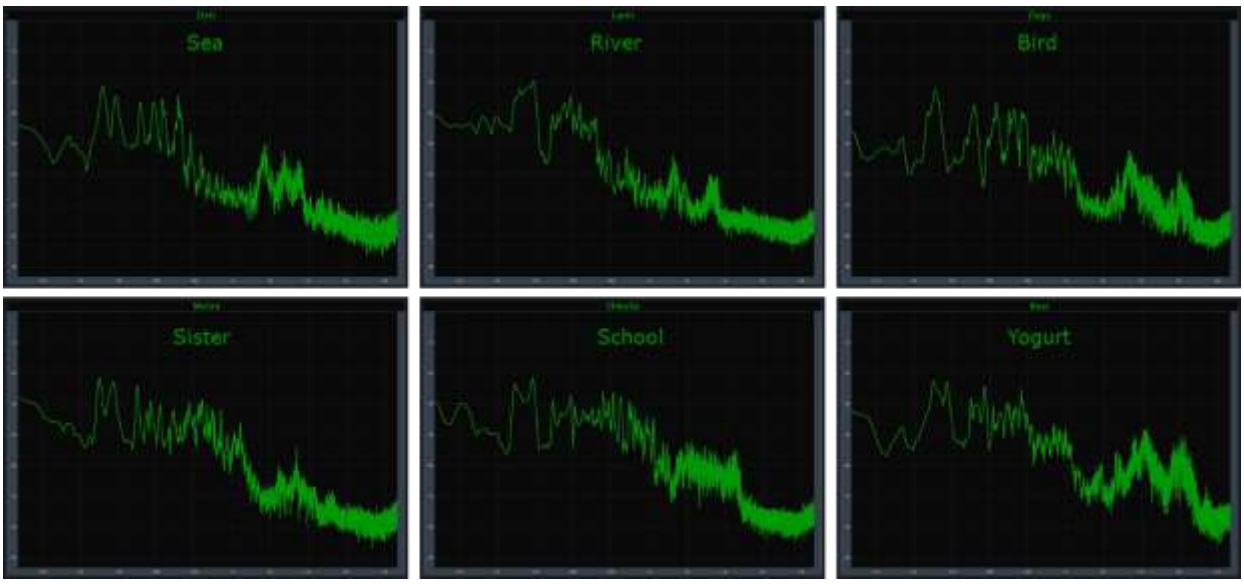

Figure 2. Sound spectral analysis of some selected words
(Sea = Deti; River = Bregu; Bird = Zogu; Sister = Motër; School=Shkolla; Yogurt = Kosi)

Each list contains 10 words (one word = 10% on the intelligibility scale).

From November 2018 to November 2019 83 adults aged 18 to 25 years old and 86 children aged 5 to 14 years old with no otological story were included in this study. They were all of Albanian origin and all spoke Albanian. All subjects or their legal representatives gave their written consent.

Examination of the external auditory channel and tympanic membrane was normal for all subjects. Tympanometry was normal for all subjects

October 2018, a SENNHEISER HDA 200 type headset, a standard audiometric booth (ambient noise < 30dBA) and an INTERACOUSTIC AT225 type tympanometry. The dissyllabic words were recorded and digitized on CD and USB stick in the Albanian State Radio Studios by an Albanian woman native speaker. (22) The CD player used was the PANASSONIC SA PMX80. The speech audiometry test was performed using the top-down method, every 3 dB, with the created wordlists. In order to achieve the vocal

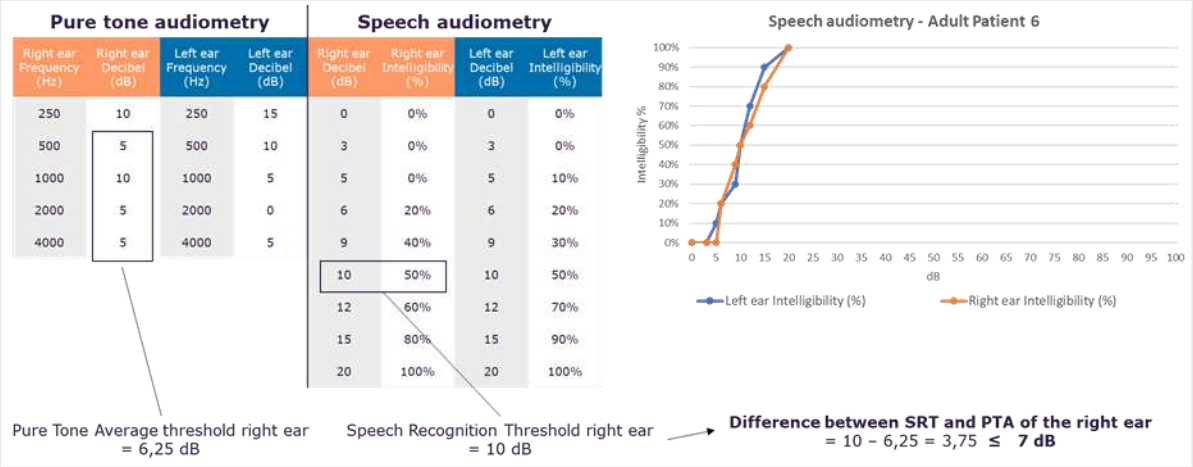

Figure 3. Example of the speech audiometry (adult patient N°6)

curve, each patient heard and repeated 7 wordlists per ear, all independent and different.

The test duration was approximately 35 min: 10 minutes of pure tone audiometry, 5 minutes of tympanometry and 20 min of speech audiometry. For each subject and for each ear, we calculated the absolute value of the difference between SRT and PTA thresholds. The PTA thresholds were obtained by calculating the average of the tonal thresholds at frequencies 500 Hz 1000 Hz, 2000 Hz and 4000 Hz.

The average of these differences was calculated within the adult and paediatric population (**Fig. 3**).

Psychometric function slope (%/dB) from 20 to 80 % was also calculated on two curves corresponding to the averaging of the curves of all the ears tested within the adult group and the paediatric group.

RESULTS

Among the adult group, there were 17 men and 66 women. 62 subjects were between 18 and 21 years old and 21 subjects were between 22 and 25 years old. Of the 166 ears tested, the difference between SRT and PTA was 3.2 ± 2.14 dB (mean ± standard deviation). Only one ear had a difference of more than 10 dB, 10 ears had a difference between 7 and 10 dB, and 155 ears had a difference of 7 dB or less in terms of absolute value between SRT and PTA.

By averaging all the curves obtained in adults, a sigmoid curve was obtained: 0% at 0 dB, 50% at 9 dB, 59% at 10 dB, and 100% at 20 dB (**Fig. 4**)

Among the children group, there were 49 boys and 37 girls. Twenty subjects were between 5 and 7 years old, 40 subjects were between 8 and 10 years old, and 26 subjects were between 11 and 14 years old. Of the 172 ears tested the difference between SRT and PTA was 3.3 ± 2.41dB, (mean ± standard deviation). Thirteen ears had a difference between 7 and 10 dB, and 159 ears had a difference of 7 dB, or less in terms of absolute value between SRT and PTA.

By averaging all the curves obtained in pediatric subjects, a sigmoid is obtained 0% at 0 dB, 26%

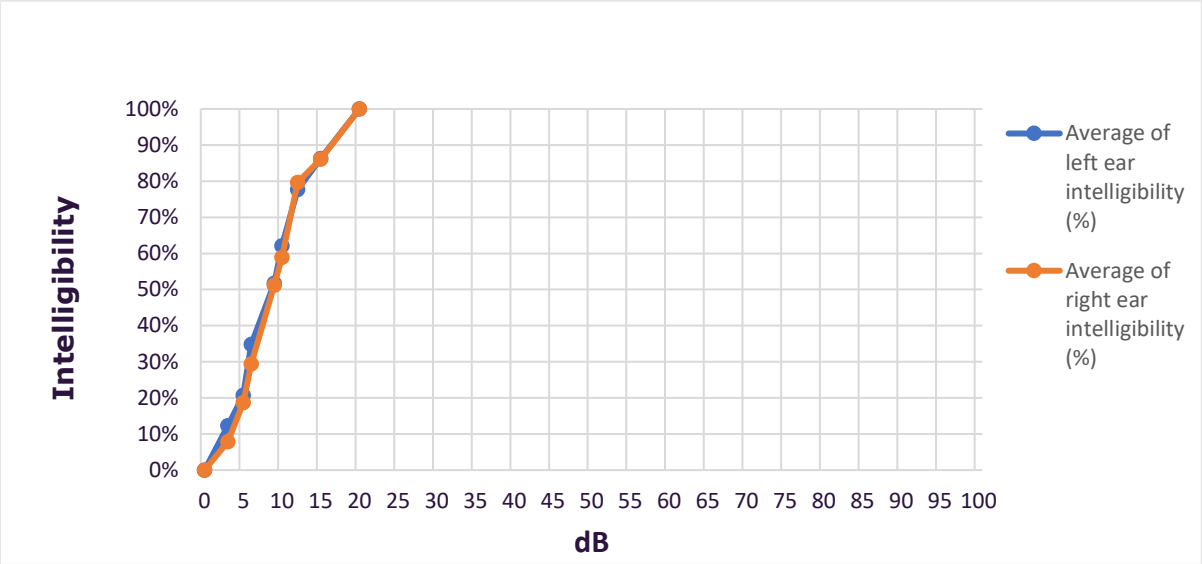

Figure 4. Normal speech audiometry - Children

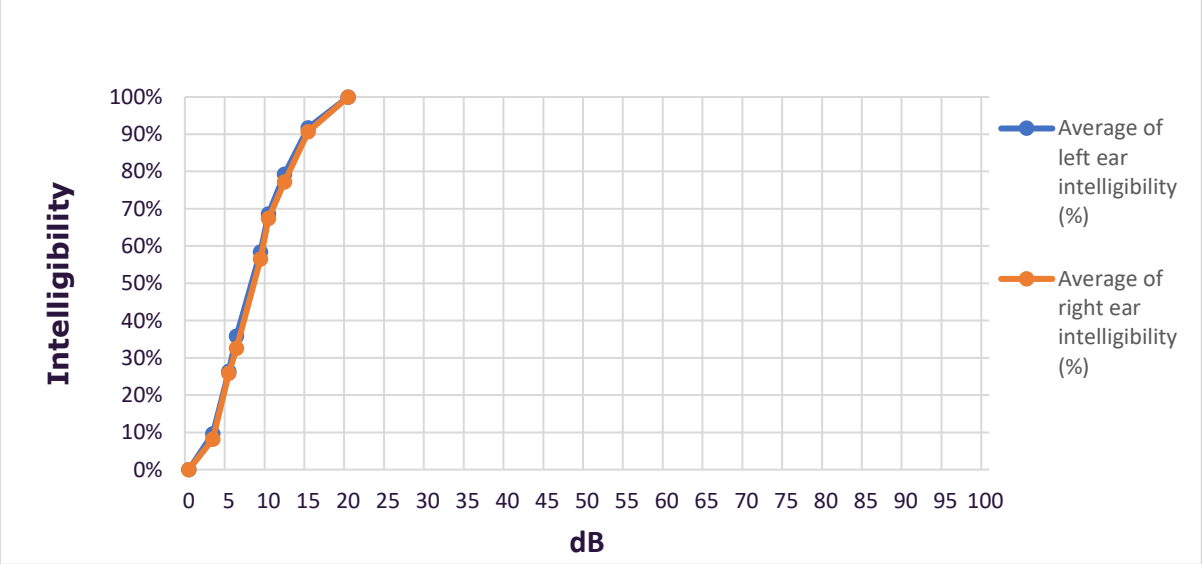

Figure 5. Normal speech audiometry - Children

at 5 dB, 50% at 9 dB, 100% at 20 dB (**Fig. 5**). Psychometric function slope from 20 to 80% was 7.5%/ dB. (23) (24)

DISCUSSION

The primary aim of the study was to develop standardized Speech Audiometry Wordlists in Albanian, that will be used in the future to better diagnose and monitor hearing and speech pathology in Albanian population (adults and children).

We have been able to develop a set of Albanian dissyllabic wordlists which are homogeneous in performance with respect to audibility and psychometric function slope for subjects with normal hearing. (22)

In other Indo-European languages, the main slopes have also been similar to those found in the present study: between 7.2%/dB, and 10%/dB in English (Hudkins & Hawkins, 1947; Wilson & Strouse, 1999), between 9.8 and 10.1%/dB in Polish (Harris, 2004), between 9.7 and 11.1%/dB in Spanish (Nissen, 2005), and around 13%/dB in Italian (Puglisi and al, 2015). (24)

CONCLUSION

Speech audiometry in Albanian language can be performed with these wordlists studied in this research. To our knowledge, our work is the first dealing with the speech audiometry in the Albanian language. Since this study was performed on children and adults with normal hearing, it would be useful in the future to conduct further studies with patients with hearing loss of various levels

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Conflict of Interest Disclosure:

The authors declare that they have no conflict of interest.

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Altered Cerebellar Metabolic Parameters in Bromazepam Treated Rats: Implications of Gradual Cessation Protocol

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Abstract

Objectives: Many reports avail on the physiological implications of benzodiazepine and bromazepam use and misuse. The aim of the study was to investigate the effect of gradual cessation protocol of bromazepam administrations on cerebellar metabolisms in female rats.

Methods: Twenty-five female rats weighing 150-160g were randomly divided into five groups of five rats each. Administration of a single daily oral dose of 1.15mg/kg body weight of bromazepam was done for nine days. Cessation was thereafter done three, six and nine days respectively. Cerebellar levels of glycogen, glucose and lactate, blood/cerebellum glucose ratio, cerebellar glucose/glycogen ratio, ataxia index and other parameters were determined.

Results: Bromazepam administration caused significant reduction in cerebellar glycogen. Bromazepam-induced depression in glycogen content was also observed 3days after cessation. However, restoration of the glycogen occurred and peaked 6 days after cessation. Plasma/ Cerebellar glucose ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Cerebellar glucose/glycogen ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Bromazepam administration significantly increased ataxia index when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Ataxia index correlated negatively

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with cerebellar glycogen ($r = -0.712$, $P<0.05$) and positively with cerebellar glucose/ glycogen ratio ($r = 0.917$, $P<0.05$) respectively.

Conclusion: The results of the study indicated the adverse but time-dependent reversible effects of bromazepam on cerebellum metabolic parameters in adult female Wistar rats.

Keywords: Cerebellum, Lactate, Glycogen, Bromazepam, Coordination, Ataxia Index

INTRODUCTION

Cerebellum is a part of rhombencephalon and it is situated in the posterior cranial fossa. It averagely weighs 150g in adult male (1). Like other brain areas, its outer part is made of gray mater formed from nerve cell bodies and inner gray mater made of nerve fibers (2). Functionally, cerebellum consists of cerebrocerebellar, spinocerebellum and vestibulocerebellum. Cerebellum plays role in balance and posture. It is concerned with motor coordination, a process which involves motor planning and time-based (1) integration between sensory input and motor output (2). Cerebellar is also a center for motor learning. Increase in cerebellum activities occurs when the position of head in space changes (1). Besides this, exposure to chemicals also affects cerebellar functions. Drugs with documented influences on cerebellum include alcohol, antipsychotic drugs and sedatives including bromazepam.

Bromazepam is a moderate lipid-soluble benzodiazepine with characteristic bromine and pyridine rings at positions 7 and 5 respectively (3). Ratified for public use in 1974, it is available in many countries except United States of America. As a mood-altering medicine, bromazepam is generally employed as anti-anxiety, anticonvulsant and hypnotic drug (4). Like alcohol, bromazepam acts by binding with GABA A receptors thereby enhancing the inhibitory effects of Gamma Amino Butyric Acid in the brain and leading to reduction in CNS activity (5). Common side effects associated with bromazepam include drowsiness, dizziness, attenuated working memory, impaired attention and motor coordination with severity being dose-dependent (6).

Like other drugs, bromazepam is degraded by hepatic cytochrome P-450 system into hydroxybromazepam (6). Studies on the likely effect of overdose or long-term administrations of the drug indicated indistinct speech, depressed respiratory function, cognitive deterioration, impaired attention, coma, decreased libido, reduction in hemoglobin and packed cell volume, decreased sperm count and percentage live sperm cells and elevated percentage of dead spermatozoa and severe abnormal sperm morphology (7), reduced LH, FSH and testosterone secretions (8). Others include psychomotor function and ataxia.

Ataxia is a condition in which there is impaired coordination of voluntary movements (1). It represents deviation in the functionality of neural structures involved in coordination. Physiological functions impaired include motor precision, locomotion, articulation, vision, swallowing among others. These include the vestibular apparatus, cerebral cortex, and cerebellum. While vestibular ataxia is motor incoordination resulting from abnormality in vestibular apparatus, sensory ataxia refers to incoordination that stems from loss of proprioceptive inputs to the brain or derangement of brain areas such as parietal lobe and thalamic nuclei which are involved in proprioception.

Cerebellar ataxia is motor incoordination that occurs when cerebellum malfunctions (2).

Many studies have identified likely mechanisms of chemical induced ataxia. For instance, diazepam was shown to reduce cyclic guanosine monophosphate (cGMP) in cerebellar slices dose-dependently (9). Administration of diazepam dose just below the toxic level enhanced malondialdehyde levels in cerebellum and brainstem and decreased mitochondrial glutathione reductase level (6). Castro *et al.*, (2009) (3) showed increased lipid peroxidation in the cerebral cortex and cerebellum and high level of carbonyl production in the striatum of rodent brain. Diazepam was shown to reduce serum glucose (10) and its prolonged use reported to result in decreased packed cell volume and hemoglobin concentration in rats (11). Chronic administration of diphenylhydantoin reduced brain metabolisms using [14C] deoxyglucose technique (12) in humans. Using 18-fluorodeoxyglucose, diazepam decreased whole brain metabolic rate without causing alteration in specific region.

In addition to non-invasive glucose uptake procedure, cerebellar lactate, cerebellar glycogen and glucose levels, glucose and glycogen ratio and plasma and cerebellum glucose ratio are indices of cerebellar metabolisms. The aim of the study was to determine the effect of gradual cessation protocol of bromazepam administrations on cerebellar lactate, cerebellar glycogen and glucose levels, glucose and glycogen ratio and plasma and cerebellum glucose ratio in female rats.

MATERIALS AND METHODS

Animal Care and management

Twenty-five female Wistar rats weighing between 150g-160g were used for the study. They were obtained from the Animal house of the Department of Physiology, University of Benin, Nigeria. They were housed in standard cages designed as previously reported (13-15) at room temperature and 12hr light/12hr dark cycle. The animals were acclimatized for 1 week. All rats were fed pelletized grower mash (standard chow) and distilled water *ad libitum.*

Ethical certification

The study (MED/294738) was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats. Consent and unwritten approval of the Research and Ethics Committee of the above University and department were received before the study.

Reagents and animal treatments

A sachet of bromazepam (3 mg, manufactured by May and Baker, Nigeria) was purchased from a registered pharmaceutical company in Auchi. A tablet of bromazepam was dissolved in 5 milliliters of distilled water to form a stock solution. An oral therapeutic dose of 1.15 mg/kg body weight was administered singly between 8.00 am and 10.00 am once per day.

Study design

The rats were randomly divided into five groups of five rats per group.

Control group (CTRL): received 0.3ml of distill water once per day.

Bromazepam-treated group (9d BR): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days.

Bromazepam (BR) withdrawal group I (3d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 3-day cessation.

Bromazepam withdrawal group II (6d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 6-day cessation.

Bromazepam withdrawal group III (9d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 9-day cessation.

Evaluation of body weight

Body weight was measured before and in the course of the study and prior to euthanasia using weighing scale. Change in weight was calculated as (Final weight – Initial weight)/ Initial weight X 100%.

Evaluation of fasting blood glucose

Following twelve hours of overnight fasting, blood glucose level was assessed using glucometer (Roche, Germany).

Ataxia index

The beam apparatus was designed according to the method of Tinh *et al.,* (2011) (16) and consisted of 1.1m beam with a flat surface of 30mm width, 40cm above the base level. All rats were allowed to tread the beam during the training session just before the coordination evaluation was done. Ataxia index was scored as the time taken for rats to tread from one end of the beam to the other end. In cases where rats turned towards the underside of the beam, the test was repeated after 20 minutes of rest. Where it became consistent, a value of 120 seconds was awarded. Ataxia index was conducted at the end of the study.

Plasma and tissue preparations

The duration of the study was 18 days. At the end of the experiment, rats were euthanized through cervical dislocation between 8-10am after 12 hours overnight fasting. Blood was collected into lithium heparin bottle for packed cell volume and hemoglobin determinations. Whole cerebellum was excised and preserved in Phosphate Buffer solution for biochemical analysis.

Determinations of packed cell volume and hemoglobin concentration

Packed cell volume

It was based on the application of centrifugal force (at 12,000RPM) to recover blood cells from anticoagulant blood in a tube.

Hemoglobin

Hemoglobin concentration was measured using the popular Sahli's method.

Determination of cerebellar catalase

Catalase activity was determined according to the method Sinha (1971) (17).

Principle

The method was based on the fact that dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The Chromic acetate produced was measured colorimetrically at 570nm.

Cerebellar metabolic parameters

Cerebellar glycogen assay

Principle

Degradation into protein and free saccharide occurred when the tissue was placed in boiling solution of potassium hydroxide. In contrast, glycogen was stable in the alkali solution. After potassium hydroxide treatment, glycogen was precipitated with 96% ethanol, washed, diluted and hydrolyzed in H_2SO_4. Glycogen hydrolysis produced free glucose, which was determined by enzymatic reaction in the presence of glucose oxidase. Glucose was converted by glucose oxidase to gluconic acid. The by-product of this reaction was H_2O_2. The hydrogen peroxide formed reacted under catalysis of peroxidase with phenol and 4-aminophenazone to form red-violet product suitable for photometric determination.

Cerebellar glucose assay

Principle

Glucose oxidase (GOD) catalyzed the oxidation of glucose to gluconate. The hydrogen peroxide (H_2O_2) produced was detected by a chromogenic oxygen acceptor, phenol, 4-Aminophenazone (4-AP) in the presence of peroxidase using standard laboratory procedure.

Cerebellar lactate assay

It was done using Enzyme Linked Immunosorbent Assay.

Statistical analysis

Statistical analysis was done using One-way ANOVA at five rats per group. Pairwise comparison was done using Least Square Difference (LSD).

RESULTS

Effect of Bromazepam and its gradual cessation on cerebellar metabolic parameters

Administration of bromazepam for nine days (Table 1) significantly reduced cerebellar glycogen when compared with control group. There was also a significant reduction in cerebellar glycogen three days after cessation when compared with control group. When compared with bromazepam treated rats, bromazepam cessation for three days, six days and nine days significantly increased cerebellar glycogen respectively. When compared with 6-day cessation group, bromazepam cessation for three and nine days respectively decreased cerebellar glycogen.

Bromazepam treatment for nine days significantly decreased cerebellar glucose when

compared with 3 days, 6 days and nine days bromazepam cessation respectively.

Cerebellar lactate significantly reduced 6 days and 9 days after bromazepam cessation when compared with 3 days-bromazepam withdrawal group respectively.

Plasma/ Cerebellar glucose ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day bromazepam cessation groups respectively.

Cerebellar glucose/glycogen ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day bromazepam cessation groups respectively.

Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. * represents significant difference (P< 0.05) from control. abcd represent significant difference from 9d BR, 3d BR-wd, 6d BR-wd and 9d BR-wd respectively.

Effect of Bromazepam and its gradual withdrawal on Ataxia Index

Bromazepam administration for nine days significantly increased ataxia index when compared with control, 3-day, 6-day and 9-day cessation groups respectively (Figure 1).

Table 1. Effect of Bromazepam and its gradual withdrawal on cerebellar metabolic parameters

Parameters	**Groups**				
	CTRL	**9d BR**	**3d BR-wd**	**6d BR-wd**	**9d BR-wd**
Cerebellar Glycogen (PPM)	33.700 ± 2.435	5.300 ± 0.095*bcd	26.900 ± 2.593*	37.800 ± 1.961bd	29.400 ± 1.804
Cerebellar Glucose (mg/dl)	3.940 ± 0.114	2.686 ± 0.40bcd	4.660 ± 0.680	5.050 ± 0.621*	5.230 ± 0.604*
Cerebellar Lactate (mmol/L)	0.336 ± 0.004	0.310 ± 0.0251	0.350 ± 0.016	0.300 ± 0.000[b]	0.288 ± 0.022[b]
Plasma/Cerebellar Glucose Ratio	18.147 ± 1.094	38.969 ± 6.478*bcd	21.649 ± 2.689	14.490 ± 1.515	13.550 ± 1.645
Cerebellar Glucose/Glycogen Ratio	0.121 ± 0.012	0.512 ± 0.084*bcd	0.170 ± 0.010	0.133 ± 0.012	0.179 ± 0.215

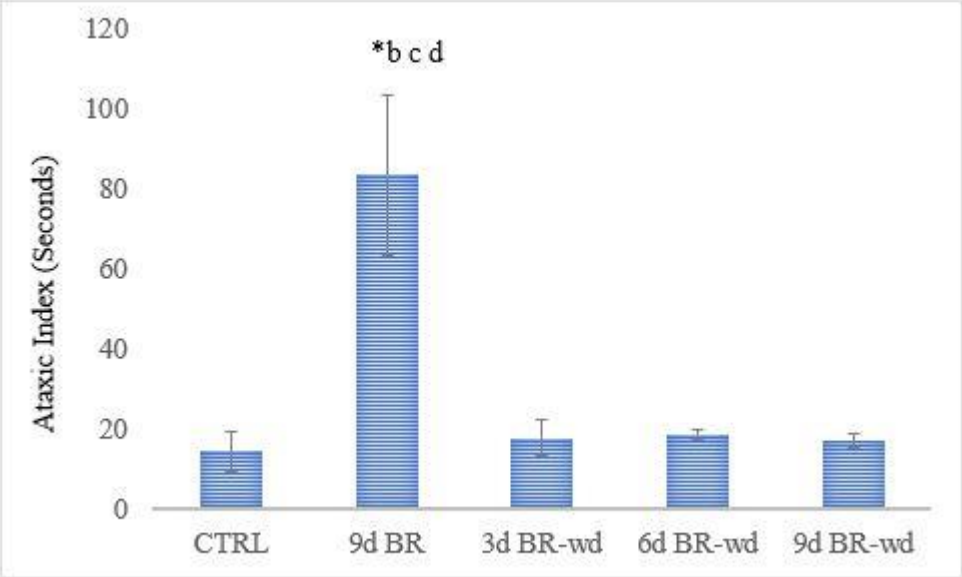

Figure 1. Effect of Bromazepam and its gradual withdrawal on ataxic index. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. * represents significant difference (P< 0.05) from control. abcd represent significant difference from 9d BR, 3d BR-wd, 6d BR-wd and 9d BR-wd respectively.

Correlation between ataxia index and cerebellar metabolic parameters

There was a significant strong negative correlation between cerebellar glycogen and ataxia index (Table 2). There was also a significant strong positive correlation between cerebellar glucose/glycogen ratio and ataxia index.

Effect of Bromazepam and its gradual withdrawal on Red Blood Cell

Bromazepam administration for nine days caused a significant decrease in packed cell volume and hemoglobin concentration (Table 3) when compared with control, 3-day, 6-day and 9-day cessation groups.

Table 2. Correlation between ataxia index and cerebellar metabolic parameters

Pearson correlation	**Ataxia index**
Cerebellar glycogen	- 0.712*
Cerebellar glucose/glycogen ratio	0.917*
Cerebellar lactate	0.056
Blood/cerebellar glucose ratio	0.357

Effect of Bromazepam and its gradual withdrawal on %weight change

Percentage weight change increased significantly in 9-day cessation group when compared to control group (Figure 2).

Table 3. Effect of Bromazepam and its gradual withdrawal on Red Blood Cell

Parameters	Groups				
	CTRL	9d BR	3d BR-wd	6d BR-wd	9d BR-wd
Packed cell volume (%)	46.0 ± 0.949	39.5 ± 0.158*	40.5 ± 0.158*	40.5 ± 0.160*	46.7 ± 0.967
Hemoglobin (g/dl)	15.00 ± 0.316	13.22 ± 0.053*	13.50 ± 0.053*	13.49 ± 0.0526*	15.60 ± 0.322

Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. *represents significant difference (P< 0.05) from control.

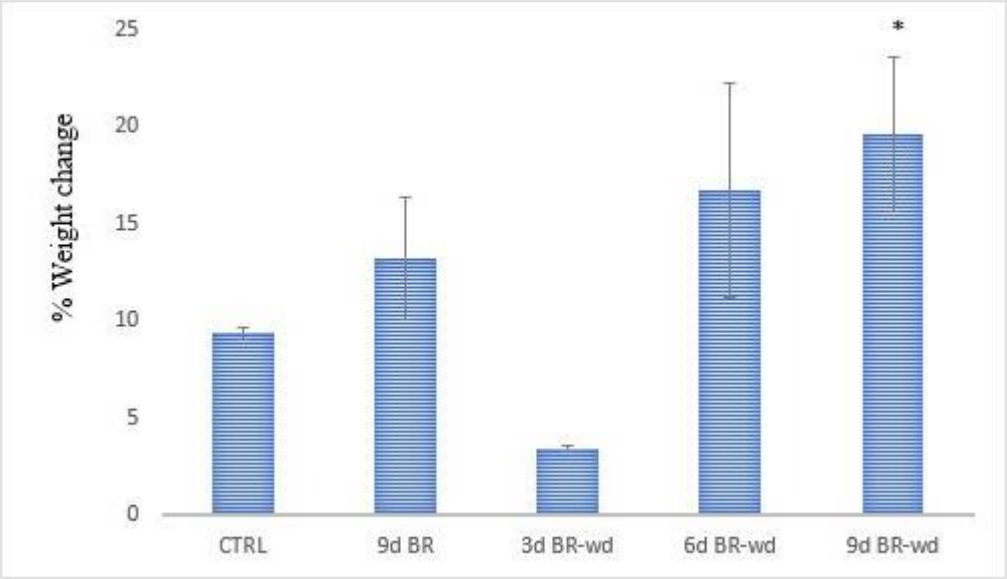

Figure 2. Effect of Bromazepam and its gradual withdrawal on % weight change. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

Effect of Bromazepam and its gradual withdrawal on cerebellar catalase

Cerebellar catalase was unaffected by bromazepam or cessation pattern (Figure 3).

Effect of Bromazepam and its gradual withdrawal on fasting blood glucose

When compared with control group, fasting blood glucose significantly increased in rats administered bromazepam for nine days and in 3-days cessation group (Figure 4).

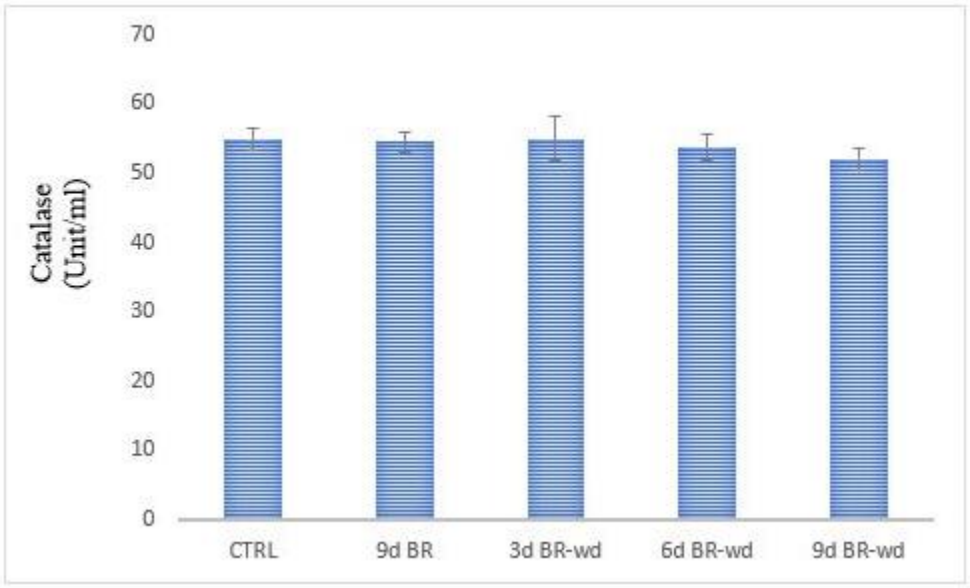

Figure 3. Effect of Bromazepam and its gradual withdrawal on cerebellar catalase. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

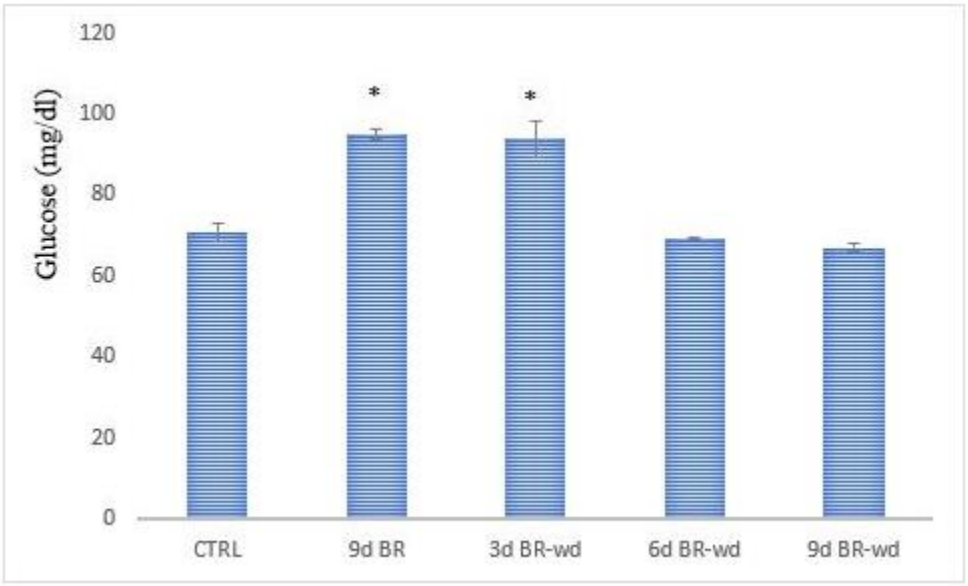

Figure 4. Effect of Bromazepam and its gradual withdrawal on fasting blood glucose. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

DISCUSSION AND CONCLUSION

The study investigated the effect of bromazepam administration and gradual cessation protocol on cerebellar metabolisms in female Wistar rats. Like other body cells, glucose is an important substrate for Adenosine Triphosphate (ATP) production (18). Using non-invasive 18-fluorodeoxyglucose, works have shown diazepam depressed brain metabolisms (19-22). However, few data exist on the effect of diazepam on cerebellar metabolisms. It was observed from the study that despite the fact that bromazepam administration for nine days did not significantly affect cerebellar glucose levels, an increase was noticed following 6 days and 9 days cessation groups when compared with control group.

In addition to evaluation of glucose utilization using positron emission tomography (PET) and other non-invasive techniques, blood/cerebellum glucose ratio used in this study tends to relate plasma glucose level to cerebellar glucose level. A high blood/tissue glucose ratio reflects reduced tissue glucose uptake. In diabetes mellitus, Cushing syndrome, acromegaly and stress (23), a high blood glucose alongside reduced glucose uptake is not impossible. The result of blood/cerebellum glucose ratio indicated that bromazepam treatment elevated the ratio when compared with healthy control rats implying that bromazepam reduced cerebellar glucose uptake. Fortunately, this trend concurred with findings from human and animal studies using non-invasive method (21,22). The only salience is that this is the first study where the ratio will be used in evaluating cerebellar glucose uptake. The present study showed that the highest blood/cerebellum glucose ratio occurred when rats were treated for nine days with bromazepam. Contrary to previous studies (10), we observed that bromazepam administration for nine days resulted in increased fasting blood glucose level when compared with health control rats. Metabolic demand is known to reduce during sleep with characteristic decrease in brain glucose uptake and utilization and attendant reduction in glucose-production mechanisms evidenced by lowered blood glucose. We have no skepticism about the possibility of fasting blood glucose increasing even despite bromazepam- induced depression of brain activities. This increase in fasting blood glucose might be a result of dawn effect and Somogyll rebound (24), These are well known mechanisms that come into play to mitigate hypoglycemia and they are often associated with release of hyperglycemic hormones. In the present study, we also observed that there was an increase in fasting blood glucose three day cessations.

Another finding of the study was the reduction in cerebellar glycogen level three days of bromazepam withdrawal when compared with healthy rats. Glycogen is a stored form of glucose and an important energy reserve. Depression in its level in the body tissue may result in decline in glycogenolysis and consequent dependence on other energy yielding alternative such as gluconeogenesis. Apart from derivation of glucose from amino acids and fatty acids which

occur virtually in all body tissues, lactate is another non-hexose energy source. A transport protein known as monocarboxylate transporter-1 is known to be responsible for lactate uptake by the brain. As far as the study was concerned bromazepam administration caused no significant decrease in cerebellar lactate level. However, 3-day cessation was characterized by a significant increase in cerebellar lactate level when compared with 6-day and 9- day cessation groups. This implied that even though, bromazepam administration for 9days did not affect cerebellar glycogen level, peak lactate level occurred three days cessation. We also noticed that glycogen restoration following bromazepam challenge peaked 6 days cessation.

The cerebellar glucose/glycogen ratio used in the study represented cerebellar glycogen synthesis level with a high ratio representing deficient glycogen synthesis. Exposure of female rats to bromazepam for nine days caused an increase in cerebellar glucose/glycogen ratio. This result corroborated the outcome of glycogen test in which bromazepam treatment for nine days was found to suppress glycogen content and glycogenolysis. Cerebellar glucose/glycogen ratio was found to reach a zenith level in bromazepam treated group when compared to other groups except healthy control group.

As far as the study was concerned, coordination test was carried out using beam balance method. High ataxia index score occurs when the rats are unable to tread the bar or move towards the underside of the bar after repeated trials and this typically is seen in fear, anxiety and muscle weakness which culminate in poor motor performance. In bromazepam treated rats, high score was obtained in a general consensus. In addition, we observed that ataxia index correlated negatively and positively with cerebellar glycogen and cerebellar glucose/glycogen ratio respectively. This implied that a rise in ataxia index was associated with a decreased cerebellar glycogen and impaired glycogen synthesis respectively. Therefore, in the study, bromazepam-induced ataxia may have been due to reduced cerebellar glycogen and increased cerebellum glucose/glycogen ratio. In addition, ataxia index was lower in all cessation groups when compared to bromazepam treated group.

Moreover, we observed that there was a reduction in weight three days after bromazepam withdrawal. This change in weight might be due to bromazepam cessation effect and readjustment to bromazepam-free life. Weight change recovered fully after nine days of bromazepam withdrawal. We also observe that packed cell volume and hemoglobin concentration were decreased in bromazepam treated rats. Reduction in hematocrit has been previously reported with diazepam use at therapeutic dose (11). Catalase plays in antioxidant defense (25-27). Specifically, it converts hydrogen peroxide into non-toxic water. Catalase analysis was conducted in the study to ascertain if bromazepam use and withdrawal have any influence on cerebellar antioxidant homeostasis. The results showed that bromazepam administration and gradual

withdrawal had no significant effect on cerebellar antioxidant balance.

Bromazepam is a commonly used and abused drug with anti-anxiety, anticonvulsant and hypnotic effects (4, 9). The present study demonstrated not only the possibility of recovery from bromazepam effects but also the recovery pattern. In conclusion, the results of the study indicated the adverse but time-dependent reversible effects of bromazepam withdrawal on cerebellum metabolism in adult female Wistar rats.

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Conflicts of Interest statement

The authors declare no conflict of interest.

Authors' contributions: All authors contributed equally.

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Association of adverse pregnancy outcome with the values of serum biomarkers of Quadruple test

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Abstract

Background: Prenatal test includes prenatal screening and diagnosis that aims to find different changes in fetus and mother during the pregnancy. Prenatal screening is focused in finding any possible pathology in the wide population using some noninvasive methods.

The changes of utero-placental blood flow lead to utero-placental insufficiency, which will be manifested by pregnancy induced hypertension, preeclampsia, intrauterine growth retardation or/and small for gestation age fetus, preterm birth, etc.

Aim: of this research is to perform a prenatal screening of serum biomarkers from Quadruple test in the second trimester of pregnancy, in order to predict early diagnosis of eventual adverse pregnancy outcome.

Material and method: This prospective study is realized in the Special Hospital for Gynecology and Obstetrics "Mother Theresa", Skopje, during the period November 2019 to June 2021. It includes 673 pregnant women, between 18-23.6 gestational weeks, followed up and monitored till delivery. We followed up the values of serum biomarkers from Quadruple test, fetal biometry, quantity of amniotic fluid and gestational week and bodily measures of the fetus in delivery.

Statistical processing: It was conducted a statistical analysis of maternal characteristics in research group and the group with no adverse pregnancy, determining the variables which significantly associate with adverse pregnancy outcome.

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Results: Within 673 respondents, 523 (77.7%) had favorable pregnancy outcome, while 150 (22.3%) of pregnant women had adverse pregnancy outcome (they made up the research group). From the group with no adverse pregnancy, 48 (32%) had preeclampsia, 32 (21.3%) had pregnancy induced hypertension, 20 (13.3%) had small fetus according to the gestational age, and 50 (33.3%) had intrauterine growth retardation.

Conclusion: Inhibin A as a single marker for adverse pregnancy outcome was the best predictor for differentiation of pregnant women with adverse and favorable pregnancy outcome.

Keywords: Quadruple test, pregnancy outcome, Inhibin A

INTRODUCTION

Prenatal screening is focused in finding any possible pathology in wide population using some noninvasive methods. Placenta and feto-placental circulation has a crucial role in perinatal growth of the fetus and the pregnancy outcome. (1) During pregnancy, inhibin A is mainly derived from the placenta and regulates the implantation and differentiation of embryos. (2) The fetal compromise and the utero-placental blood flow changes can result to utero-placental insufficiency. Placenta, produces several specific proteins that flow in the maternal serum, in different quantity and they have a very important role in normal development of the fetus. (3) According to the literature data, there is a correlation between the maternal serum protein values and the pregnancy outcome, thus the prediction of potential placental insufficiency is very important. A shallow implantation leads to pregnancy induced hypertension, preeclampsia, intrauterine growth retardation, preterm birth, etc. The risk of pregnancy induced hypertension is due to placental ischemia, restrictive changes in intrauterine fetal growth and is an indication for preterm delivery. (4)

Usually, preeclampsia is found after the 20^{th} gestational week, in previously normotensive women, but it can be found also during delivery and after it (ACOG, 2020). The certain etiology isn't known, but it is met usually in women who have previous preeclampsia, hypertensive disease, gestational and familiar hypertension, in oldest pregnant women (up to 40 years), in black women, obese pregnant women, multiple pregnancies, etc. Pathophysiology is due to immunological and genetic factors, placental ischemia, oxidative stress and other factors that result in spiral arteries abnormality, dysfunction or inadequate trophoblastic invasion and shallow implantation. Final result is placental hypo perfusion and ischemia, producing different substances that enter the maternal blood circulation. Except high blood pressure, as a first sign of preeclampsia, appearance of proteins in urine, more than 0.3 g./24h and joint and face swelling are the other accompanying signs. If not diagnosed on time, it can lead to eclampsia, a state of high risk for both mother and baby, and may have fatal end. (5) A study by Park Hea Ree, (2021) concluded that Inhibin A and other second-trimester serum markers may be useful for early detection of preeclampsia. (6)

Intrauterine growth retardation is a complex complication or fetal growth restriction under the 10^{th} percentile, accompanied with other pathological restrictions and perinatal risk, due to shallow placentation. In case of IUGR (Intrauterine Growth Retardation), an induction of preterm delivery is indicated very often. (7)

The cause of preterm delivery is utero-placental ischemia, preeclampsia, preexisting hypertension of the pregnant women, gestational diabetes, obesity or malnutrition, cervical insufficiency, infections, etc. (8)

The quadruple marker test or the second trimester screen is a prenatal test that measures levels of four substances in pregnant women's blood:

- Alpha-fetoprotein (AFP), a protein produced by the fetus,
- Human chorionic gonadotropin (HCG), a hormone produced by the placenta,
- Estriol, a hormone produced by the placenta and the fetus' liver,
- Inhibin A, a hormone produced by the placenta.

The same biomarkers are used as indicators and predictors that refer to risk, not just for the numeric chromosomic aberration but also to correlation with other pathophysiological fetal changes, pregnancy outcome, preeclampsia, pregnancy induced hypertension, preterm birth, intrauterine growth retardation, etc. Down syndrome biochemical markers levels are altered in those patients who subsequently developed preeclampsia and may be a useful screening test for preeclampsia. Inhibin-A is the most predictive marker and correlates with the severity of subsequent preeclampsia and inversely with the week of occurrence of preeclampsia. (9)

The aim of this study was to define the role of biomarkers of Quadruple test in prediction of adverse pregnancy outcome.

MATERIALS AND METHODS

This prospective study was realized in a Special Hospital for Gynecology and Obstetrics "Mother Theresa", Skopje, from November 2019 to June 2021. It includes 673 second trimester pregnant women, followed till delivery. Firstly, were taken the bodily measures and blood pressure, after that was taken 2 ml venous blood for Quadruple test and ultrasound for fetal biometry and amount of amniotic fluid. All participants were monitored till end of pregnancy by regular measuring blood pressure and biochemical analysis. Inclusion criteria were: singleton pregnancy, age at least 18 years old of pregnant women, gestational age 18-23.6 week, previously excluded fetal anomalies by ultrasound. Exclusion criteria were: twin or multiple pregnancy, fetus mortus in utero, findings of fetal anomalies, pre-existed hypertension, other diseases in pregnant woman (diabetes, autoimmune diseases, etc), pregnant women that used Aspirin.

Statistical processing was done by conducting a statistical analysis of maternal characteristics in all patients included in the study and determining the variables which significantly associate with adverse pregnancy outcome.

RESULTS

From 673 respondents, 523 (77.7%) had favorable pregnancy outcome, while 150 (22.3%) of pregnant women had adverse pregnancy outcome (they made up the research group). From the group with no adverse pregnancy, 48 (32%) had preeclampsia, 32 (21.3%) had pregnancy induced hypertension, 20 (13.3%) had fetus small for gestational age, and 50 (33.3%) had intrauterine growth retardation. (Table 1)

Table 1. Mother's characteristics in the two groups.

Mother's characteristics	Statistical parameters	Adverse pregnancy outcome	No adverse pregnancy outcome	p-level
Age Years	mean ±SD	27.3 ± 3.8	27.8 ± 4.5	t=1.22
	min – max	22 – 37	18 – 48	p=0.22 ns
BMI kg/m^2	mean ±SD	28.10 ± 2.8	27.02 ± 3.8	t=3.22
	min – max	23 – 34.5	18 – 45.1	**p=0.0014 sig
TA systolic (mmHg)	mean ±SD	132.50 ± 10.8	124.72 ± 9.4	t=8.61
	min – max	105 – 155	102 – 145	***p=0.000000 sig
TA diastolic (mmHg)	mean ±SD	85.67 ± 7.5	78.77 ± 6.2	t=11.47
	min – max	70 – 105	64 – 95	***p=0.000000 sig
Gestational week /in second trimester	mean ±SD	21.1 ± 1.0	20.9 ± 1.3	t=2.05
	min – max	18.6 – 23	17.1 – 23.6	*p=0.041 sig
Gestational week /delivery	mean ±SD	37.5 ± 1.1	39.7 ± 1.05	t=21.9
				***p=0.000000 sig

t(Student t-test) *p<0.05; **p<0.01; ***p<0.0001

Table 1 presents pregnant women with adverse and no adverse pregnancy outcome were mean age was 27.3 ± 3.8 and 27.8 ± 4.5 years, in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome, respectively, without statistical significance (p=0.22)

The body mass index has a significant higher value in the group with adverse pregnancy outcome than in the group with no adverse pregnancy outcome (difference value was 28.10 ± 2.8 vs 27.02 ± 3.8, p=0.0014).

The systolic and diastolic pressure were significantly higher in the group with adverse pregnancy outcome comparing to the group with no adverse pregnancy outcome (p<0.0001); 132.50 ± 10.8 and 124.72 ± 9.4 mean values for systolic pressure, respectively in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome; 85.67 ± 7.5 and 78.77 ± 6.2 mean values of diastolic pressure, respectively in research and control group.

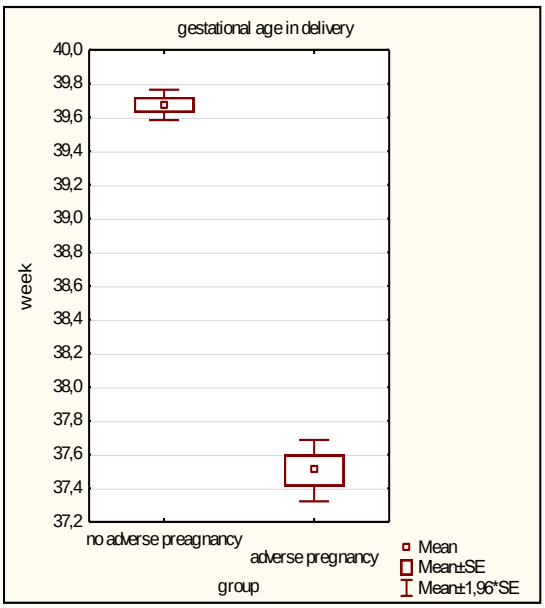

Figure 1. Graphic presentation of gestational week in the group with no adverse pregnancy outcome and the group with adverse pregnancy outcome in delivery

Figure 1 presents the gestational week at delivery for both groups included in the study. The gestational week was significantly different in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome (21.1 ± 1.0 vs 20.9 ± 1.3, p=0.041, and 37.5 ± 1.1 vs 39.7 ± 1.05, p<0.0001, consequently).

adverse and no adverse pregnancy outcome doesn't show any statistical difference in relation to parity (p=0.62). It was a domination of pregnant women with one delivery in both groups - 56% and 55.4%, respectively in groups with adverse and no adverse outcome.

Pregnant women from the group with adverse and

Table 3. Fetal body measures in delivery in both groups

Obsterical caracteristics	Statistical parameter	**Adverse pregnancy outcome**	**No adverse pregnancy outcome**	**p-level**
Body weight (gr)	mean ±SD	2596.5 ± 364.4	3441.5 ± 344.6	t=26.13
	min - max	1460 – 3450	2340 – 4520	***p=0.000000 sig
Body length (cm)	mean ±SD	47.0 ± 1.5	50.7 ± 1.7	t=24.01
	min - max	45 – 52	46 – 55	***p=0.00000 sig

Table 2. Parity in pregnant women in both groups

Parity	**Groups**	
	Adverse pregnancy outcome n(%)	**No adverse pregnancy outcome** n(%)
1	84 (56)	289 (55.36)
2	43 (28.67)	160 (30.65)
3	14 (9.33)	55 (10.54)
4	7 (4.67)	15 (2.87)
5	2 (1.33)	2 (0.38)
6	0	1 (0.19)
p-level	X^2=3.52 p=0.62 ns	

X2(Pearson Chi-square)

Table 2. presents the newborn's body measures in both groups included in the study. Newborns, that have adverse pregnancy outcome, has significantly lower bodily measures comparing to those from mother with no adverse pregnancy outcome (2596.5 ± 364.4 vs 3441.5 ± 344.6 gr/cm, and, 47.0 ± 1.5 vs 50.7 ± 1.7 cm; p<0.0001, respectivly).

Table 3 presents pregnant women in both groups included in the study. Pregnant women with

no adverse pregnancy outcome has significantly different values for serum biomarker Inhibin A, (p<0.0001). Median serum concentration of this biomarker were significantly higher in the group with adverse pregnancy outcome in relation to the group with no adverse pregnancy outcome – 489 vs 203.7 pg/mL, and 1.12 vs 0.45 MoM, (table 4).

Table 4. The value of Inhibin A in the group with adverse pregnancy outcome and the group with no adverse pregnancy outcome

Variable	calculated parameter	Groups		p-level
		Adverse pregnancy outcome	No adverse pregnancy outcome	
Inhibin A (pg/mL)	mean ±SD	520.22 ± 269.3	241.96 ± 138.4	Z=12.07
	median (IQR)	489 (267 – 659)	203.7 (156 – 294)	***p=0.00000 sig
Inhibin A (MoM)	mean ±SD	1.19 ± 0.6	0.54 ± 0.3	Z=12.56
	median (IQR)	1.12 (0.62 – 1.46)	0.45 (0.34 – 0.64)	***p=0.000000 sig

Z(Mann-Whitney U Test) ***p<0.0001

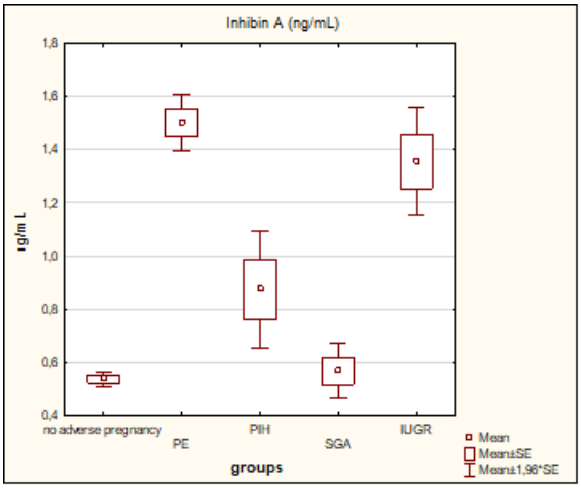

Figure 2. Inhibin A in all groups

Figure 2. presents the values of Inhibin A in pregnant women with adverse pregnancy outcome and the group with no adverse pregnancy outcome. Serum Inhibin A has the highest medial values in group with preeclampsia (1.37 MoM), followed by the group with IUGR (1.16 MoM), PIH– (pregnancy induced hypertention) (0.81 MoM), SGA (small for gestational age)(0.55MoM) and the lower values were measured in the group with no adverse pregnancy outcome (0.45 MoM). There were confirmed statistically significant higher values of Inhibin A in group with preeclampsia comparing to the group with no adverse pregnancy outcome, PIH versus group with no

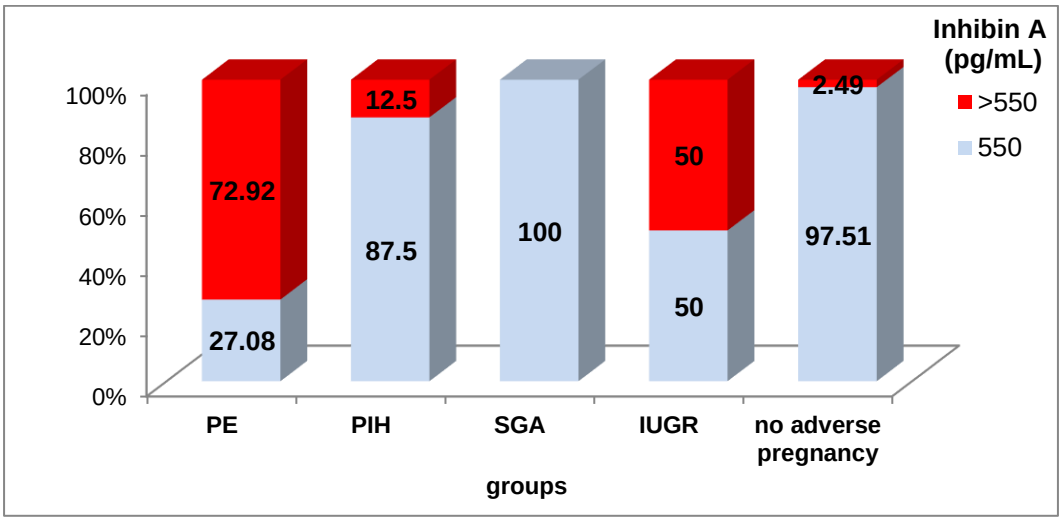

Figure 3. Inhibin A in all research groups in relation to the group with no adverse pregnancy outcome

adverse pregnancy outcome and IUGR versus group with no adverse pregnancy outcome.

Figure 3 represent the values of Inhibin A in the groups with adverse pregnancy outcome in relation to the group with no adverse pregnancy outcome.

Pregnant women with PE, significantly less frequently than women from the group with no adverse pregnancy has decreased values of uEstriol (68.75% vs 82.41%), while pregnant women with SGA more frequently than pregnant women from the group with no adverse pregnancy outcome has decreased values of uEstriol. (fig. 4).

Medial values of serum HCG were 50 780, 20 844, 33 172, 50 780 and 24 113 IU/mL, respectively in groups with PE, PIH, SGA, IUGR and the group with no adverse pregnancy outcome. In relation to the group with no adverse pregnancy outcome, the values were significantly

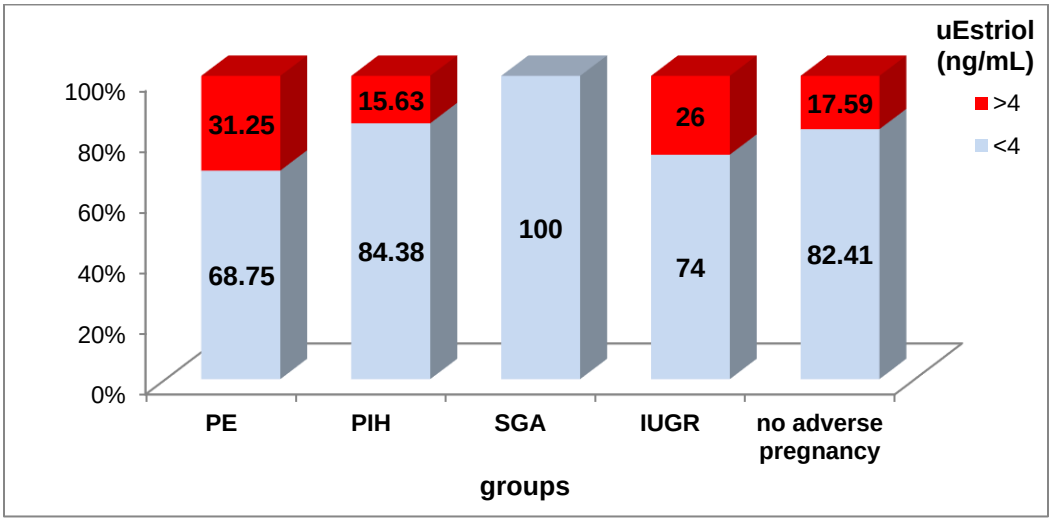

Figure 4. Presentation of uEstriol in all groups

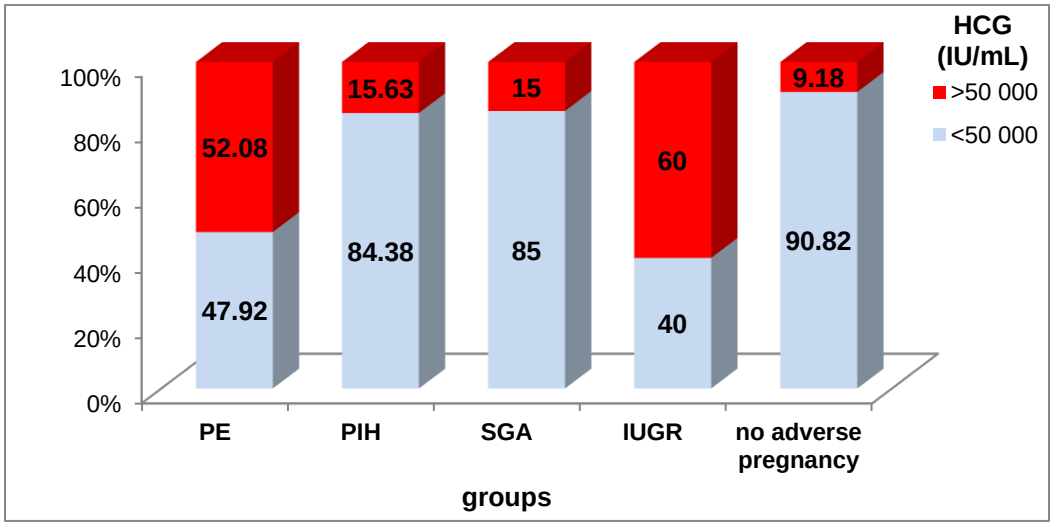

Figure 5. Presentation of HCG in all groups

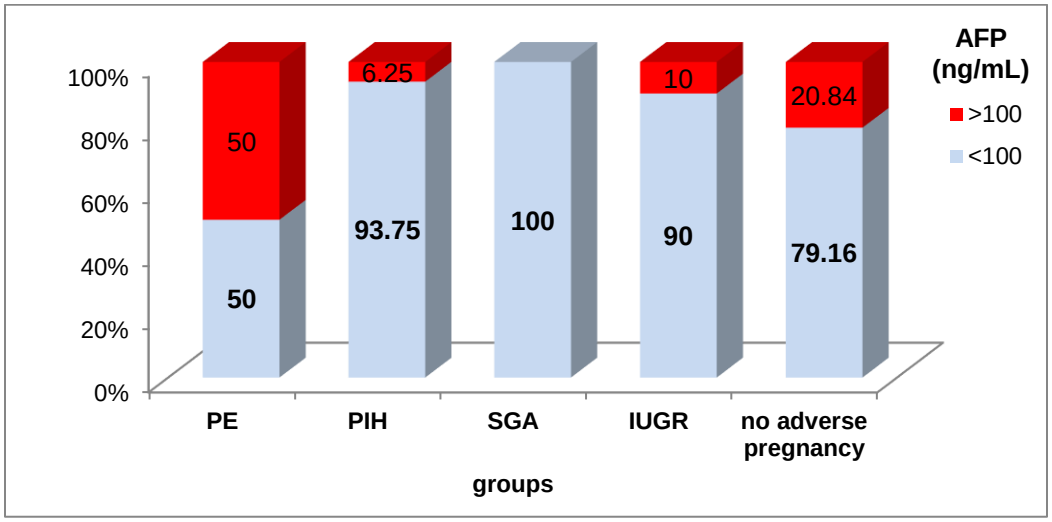

Figure 6. Presentation of AFP in all groups

higher in pregnant women with PE (p<0.0001), in pregnant women with SGA (p=0.0019) and in pregnant women with IUGR (p<0.0001), (fig. 5).

Alpha-fetoprotein (AFP) has significantly higher mean serum concentration in group with PE comparing to the group with no adverse pregnancy outcome (94.32± 47.5 vs 78.09±38.1,p=0.0059), non significantly higher in group with IUGR comparing to the group with no adverse pregnancy outcome (81.68±23.1 vs 78.09±38.1,p=0.51), non significantly lower in group with PIH comparing to the group with no adverse pregnancy outcome (74.83± 41.2 vs 78.09±38.1,p=0.64) and non significantly lower in group with SGA comparing to the group with no adverse pregnancy outcome (75.04± 16.7 vs 78.09±38.1,p=0.51) (fig. 6).

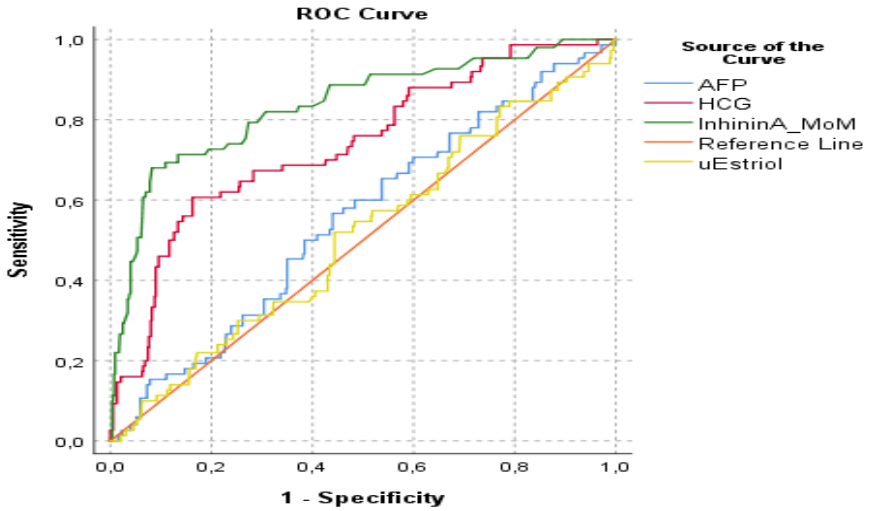

Figure 7. Performances of serum biomarkers of Quadruple test

Figure 7. presents the results for performances of serum biomarkers of Quadruple test. According to the size of area under the ROC curve, Inhibin A as a single biomarker has the best differential ability in diagnosing pregnant women with adverse pregnancy, AUC=0.701.

The combination of Inhibin A, uEstriol, HCG and AFP has the biggest area under the ROC curve (AUC=0.792) that is, this combined model represents the test with the best differentiation ability for pregnant women with adverse and favorable pregnancy outcome

DISCUSSION AND CONCLUSIONS

This study analyzed biomarkers from Quadruple test; Inhibin A, HCG, AFP and uEstriol and fetal biometry in second trimester of pregnancy and their relation to pregnancy outcome. According to Chowdhary et al. (2017), the use of Inhibin A as a predictor for IUGR has a great importance. Increased value of maternal serum Inhibin A in second trimester of pregnancy has an important correlation with abnormal placentation. (10) In our study the level of Inhibin A in serum of pregnant woman has the highest values in group with preeclampsia, followed by the group with IUGR, PIH and SGA. More frequently the increased serum Inhibin A values in pregnant women with adverse outcome versus those with no adverse pregnancy outcome, statistically was confirmed as significant, $p<0.0001$, while the performances of the test showed a good differentiation ability.

It is confirmed by Mazhari (2018) and Sanayukta (2019), that the serum level of HCG in second trimester of pregnancy is a good predictor for PIH. (11), (12) In our study, median values of HCG in serum were significantly higher in the group with preeclampsia, followed by the group with SGA, IUGR versus control group. More than half of respondents with preeclampsia and IUGR (52.1% and 60% respectively), has increased level of serum HCG, while in other groups, that was much lower, 15.6% in PIH, 15% SGA and 9.2% in the control group.

According to Hu et al. (2020), increased level of AFP in maternal serum is associated with a big risk for adverse pregnancy outcome. (13) This was confirmed partially in our study, because just women with preeclampsia has significantly higher values of AFP, comparing to pregnant women with PIH, IUGR and control group. Also, our study didn't find any statistically significant difference between median values of uEstriol, as a single biomarker in all four research groups and control group. The area under ROC curve showed that uEstriol has a weak discriminatory ability, thus this biomarker, independently does not allow a prediction of pregnancy outcome.

The results of Quadruple test represented significantly higher values of Inhibin A, in the group with adverse pregnancy outcome (median 1.12 vs 0.45 MoM), and significantly higher values of HCG in the same group (median 45684.5 vs 24113), while uEstriol and AFP values were non significantly higher in the group with adverse pregnancy outcome.

Inhibin A has increased serum level in the group with preeclampsia (659.2 pg/mL), followed by the group with IUGR (524.65pg/mL), PIH (376.2 pg/mL) and SGA (237.pg/mL), while the lowest serum level of Inhibin A has CG (203.7pg/mL). It was found a statistically significance between groups with PE versus CG, PIH versus CG and IUGR versus CG.

uEstriol has no statistically significant difference in median values of uEstriol in four research groups comparing to the control group 3.64, 2.92, 2,71, 3.04 vs 2.85, respectively for PE, PIH, SGA, IUGR and CG).

The results for validity of using serum biomarker in our study, for diagnosing adverse pregnancy outcome, showed that Inhibin A as a single marker has the best diagnostic performances (AUC=0.701, sensitivity 65%, specificity 76%). These biomarkers may be predictive of adverse pregnancy outcome, first of all in prediction of preeclampsia, but the prediction value is low.

Combination of Inhibin A, u Estriol, HCG and AFP has the biggest area under the ROC curve (AUC=0.792), so this combination of test represents the test with the best ability of differentiation between pregnant women with adverse and no adverse pregnancy outcome.

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Conflict of interest: No conflict of interest.

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The Role of Oral Corticosteroids in the Management of Children with Acute Respiratory Diseases

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Abstract

Respiratory diseases are the most frequent reason for children's visits to the paediatrician. They account for about 25% of paediatric consultations, 10% of which are for asthma, the others are for bronchiolitis, bronchitis and respiratory infections. Asthma is the most prevalent chronic disease of childhood and accounts for a substantial proportion of hospitalizations among children, whereas viral bronchiolitis is the leading cause of hospitalization in the first year of life. In preschool children, wheezing and croup are common respiratory conditions. Corticosteroids are often prescribed as they prevent or suppress inflammation in response to allergic or viral triggers. Oral corticosteroids are often used to treat some acute respiratory diseases. We discuss here about the management of bronchiolitis, croup, wheezing, and asthma.

Keywords: Bronchiolitis, Croup, Preschool Wheezing, Asthma, Corticosteroids

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INTRODUCTION

Acute and chronic respiratory diseases represent a global public health problem because of their increasing prevalence and severity worldwide (1). This can be attributed to several factors: 1. the significant increase in the prevalence of early allergen sensitization in childhood; 2. the frequent recurrence of viral infections; and 3. the increased survival of extremely preterm children born with bronchopulmonary dysplasia. All these factors contribute to the increased risk of acute manifestations becoming chronic. (1) In this article, we discuss issues related to the treatment of the acute respiratory diseases and to asthma, focusing on corticosteroids. We do not address forms of respiratory diseases, such as pneumonia, that have a different pathogenic basis and therefore require a very specific approach.

PHARMACOLOGICAL ASPECTS OF CORTICOSTEROIDS

Since their identification in 1935, corticosteroids application in pharmacotherapy has found a variety of uses. It is about 3 groups of steroids synthesized in the adrenal gland, respectively: 1. Mineralocorticoids in the glomerular zone, 2. Glucocorticoids in the fascicular zone, 3. Androgens in the reticular zone. (2)

Regulation, synthesis and release of the steroids are closely related with the HPA (Hypothalamus-Pituitary-Adrenal) axis. Among adrenocorticosteroids, glucocorticoids have a special position due to their involvement in a number of pathologies and their multiple effects, either therapeutic or side effects. (3,4,5)

The secretion of glucocorticoids is based on a circadian intensity, having the higher release during the morning hours and the minimal release on night hours. (6)

The pharmacological effects of glucocorticoids can be summarized in three categories:

1. Physiological effects, including the metabolic and facilitating effects.
2. Pharmacological effects, which consist of anti-inflammatory effects.
3. Immunosuppressive and anti-anaphylactic effects, anti-shock effects, hematological effects and side effects, which consist of effects on the level of muscle, bone, skin, eyes, metabolic effects, effects on central nervous system, gastrointestinal tract, cardiovascular and renal system. (7,8)

Important side effects for which glucocorticoids are blamed of are the growth inhibition in children and osteoporosis caused by the effect on calcium metabolism or by acting directly on the osteoblasts (9,10).

Glucocorticoids are classified into natural drugs (hydrocortisone, cortisone) and synthetic ones (prednisone, prednisolone, dexamethasone, betamethasone, etc.) (11).

Another classification is based upon their time-course activity: short acting (cortisone, hydrocortisone), intermediate acting (prednisone, prednisolone) and long acting (betamethasone, dexamethasone) glucocorticoids. (12, 13)

There are several pharmacokinetic aspects that a health professional should consider when prescribing these drugs. Glucocorticoids are mainly of lipophilic nature and are well absorbed during both oral and parenteral administration, but the route of administration that reaches the highest compliance in patients is the oral administration. These drugs are strongly bound to plasma proteins and represent a half-life of about 2-4 hours. The biotransformation process is of particular importance because through this process occurs the activation of several representatives (cortisone in hydrocortisone and prednisone in prednisolone). Glucocorticoids are mostly excreted through renal excretion. (14,15,16)

A serious concern with the administration of glucocorticoids is the suppression of the HPA axis, which is the base of a considerable number of side effects. The suppressive effect depends on the given drug, the dose, the administration time and the persistence of the treatment. (17)

Several studies are carried out in order to document the beneficial and adverse effects of different representatives of glucocorticoids. Studies demonstrated that application of chronotherapy in the dosing regimen of prednisolone resulted in better clinical outcomes with fewer side effects. (18)

Other studies indicated that the variations of concentration profiles depending on the time for both cortisol and ACTH were more closely to normal profiles when therapeutic doses of prednisolone were applied. Meanwhile, administering the betamethasone equivalent has made a more significant inhibition of the normal performance of these two hormones. It is considered that the suppression of HPA axis is developed by the use of betamethasone in physiological concentrations and of prednisolone in high concentrations for at least 2 weeks. (19)

The effect of drug, dose and continuation of therapy on bone metabolism has been investigated and was shown that the inhibition of intestinal calcium absorption is present during the use of betamethasone in both short-term therapy and low doses, while the inhibition is present with the use of prednisolone only at high doses. (20,21).

Recommendations regarding the use of glucocorticoids, which aim to minimize the suppression of the HPA axis, consist of:

• Using Systemic corticosteroids only in clear indications.

• Prefer the analogs with average duration of action and those protecting the steroids (steroid sparing drugs).

• Use the minimal effective dose for as short as possible by administering the drug as a single dose in the morning or on alternate days.

• Make slow discontinuation after the chronic therapy. (22, 23, 24, 25,26,27).

FREQUENT RESPIRATORY DISEASES IN CHILDHOOD

BRONCHIOLITIS

Epidemiology, clinical presentation, and treatment of bronchiolitis

Bronchiolitis is the most common viral infection of lower respiratory tract in children in their first year of life. The main etiologic virus is the Respiratory Syncytial Virus (RSV), which mainly affects children in their first 6 months of life reaching the peak in the first 30 to 60 days of life and in premature infants. RSV constitutes 60-80% of the etiologic factors (28,29) and has a seasonality nature from November to April (30). Also other viruses as human rhinoviruses, parainfluenza, metapneumovirus, etc, are frequently involved in bronchiolitis. Its clinic is preceded by a respiratory syndrome with sub febrile temperature, rhinorrhoeaand further reveals the typical clinical repertoire with cough, tachypnoea or apnoea (in premature infants), respiratory distress, expiratory wheezing and inspiratory crackles, reduction of oxygen saturation (SatO2) and dehydration due to feeding difficulties (31). Disease severity must be assessed to identify children who require hospital admission (Table 1)

Table 1. Criteria for hospitalisation (1)

	Ambulatory treatment or out of the hospital	Under observation	Hospitalization
Respiratory distress	With or without light retraction of thoracic walls	Nasal flaring, thoracic retraction	Moderate to severe respiratory insufficiency
O2 saturation	SaO2 > 95% , no need for extra O2	SaO2 90-95%	Persistent saturation <92%, O2 therapy is necessary
Feeding	Normal or slightly decreased	50-70% of normal feeding	<50%, does not feed, dehydration
Gestational age	Gestational age > 37 weeks, after birth age >12 weeks		Gestational age < 37 weeks, after birth age 6-12 weeks
Reaction and consciousness	Good reaction and the child is wake up (alert)		Weak reaction or no reaction
Socio-economic factors	Careful parents and collaborative, near to the hospital		No collaborative parents, distance from the hospital
Presence of the risk factors	Without risk factors	Pulmonary dysplasia, FC, cardiopathy, genetic defect, neuromuscular disease, Immunodeficiency.	Pulmonary dysplasia, FC, cardiopathy, genetic defect, neuromuscular disease, Immunodeficiency.

The management of bronchiolitis largely depends on the severity of the condition. Supplemental oxygen should be administered if O2 saturation levels are persistently below 90-92% at ambient air, Oxygen may be administered by means of nasal prongs, face masks or HFNC. In children who cannot maintain oral hydration is recommended rehydration with intravenous

fluids or with nasogastric tubes. Nebulizing hypertonic saline may help to decrease airway edema and improve mucociliary clearance (31). There is no convincing evidence in most of the guidelines for nebulized adrenaline, salbutamol, ipratropium bromide, antibiotics, antivirals, or inhaled and systemic corticosteroids (32). Oral salbutamol is not recommended. Systemic corticosteroids are indicated only in severe cases requiring hospitalization (33,34,35). In a randomised trial, corticosteroids in combination with salbutamol reduce 31% prolongation of symptoms especially in atopic children (36).

LARYNGOTRACHEITIS

Etiology, clinical history, and treatment of laryngotracheitis in children

Laryngotracheitis is one of the most frequent causes of acute respiratory distress in young children. The disease mainly affects children aged between 6 months and 3 years. The aetiology may be of viral origin (Parainfluenza 1, 2, 3 & 4, respiratory syncytial virus, Influenza A, B, Human metapneumoniae virus, Adenovirus) or bacterial one (H. influenza) (37). It is characterized by inspiratory stridor, severe barking cough, shortness of breath, hoarseness. The most affected age results to be 6m-3y (the highest incidence - 2nd year). According to Westley, (the clinical scoring system of croup), the gravity of croup clinical forms is scored based on the evaluation of stridor, retraction of the chest wall, the presence of cyanosis, the level of consciousness, and the air entry with points. Mild croup: WCS $\leq$ 2, Moderate croup: WCS: 3-5, Severe croup: WCS 6-11, Respiratory Insufficiency: WCS> 11(37,38).

Management: The child should be examined in the quietest conditions preferably in the parent's arms. In the mild form there is no need for special therapy except of informing the parents, the follow-up is performed as outpatient. Conventionally, croup is treated with corticosteroids and epinephrine. Dexamethasone and prednisolone are the most effective for mild-to-moderate croup, recommended dosages for oral prednisolone 1mg/kg and dexamethasone 0.6mg/kg (if oral administration is impossible, apply im), the child is monitored and followed for 2-4 hours: In case of deterioration, the patient is hospitalized, otherwise is sent home with the respective recommendations. The severe cases should always be hospitalized, and if child is in respiratory distress is administered oxygen therapy, nebulized epinephrine is applied 0.5 ml/kg (or adrenaline 1: 1000, 4 ml insoluble sol. under O_2 therapy) (39). It is important to maintain the child's hydration. He/she should be observed in hospital conditions and in cases of improvement, for a further period of 3-4 hours for any possibility of recurrences. (39)

PRESCHOOL WHEEZING

Classification, diagnosis and treatmentof Preschool Wheezing

Wheezing is one of the most common symptoms of child presentation for paediatric consultation. 1/3 of preschool children are presented with

wheezing before 5 years of age (1 in 3 children have at least one episode with wheezing before the age of 5 years old). The wheezing clinical phenotypes are very heterogeneous, while there is few evidence of its physiopathology and treatment. The most important risk factor for persistent symptoms at school age is the atopy: the more allergens and the greater the level of sensitization, the greater the possibility that wheezing persists even in school age (40)

Classification of the preschool wheezing

In 2008, ERS task Force proposed classification **according to the etiologic stimuli**:

1. **Episodic wheezing vs Multiple-trigger wheeze**: Episodic wheezing (viral) which lasts for a short period of time, is accompanied by a viral infection of upper respiratory tract, and there is no evidence of wheezing between the episodes. Multiple-trigger wheeze (wheezing from multiple stimuli), children make severe exacerbations, have also symptoms between episodes. Important stimuli are considered the tobacco smoke, exposure to allergens, physical strain, such as weeping, laughter, etc. (41). Several other classifications of wheezing have been proposed but still there is no complete consensus on the classification and used terminology (42,43,44):

2. Atopic wheezing vs.non atopic wheezing: Atopic wheezing (or allergic asthma): if the patient does ≥ 3 episodes with dyspnoea and wheezing AND sensibility confirmation to inhaled or nutritional allergens. Non atopic wheezing (viral) is considered when thepatient does ≥ 3 episodeswith dyspnoea and wheezing, which happens during the viral infections, AND there is no evidence for allergy from pneumoallergens or nutritional allergens. (41)

3.Wheezing **by frequency and gravity**: Mild form of Wheezing is not common, in cases when no affects on the daily life, and have rare episodes

Table 2. Aetiologies of Atypical wheeze

Warning sign	Possible underlying causes
Persistent symptoms from birth	Tracheobronchomalacia and PCD
Productive wet cough as a main symptom	PCD, CF, immune deficiency and TB
Never completely symptom free	Tracheobronchomalacia, vascular ring, foreign body aspiration and neonatal chronic lung disease
Failure to thrive	CF and immune deficiency
Recurrent pneumonia	CF and immune deficiency

(<1 episode /month). Severe form of Wheezing, is common, in cases when there is considerable impact on daily life (presentation in the urgency or hospitalization), as well the episodes are frequent (≥2/ months). (41)

Diagnostic approach to wheezing… Wheezing is a non-specific symptom, which occurs in several diseases. The initial assessment aims to exclude serious pathologies which are presented as "Atypical Wheeze" (40). After the exclusion of aetiologies as in below table, the majority of pre-school children with wheezing have "Typical Wheeze". In children where "typical wheeze" is confirmed, the only useful diagnostic test is skin prick test and/or specific Ig E for atopic wheezing confirmation (allergic pre-school asthma). Allergic asthma is the most common chronic childhood disease, which begins in early childhood (1/2 of asthma patients refer for symptoms since in childhood). The most important risk factor considered is the atopy (45).

Treatment of wheezing & pre-school asthma

It is recommended to use short-acting beta2 agonists (as needed) for all patients with wheezing exacerbation. Prophylactic therapy with ICS is recommended in children who have 1.recurrent episodes of wheezing, 2.frequent and/or severe episodes, 3.persistence of the symptoms even between episodes (40,45). OCS (oral corticosteroids) are more effective in children with asthma compared to pre-school children with acute wheezing episodes. In pre-school children with wheezing, it is recommended to use OCS in cases of hospitalization need, O_2need and in atopic wheezing (1): prednisolone 1-2 mg/kg/day max. Dose of 20mg/day in children ≤2 years old, and 30 mg/day in children 2-5-year-old (Evidence A). It is recommended the treatment duration of 3-5 days, it may be discontinued immediately. (Evidence D) (45).

ASTHMA CRISIS

Treatment of Asthma crisis

Asthma is the chronic disease with the highest prevalence in children> 5 years old, affects 5-20% of children of school age. Asthma is characterized by chronic inflammation of respiratory airways, bronchialhyperactivity, and variable expiratory obstruction. Clinically is presented with wheezing, cough, dyspnoea, chest tightness. Factors that can provoke asthma crisis are the effort, allergens, atmospheric changes, viral infections, etc. (45)

Pharmacological treatment of crisis: It is recommended O_2 therapyif SatO2 ≤ 92% in air. Short-acting beta2 agonists (SABAs) 2-4- 10 puffs Salbutamol 100 mcg (through pMDI + aero chamber). If needed repeat every 20-30 minutes during the first hour of the treatment and then every 1-4 hours as needed. Ipratropium bromide (anticholinergic) is given together with salbutamol, to treat children with a moderate-to-severe asthma attack who respond poorly to SABAs.250mcg/dose, its use improves FEV_1 and clinical scoring. In the asthma crisis, which does not respond to SABA therapy, it is recommended

to use Corticosteroids. The use of CS is associated with a rapid improvement in pulmonary function, shortens the time of hospitalization, minimizes the number of hospitalization, reduces the chances of relapse after emergence, and reduces the need for SABA. Current guidelines such as Gina 2020, British guidelines 2019, recommend oral use of CS;ivCS are recommended only when the condition is severe and the child cannot drink from the mouth (33,34). CS in children <12 years old: **Prednisolone:** 1-2 mg/kg/day, divided into 2 doses (max: 60 mg/day), until PEF is 70% of the estimated one, with a treatment duration of 3- 10 days. The duration of treatment is determined based on the removal of symptoms or PEF 80%, usually requires 3-10 days of treatment (on average 5 days); It may be neededfor a longer-term treatment. In cases where longer-term treatment is required, the use of prednisolone 0.25-2 mg/kg/day is recommended, a single dose in the morning or on an alternative day (as far as asthma control is concerned); Dose max: 60 mg/day. Use of IV methylprednisolone (1-2 mg/kg/6-8 h, up to max dosage 40 mg) is reserved for severe crises and children who cannot receive oral medication (45,46,47). Dosage of CS in children ≥12 years old: **Prednisolone:** 40-80 mg/day, 1-2 times/day until PEF 70% of the estimated, with a treatment duration of 3-10 days; In cases where longer-term treatment is required, prednisolone 7.5-60 mg/day, single dose in the morning or on alternate day as needed for asthma control. (45)

COMPLIANCE OF DRUGS IN CHILDREN

Drug compliance/adherence is a major concern nowadays. It can directly influence the therapy outcomes. Trying to address this problem, doctors are advised to apply measures that help in minimizing the non-adherence such as: counselling, simplified regimens, use appropriate dosage forms ecc. (48)

The advantage of using oral corticosteroids and solution form

- They are well absorbed
- They act as fast as the parenteral forms
- They achieve good results since the first doses
- They have fewer side effects (36).
- They are applied easily without causing pain to the child
- The effect is realised in short term period, which is not associated with the “rebound” phenomenon
- It affects slightly the hypothalamus – pituitary axis. Minimal effect in the child growth (in bone metabolism, bone mineralization and adrenal gland function)
- Oral solution of prednisone has good absorption (peak concentrations 1-2 hours after administration: 20% higher and 15 minutes earlier than tablets), avoids swallowing difficulties in children, has a better compliance/adherence (37).

CONCLUSIONS

Glucocorticoids represent an important class of drugs being used successfully in the treatment of different respiratory syndromes in childhood. They can be effective in respiratory diseases as acute laryngotracheobronchitis, bronchiolitis, asthma and respiratory insufficiency. The physician should closely monitor their use aiming to achieve the best therapeutic effects and to avoid eventual side effects.

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Conflict of interest:

None declared.

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Systemic Treatments of Acne Vulgaris – 4 Case Series and Review of Literature

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Abstract

Acne vulgaris is a common disease among both adolescents and adults (adult acne). We present four different clinical cases classified as moderate-severe acne vulgaris treated with systemic treatments based on European protocols and review of the literature. Acne vulgaris is a disease that affects a lot the quality of life of the patient and either of the whole family. Treatment with systemic therapies based on the gravity of acne, the conditions of the patient and their preferences and tolerability of the medication have to be taken in consideration for good results.

Keywords: Acne vulgaris, systemic treatments, doxycycline, isotretinoine, oral contraceptive, spironolactone.

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INTRODUCTION

Acne vulgaris is a common disease among adolescents and adults (adult acne). It is a chronic inflammatory disease of pilosebaceous unit. The pathogenesis is complex resulting from increased sebum production induced from androgen, inflammation, and colonization by Corynebacterium (Propionibacterium acnes). (1,2) We present four clinical cases classified as moderate - severe acne vulgaris treated with systemic treatments based on European protocols and review of the literature. Acne vulgaris depending on clinical condition is classified as: mild, moderate, severe. The cases with moderate and severe conditions need systemic treatment combined with local treatment. Trigger factors have to be discussed carefully with the patient in a way to minimize the possibility to exacerbate acne. Oral antibiotics is an option of treatment of papular- pustular acne but always needs to combine oral antibiotics with local treatments to decrease antibiotic-resistant organisms. (1, 2, 3) Another effective therapy option is oral isotretinoin but doctors and patients have to take in consideration side effects and teratogenicity of this medication. Alternatives of systemic acne treatments are oral contraception and spironolactone. Every treatment has to be tailored for every case and for specific needs of the patients. Treatment with systemic therapies based on the gravity of acne, the conditions of the patient and their preferences and tolerability of the drug have to be taken in consideration before the treatment.

In this paper we discuss four different cases and systemic treatment recommended for these patients and we discuss the relevant literature.

Case 1

We present a 17 years old male who has been suffering from acne vulgaris since he was 12 years old. In the clinical examination were noticed papule/ pustular elements, black/white comedones and some acne scars remained from previous acne elements. The elements were localized on forehead, cheeks, chin and the upper part of the trunk. Time after time he was treated locally with salicylic acid washable gels, topical antibiotics (erythromycin and clindamycin) without significant improvement. The condition has affected a lot his quality of life and either the life of his parents. Both parents have had acne vulgaris during their adulthood.

Referring to the acne classification the case was classified as moderate acne vulgaris and the treatment recommended for this situation based on acne guidelines were oral antibiotics.

We started the treatment as follows: a- Doxycycline 200 mg/day, for 10 days, lowering the dose to 100 mg/day for 20 days and maintenance dose 50 mg/day for 2 months.

b- Local treatment with topical benzoic peroxide and retinoid.

c- Salicylic Acid washing gel.

The follow up was done every 4-6 weeks for 4 months.

In every session the clinical condition was significantly improved with the absence of

papules/ pustules and remaining few comedonic elements. The sebum production was significantly lowering.

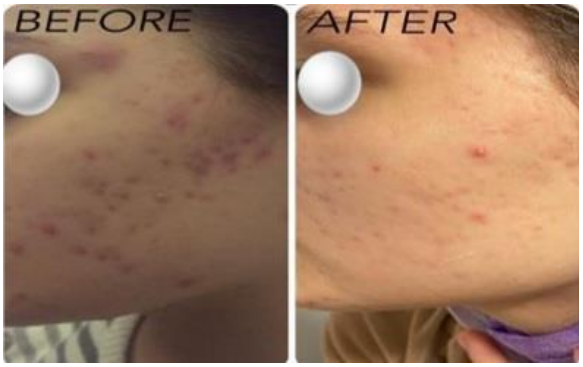

Figure 1. Case 1

Case 2

A girl 22 years old was treated with different local treatments and oral antibiotic (doxycycline) unsuccessfully for four years. The clinical condition was worsening and the stress of the patient and the whole family was at high level. In clinical examination there were evident nodular elements, papule/ pustules on the face and on the trunk. Patient was complaining for pain of the elements, esthetic issue and scars. The weigh was 62 kg.

Referring to the acne classification the case was classified as moderate- severe nodular acne vulgaris and the treatment recommended for this situation based on acne guidelines was systemic retinoid.

We decided to treat the patient with isotretinoin systemic therapy. Before the treatment we performed the blood tests for liver enzymes and lipids profile in the blood. The patient signed a consent paper that she has been informed for the retinoid risks in pregnancy and we decided to explain the contraception procedures.

The treatment at the starting point was:

a. Oral isotretinoin 30 mg/day for 30 days, increasing the dose 60 mg/ day for the next 6 months.

b. Local antimicrobial agent (erythromycin) for 6 weeks

c. Topical benzoic peroxide

d. Salicylic Acid washing gel.

After 8 weeks of treatment the improvement in the reducing number of papules/ pustules and the dimensions of nodules was noticed. One of the evident side effects of the treatment was cheilitis, and for this we recommended a lip balm. The lab tests were re-done after two months of treatment and no any alteration was noticed. We continued the treatment for 7 months with a significant improvement without any other side effects. The result was really good and after this the planning protocol was to treat remaining acne scars.

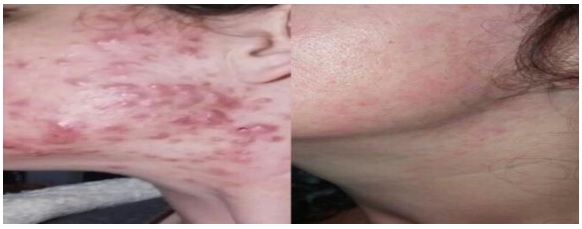

Figure 2. Case 2

Case 3

A.M, 33 years old patient presented in our clinic with acne on face for 1 year. She referred that 4 years ago she was treated for polycystic ovary. She was treated for acne with doxycycline 100 mg-day and topical erythromycin and benzoyl

peroxide for several weeks, but without any improvement. In clinical examination there were evident nodular elements, papule/ pustules on jawline distribution. Referring to the acne classification the case was classified as moderate-severe nodular acne vulgaris. Based on her anamnesis she was recommended to have a gynecological ultrasonography, which resulted in multiple cysts in the ovaries (polycystic ovary). 8 weeks after starting treatment with oral contraceptive (a combined preparation of ethinyl estradiol and drospirenone) patient referred to have considerable improvement of acne. Actually, the patient is in the sixth month of treatment with oral contraception and is being followed up.

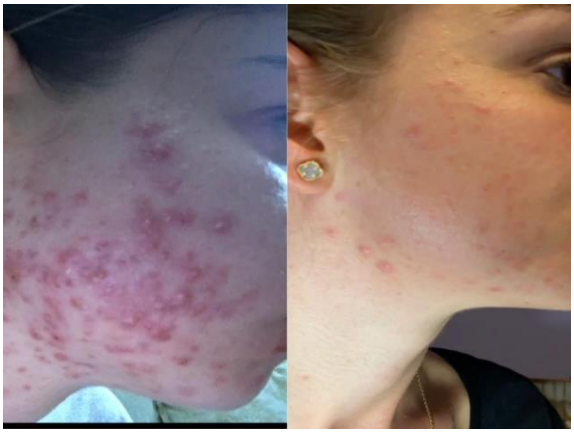

Figure 3. Case 3

Case 4

A female 36 years old has been treated for many years for acne vulgaris but not with a satisfying result. She refers that has used retinoids 10 years before but actually she does not want to use anymore retinoid. The gynecologic structure was not polycyclic structure. In clinical examination there were evident comedonal elements, papule/ pustules on all face. Referring her experience with acne vulgaris, anamnesis and her gynecologic condition we decided to treat the patient with oral spironolactone 50mg / day for the first month and 100mg / day for the next 2 months. Based on the protocol we monitored potassium and creatine levels 1 week after initiation of the therapy and then monthly for 3 months.

Figure 4. Case 4

After the first month there was a noticeable improvement of the condition, and the best results were seen after 3 months of full treatment.

During treatment with spironolactone the patient complained of a slight swelling and breast pain. Now she is in the 6th month of treatment for acne scars and is being followed up.

DISCUSSION

Referring the protocols of Acne treatment, oral antibiotics are prescribed as second-line therapy for patients with mild-to-moderate acne that is not adequately controlled with topical agents alone. Oral antibiotics use is a good choice in the treatment of patients with moderate-to-severe

inflammatory acne. We treat our case 1 with oral antibiotic referring the condition of the patient, his preferences and tolerability. Oral antibiotic treatment is recommended to combine with local treatment to improve the efficacy and either for resistance reason with topical retinoid or benzoyl peroxide if tolerated. (1,2,3) Referring to this protocol the treatment of our case 1 was the choice of antibiotic and local treatment. The results were noticeable improvement after 4 months of treatment. Monotherapy just with oral antibiotics is not recommended referring the last protocols and publications. (4, 5) The global problem of antibiotics resistance emphasizes the fact that the treatment must be recommended to be used for the shortest possible duration, ideally 3-4 months. (5) Local treatment during the use of oral antibiotics and after are strongly recommended. (6, 7, 8,9)

Treatment with antibiotics for limited period use may reduce and minimize the risk of inflammatory bowel disease (for tetracycline's), pharyngitis (for tetracycline's) (10), Candida difficile infection (11) and candida vulvovaginitis. The studies have shown that these side effects during antibiotic use are rare. During pregnancy antibiotics: Penicillin, erythromycin, and cephalosporin have safety profile. (12) The antibiotics that are recommended for treatment of acne vulgaris are: tetracycline class (minocycline, doxycycline, tetracycline), macrolides, azithromycin, Trimethoprim sulfamethoxazole (TMP/SMX), Penicillin and cephalosporin.

First line therapy for moderate- severe inflammatory acne referring the antibiotic use is the group of tetracycline that include: minocycline, doxycycline, and tetracycline. All the drugs within this group have an anti-inflammatory effect. (5) Doctors have to take in consideration some issues before prescribing this group of drugs: allergy, age< 8 years old, and pregnancy. One side effect or phenomenon associated with this group is pseudotumor cerebri. (5) Doxycycline is recommended in the dosage 1.7-2,4 mg/kg dose range (12) but for practical reason it is recommended to be used 50, 100, 200 mg a day. The use of doxycycline in sub antimicrobial dose (20 mg, 40 mg daily) is used successfully for anti-inflammatory effect in patients with moderate acne vulgaris. (13, 14) Doctors have to take in consideration that doxycycline is a photosensitizing medication and is more than minocycline, (5) and either the fact of gastrointestinal disturbances in high doses. (15) To minimize these side effects of the treatment patients should be advised to wear high SPF factor cream every day, to avoid sun baths during the treatment and to take doxycycline after the meal with water. (5) Another oral antibiotic that is recommended to treat acne vulgaris is minocycline that is recommended to be used 1 mg/kg. (5) For practical purposes, minocycline is generally dosed at 50 to 100 mg twice daily. Referring a study by Strauss et al treatment with minocycline was thought to be superior to doxycycline in reducing Proponium acnes. (16) Side effects noticed during the treatment with

minocycline are tinnitus, dizziness, and pigment deposition within the skin, mucous membranes, and teeth. (5) The hyperpigmentation is noticed in patients that use high dose and for long period. (5) In few cases are reported hypersensitivity drug reactions, drug-induced lupus. (17,18,19,20)
Oral erythromycin is another oral antibiotic that is used successfully in the treatment of acne vulgaris. Is a medication of choice in pregnant woman? (21,22). It is recommended to be used in the doses 250 to 500 mg twice daily and in combination with local treatment such as Benzoyl peroxide. (23) Doctors have to take in consideration that although erythromycin is largely considered safe for use during pregnancy, some papers reported existence of fetal cardiac malformation (24) and if used for long periods of time, hepatotoxicity is noticed in 10-15% of pregnant patients. (25,26)
Azithromycin is a medication that is tolerated better than erythromycin and is recommended in different dosage. One protocol is 500 mg, once daily for 4 consecutive days per month for 2 consecutive months. (27,28) Referring another study the protocol is 500 mg once daily for 3 days in the first week followed by 500 mg once weekly until week 10. (29) Another group of authors recommend the use of 500 mg once daily for 3 consecutive days each week in month 1 followed by 500 mg once daily for 2 consecutive days each week in month 2 and then 500 mg once daily for 1 day each week in month 3. (30) Referring one study in 2005 it was emphasized that azithromycin is as effective to treat patients with Acne Vulgaris as doxycycline. (30) Another more recent study in 2014 by Ullah et al. that compare the treatment of Acne Vulgaris with azithromycin and doxycycline showed the superiority of doxycycline. (31)
In patients with Acne vulgaris that are recalcitrant to tetracycline and macrolide it is recommended to use Trimethoprim sulfamethoxazole (TMP/SMX). Doctors when switch to this medication have to take in consideration the risk of resistance development. Referring the protocols TMP/SMX should be restricted to patients who are unable to tolerate tetracycline agents or in patients who are treatment-resistant. (5) The usual dose for patients with AV is one double-strength tablet twice daily. Side effects reported are gastrointestinal disturbances, photosensitivity, drug eruptions and Stevens-Johnson syndrome/TEN. (32,33)
Another alternative for the treatment of Acne vulgaris is Penicillin and cephalosporin especially during pregnancy or with allergies to other classes of antibiotic treatments. (5) Side effects include risk of hypersensitivity reactions (drug eruptions, anaphylaxis) and gastrointestinal disturbances (i.e., nausea, diarrhea, and abdominal distention and discomfort). (5) The recommended dosing for amoxicillin is 250 mg twice daily up to 500 mg three times daily. A study published by Fenner et al. showed that cephalexin is effective to treat patients with acne, 78% of patients noticed clinical improvement. (34) Recommended doses of cephalexin are 500 mg twice daily.

Our case 2: based on clinical condition and the anamnesis of the patient we decided to treat the patient with isotretinoin. Isotretinoin is a non-hormonal and non-microbial treatment option for moderate - severe acne vulgaris. (33) It is used successfully to treat recalcitrant resistant Acne Vulgaris or cases that relapses quickly after the antibiotic treatment. (34, 35, 36, 37,38,39) Based in protocols, isotretinoin is indicated for the treatment of patients with moderate- severe inflammatory acne that is either treatment-resistant, produces physical scarring or significant psychosocial distress. (50) Different studies shows that isotretinoin is effective in decreasing sebum production, the number of acne lesions (papules, pustules, nodules) and acne scars. (39,40,41,42,43,44,45,46,47) The starting dose recommended is 0.5 mg/kg/day for the first month and then increased to 1 mg/kg/day as tolerated up to a cumulative dose of between 120 and 150 mg/kg. (46,47,48) Some studies shows that higher cumulative doses up to 200 mg/kg may be more effective to reduce the rates of acne relapse and retreatment, but doctors have to take in consideration side effects of the medication. (50, 49) During the everyday practice in a way to minimize side effects and the medication to be tolerated well by patients are recommended low dose isotretinoin (0.25-0.4 mg/kg/day) and lower cumulative dose regimens. (34,35, 37,38, 40,48,49,50,51,52,53) It is recommended to take isotretinoin with meals, absorption is increased with fatty foods. (54,55)

In adult women, before prescribing isotretinoin it is recommended to take in consideration the contraception methods because of teratogenicity effect. Doctors should counsel women that they should not become pregnant 1 month before, during, or within 1 month after completion of isotretinoin therapy. Other side effects reported include: xerosis, cheilitis, xerophthalmia (most frequently), decreased night vision, vision changes, headache, hepatotoxicity, hypertriglyceridemia, mood changes, bone demineralization, cardiovascular risk factors, possible link to depression/anxiety/mood changes/suicidality, and possible link to inflammatory bowel disease (IBD). (31) A study by Zaenglein et al emphasize that there is insufficient data to support the link between isotretinoin use and IBD. (31) A frequent side effect during the treatment with oral isotretinoin are symptoms that mimic hypervitaminosis. A but these side effects resolve after discontinuation of therapy. (31) There is a debate referring the link between oral isotretinoin use and depression, anxiety, mood changes, or suicidal ideation/suicide is mixed. Some case reports show that isotretinoin has no negative effect on mood, memory, attention, or executive function. (31,55,56,57,58,59,60,61,62,63,64,65,66,67,69) But in the other hand there are studies that shows that 140 patients treated with isotretinoin have committed suicide while taking this treatment or within a few months of discontinuation of treatment and around 257 patients have been hospitalized for severe depression or attempted

suicide. (70) However, there are authors that argued that the number of reported cases that suffer for depression among isotretinoin users is not greater than people that suffer from depression in the general population. (71) It is recommended for doctors to monitor carefully patients under treatment with isotretinoin for depressive symptoms. The AAD working group recommends that prescribing physicians monitor patients for any indication of depressive symptoms and educate patients on the potential risks of treatment with isotretinoin.

After the first month of treatment, we did laboratory test to monitor the patient health (case 2) during the treatment with oral isotretinoin. Serum lipid profile (serum cholesterol, triglycerides), liver functions tests (transaminases) are known to increase in some patients who take oral isotretinoin (72,73,74). Some practitioners monitor laboratory test results monthly, but others only check at baseline and after dosing changes. Referring Hansen et al. recommendations for lipid panel and liver function tests are indicated to be repeated after two months and if these results are within the parameters, then no more tests are needed. (75) Pregnancy testing is required for female patients of childbearing potential at baseline, monthly during therapy, and 1 month after completion of isotretinoin treatment. The use of isotretinoin during pregnancy is contraindicated.

Our case 3: based on clinical condition and the anamnesis of the patient we decided to treat the patient with birth pill control (combined preparation of ethinyl estradiol and drospirenone). OCPs offer a valuable treatment option of women with acne. Hormonal contraception is used for treating acne vulgaris in the settings of hyperandrogenism, late-onset acne (> 25 years of age), and jawline acne distribution, acne with menstrual flare, comedonal acne with seborrhea, and acne that is resistant to conventional therapies. (78) cOCPs include an estrogen component, usually ethinyl estradiol, and a variant of progestin component. Estrogens are known to decrease sebum production and inhibit production of LH, FSH hormones. Progestin-only contraception is not used to treat acne. Synthetic progestins act at the progesterone receptor but also react with the androgen receptor to varying degrees and thus may even potentiate acne. (80) Newer synthetic generation progestins, have less activity at the androgen receptor and more specificity for the progestin receptor. These modifications were undertaken to reduce the potential risk of thromboembolic events and androgenetic side-effects. In most of the studies analyzed, total acne lesion counts decreased 40% to 60% with cOCP use and inflammatory lesion counts show greater improvement than noninflammatory lesion counts. (80) Cyproterone acetate (2 mg of cyproterone acetate and 0.35 of ethinyloestradiol) has shown variable efficacy outcomes. RadosławSłopień et al in their study emphasized that the results were visibly improved in acne in 40% and noticed a very good cosmetic effect in 26% of patients after 3 months of treatment.

Cypreterone is an analog of 17 OH-progesterone. cOCPs containing chlormadinone acetate or cyproterone acetate seemed to improve acne better than those containing levonorgestrel; however, this was based on limited clinical data. (86) Drosperinone is an analogue of spironolactone also used in the treatment of acne. DRSP 3 mg has been combined with two different doses of EE: 0.030 mg for one type of an oral contraception and 0.020mg for another medication of oral contraception. A drospirenone COC appeared to caused improvement in the facial and trunk acne (improvement > 50%) after 6 months of treatment and to be more effective than norgestimate or nomegestrol acetate plus 17β-estradiol but less effective than cyproterone acetate. (82) Dienogest significantly improved acne in 52%-66% of treated patients. (83-84) Dienogest was more antiandrogenic than both drospirenone and chlormadinone acetate. (85) EE-norgestimate was shown to be efficacious in moderate facial acne treated for 6 months, inflammatory lesions reduced by 56%, non-inflammatory by 41% and 32% achieved excellent improvement. (81) A lot of formulations of oral contraceptive medications exist, but the U.S. Food and Drug Administration (FDA) has only approved three types of OPCs for the treatment of acne vulgaris: norgestimate 0.180 mg/0.215 mg/0.25 mg - ethinyl estradiol 0.035 mg; norethindrone 1 mg- ethinyl estradiol 0.020 mg/0.030 mg/0.035 mg; drospirenone 3 mg -ethinyl estradiol 0.02 mg .(86) Cyproterone acetate is a synthetic derivative of 17-OH progesterone, approved in Europe for the treatment of acne, but is not available in the United States. Before prescribing OCPs should be considered some important contraindications: cardiovascular risk factors, severe hypertension, history of stroke or myocardial infarction, smoking combined with age > 35 years, history of migraine with focal aura, history of migraine combined with age > 35 years, current or past history of breast cancer or endometrial cancer, diabetes with complications, hepatic malignancy, abnormal liver function, hypersensitivity to OCPs, pregnancy. (87) OCPs offer an acne treatment option, but they are overlooked because dermatologists are unfamiliar with their description, their side effects. One study showed that dermatologists prescribed OCPs in only 2% of visits with female patients aged 12 to 55 years who presented for acne treatment. (80) Patients' fear of using a medication for several months/years as well as the possibility of serious side effects are another barrier to the use of OPCs. In conclusion, oral contraceptive pills can be an effective treatment option for women with acne, but understanding the risks and identifying the ideal candidates for therapy is essential.

Our case 4: based on clinical condition and the anamnesis of the patient we decided to treat the patient with spironolactone. Post-adolescent acne primarily affects females and is resistant to conventional treatment in 79-82% of cases (88). 81% of women report failures with systemic antibiotics and failures in response to treatment with isotretinoin ranging from 15 to 30% (89).

Spironolactone is used as a good alternative treatment in this population. It works as an inhibitor of the 5-alpha-reductase receptors at the sebaceous gland and reduces production of luteinizing hormone (LH) production at the pituitary level. Currently, very few studies have been performed in a limited number of patients: the studies showed that at low doses (lower than 200 mg/day over 3 months duration), spironolactone can be effective against acne. (89) P. Vargas-Mora et al 2020 recommend in acne to start dosing at 25 mg/d for 1 week and then to increase to 50 mg/d (and maintain this dose in most patients). In this study authors have not found it necessary to use doses greater than 100 mg/d. The treatment works slowly over several months. Although spironolactone has been around for almost 30 years in the US as a treatment for facial acne, its indication for acne treatment has not yet been approved by the FDA, which limits dermatologists in its description. Common side-effects in pre-menopausal women include breast tenderness/enlargement and irregular menstrual periods. These effects can be avoided by taking spironolactone with oral contraceptive pills in women of childbearing age. This medicine should not be prescribed to women who are pregnant or planning to become pregnant. Spironolactone can cause mild or serious side effects. Mild side effects of Spironolactone can include confusion, headache, itching, menstrual problems, nausea and vomiting, diarrhea, sexual dysfunction, stomach cramps, dizziness. Most of these side effects may go away within a few days or a couple of weeks. Serious side effects can include: allergic reaction, electrolyte imbalance, gynecomastia. Physician should recommend to check frequently the potassium level in women with heart or kidney problems as well as those taking medications that affect potassium levels. (90) Because of its anti-androgenic effects, spironolactone has been hypothesized to be associated with an increased risk of estrogen-sensitive cancers but there is currently no evidence to support this in human subjects (91). The benefits of using spironolactone in the treatment of acne are: Improving the quality of life in women suffering from acne; reduces the risk of developing bacterial resistance by reducing the prescription of antibiotics (92); can be used as an alternative to isotretinoin in patients of childbearing age and the peripheral hyperandrogenia that frequently occurs in women does not respond well to isotretinoin. Patients showed 73.1%, 75.9%, and 77.6% improvements on the face, chest, and back, respectively, which supports that spironolactone is equally effective in treating acne in multiple areas of the body. (91) Based on the study performed by Charny at al (2017) spironolactone has a higher efficacy than minocycline and almost the same as isotretinoin making it an effective treatment for acne in adult women. Although it has shown efficacy, its indication has not yet been approved by the FDA for acne treatment. Studies with larger groups will be needed for spironolactone to gain legitimacy as a systemic acne medication. Thus,

for many dermatologists' spironolactone remains an alternative rather than a mainstay treatment for female patients with acne. (91)

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Balo Concentric Sclerosis

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CASE

A 13-year-old female presented with a left hemiparesis. Her family history was unremarkable, and her parents stated that she complained of headaches, sensory disturbances and general weakness in the past few weeks. Physical examination showed an obese patient but was otherwise unremarkable. A brain MRI study was ordered and showed multiple high signal lesions in the white matter with the largest in the right centrum semiovale having a multilayered concentric with alternating rings of higher and lower signal intensity in all sequences (Figure 1).

MR spectroscopy (MRS) was also done and showed elevated choline peak, a finding in keeping with the diagnosis of acute Balo concentric sclerosis (BCS) due to increased number of inflammatory cells (Fig. 2). N-acetyl aspartate was low indicating an alteration of neurons and their axons. The patient underwent treatment with intravenous methylprednisolone and had complete resolution of her neurologic deficits.

BCS is a rare variant of acute multiple sclerosis first described in 1928 by the Hungarian neuropathologist, Joseph Balo. Histopathology reveals alternating bands of myelinated and demyelinated axons corresponding to the concentric rings of higher and lower signal intensity present in various MRI sequences (1). Higher T2 signal intensity rings represent demyelinated and swollen axons with lymphocytic and macrophage infiltration as well as activated microglia (all explaining the increased choline on MRS) producing cytokines and other substances. Lower T2 signal intensity rings correspond to preserved myelination (2, 3). Historically, most of these patients were diagnosed post-mortem but nowadays MRI reveals distinctive features that enable early diagnosis, avoid biopsy and guide adequate treatment.

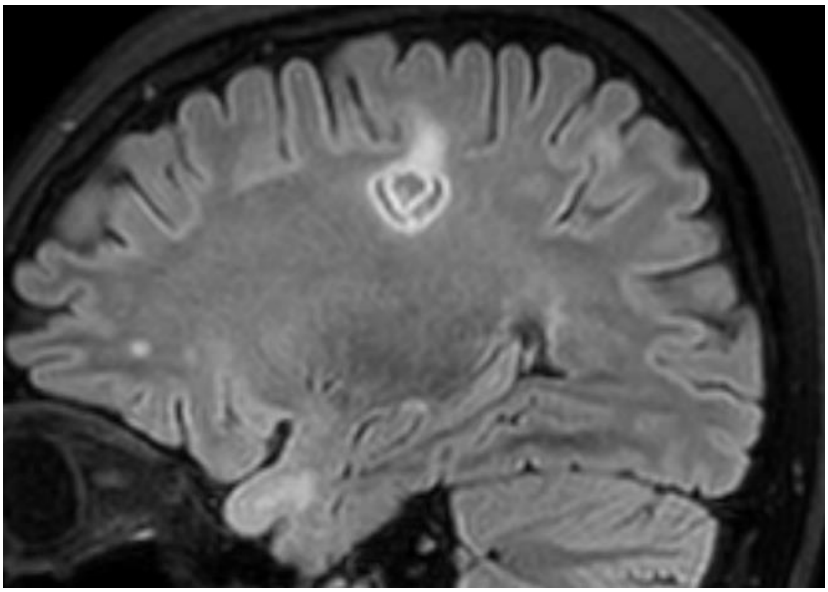

Figure 1. Parasagittal FLAIR image through the right centrum semiovale shows a multilayered lesion harboring concentric rings of high signal intensity interspersed with ones of lower signal intensity. There are additional and non-specific smaller high intensity lesions in the lower frontal and anterior temporal lobes.

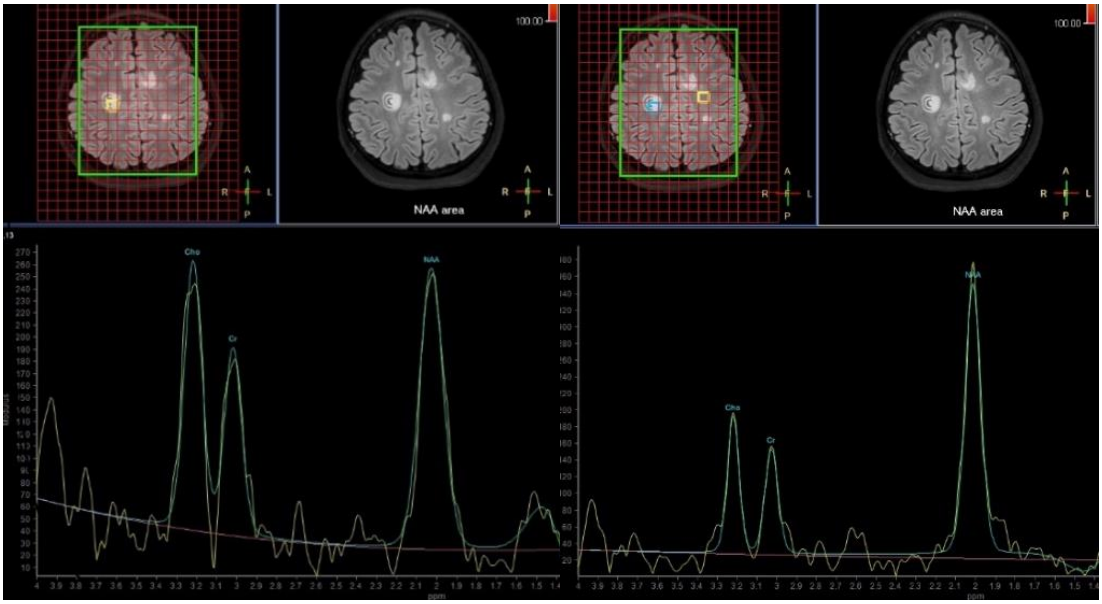

Figure 2. Multivoxel MRS of the lesion (A) shows increased choline and low n-acetyl aspartate when compared to control voxel on the opposite side (B) which shows a normal spectroscopic pattern.

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Conflict of interest:

None declared.

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Quantitative Analysis of Heavy Metals in a Hair Sample with the ICP-MS: A Case Report

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Abstract

Background: Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is a type of mass spectrometry that uses an inductively coupled plasma to determine how much of a specific element is in the material analyzed. It is a highly sensitive and specific quantitative analysis, when the concentration of each element is determined by comparing the counts measured for a selected isotope to an external calibration curve that was generated for that element.

Case Report: The aim of the study is to determine the concentration of each chemical element with ICP-MS in a hair sample of a 25-year-old woman (non-smoker, without a previously diagnosed chronic disease). The research was carried out in the UNILAB laboratory at the University "Goce Delcev"-Stip by the method of ICP-MS, Agilent7500.

Conclusion: We observed low concentration of: Cu, Zn, Ge, Se, B, Fe, Na, K, Rb, Cd, Hg, Tl, Pb, Be, Ag, Sb, Bi and U and high concentration of: Mg, Ca, S, V, Cr, Mo, Mn, Co, Li, Sr, Al Ni As and Ba. The proposed ICP-MS method for analysis of multiple chemical elements is a non-invasive method of investigation and it can be employed in routine analysis, which can extend the use of hair analysis for therapy, occupational, nutritional, and toxicological controls. Therefore, the method itself can help health professionals in identifying and detecting certain toxic elements in the body and perform early diagnosis of certain diseases.

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Keywords: Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), heavy metals, hair, biomonitoring

INTRODUCTION

Determination, i.e., detection of the presence of heavy metals in various biological samples is of great importance for the organism, because the increased concentration or presence of certain heavy metals in the body can cause some pathophysiological changes in the tissues themselves (1-5). Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is a method used to quantify the presence of heavy metals in each biological sample and it is highly sensitive and specific for analysis of multiple chemical elements. It also allows the analysis of isotopes with a low limit of detection for multiple elements in concentration from ppb (part of billion) to ppt (part of trillion). The Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method uses a high-frequency inductively coupled plasma as ionization source and a mass Spectrometer as mass/charge filter device. It is ideal for the elemental analysis of sample solutions, where the lowest detection limits are demanded. Due to the increase in the number of analyzed samples, analyzed elements and detection limits in recent years, the ICP Mass Spectrometer has become highly regarded for its speed, accuracy, and performance (6). The aim of the study is to emphasize the importance and significance of ICP-MS method as a powerful alternative to the usual bioanalytical methods. This approach extends the detection or determination of concentrations of chemical elements as biomarkers and offers the possibility of detailed analysis of the researched biological sample, in our case, hair.

CASE REPORT

The research was carried out in the laboratory of UNILAB at the University "Goce Delchev" - Stip. More than 30 elements present in a hair sample were analyzed (both macro and micro-essential and toxic elements) (Figure 1 and Figure 2). The respondent is a volunteer, a 25-year-old woman (non-smoking woman with no previously diagnosed chronic disease). The analysis was made due to the respondent's interest in the state of toxic elements in her own organism and accordance with ethical standards, with the consent of the respondent and accordance with the Declaration of Helsinki. After the collection of approximately 0,5g of human hair from the nape of the neck using stainless steel scissors (only the first 3-4 cm closest to the scalp were used), hair samples were performed without treatment (washing) in a laboratory with organic solvents (according to the International Atomic Energy Agencies recommended procedure (7,8). The hair was mineralized (dissolved) with a combination of 5 ml nitric acid and 2 ml hydrogen peroxide in a closed system – laboratory microwave, at constant temperature and pressure, and in the resulting solution were subsequently read the contents of the exanimated elements by the method of ICP-MS, Agilent7500.

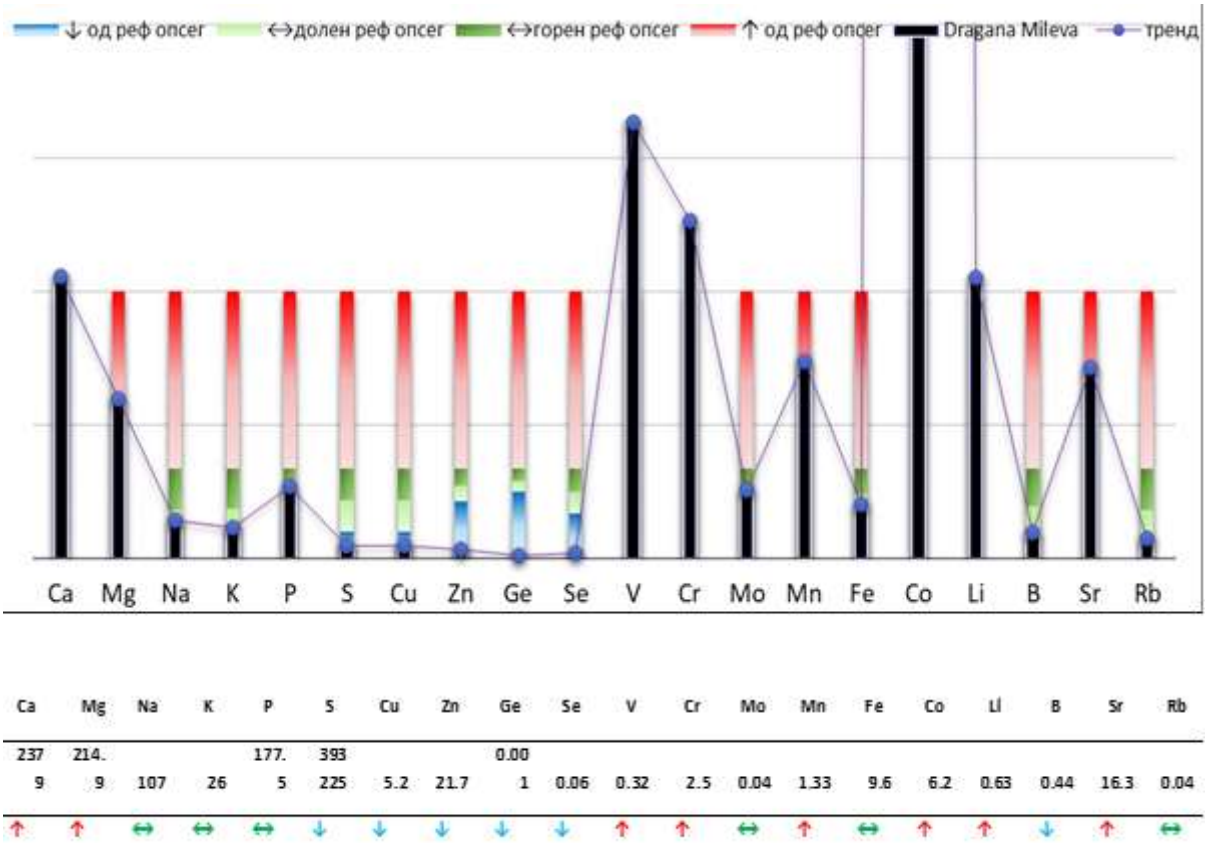
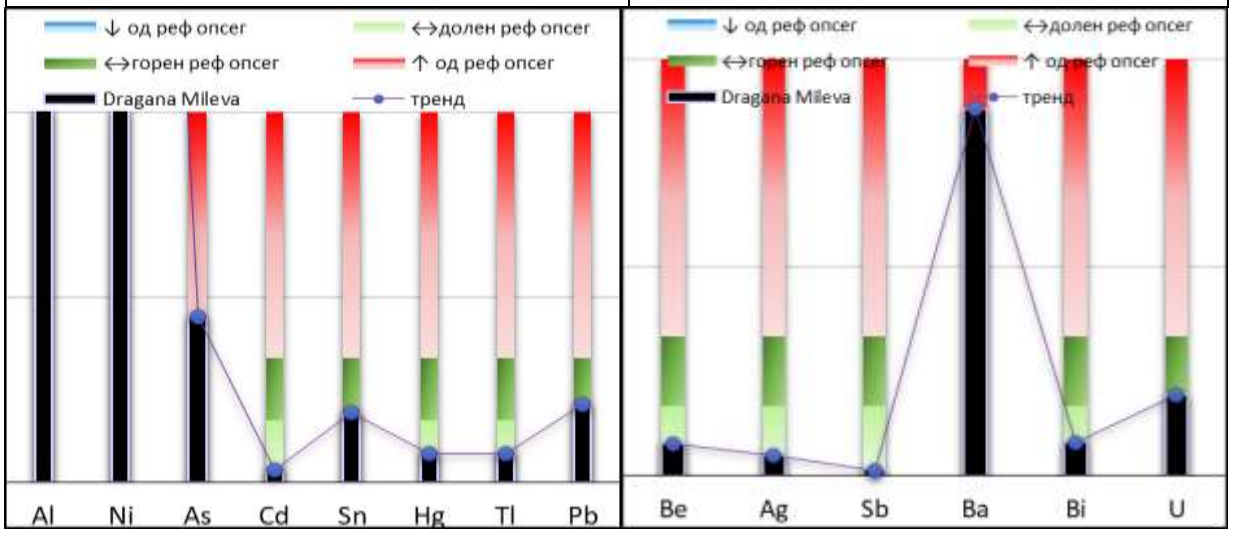


Figure 1. Relative content of essential macro and microelements in hair in terms of % reference bands and absolute content of certain elements in mg/kg.

1. Relative content of toxic elements in the hair	2. Relative content of potentially toxic elements in the hair



Al	Ni	As	Cd	Sn	Hg	Tl	Pb	Be	Ag	Sb	Ba	Bi	U
13.6	2.7	0.080	0.005	0.17	0.1	0.000	0.50	0.005	0.02	0.002	5.3	0.14	0.03
↑	↑	↑	↔	↔	↔	↔	↔	↔	↔	↔	↑	↔	↔

Figure 2. Relative content of toxic and potentially toxic elements in hair in terms of % reference bands and absolute content of certain elements in mg/kg

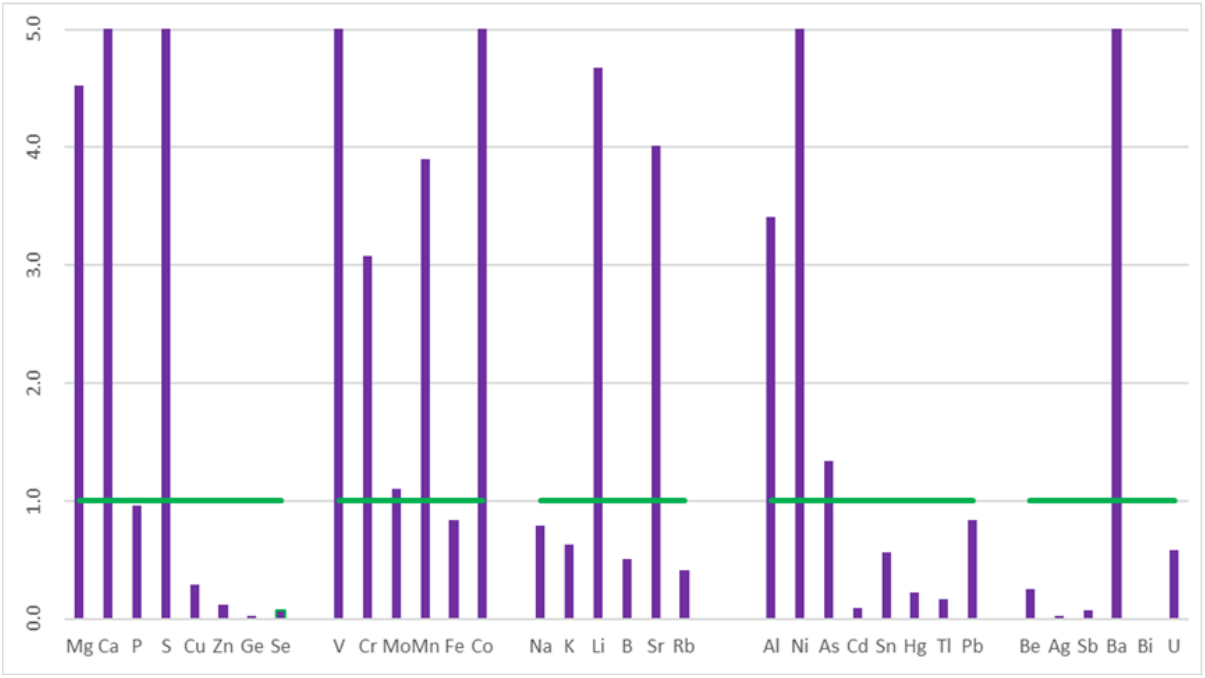


Figure 3. Metabolic profile of different chemical elements in the hair

Low content of chemical elements	**Cu, Zn, Ge, Se, Fe, Na, K, B, Rb, Cd, Sn, Hg, Tl, Pb, Be, Ag, Sb, Bi, U**
Normal content	**P**
High content of chemical elements	**Mg, Ca, S, V, Cr, Mo, Mn, Co, Li, Sr, Al, Ni, As, Ba**

The results were compared with trace element content in hair of reference man (9) and with few literature data (10-14). According to these, in the hair sample of the respondent in our study, we conclude: (Figure 3):

- low content of: Cu (5,3<24 mg/kg), Zn (21,7<180mg/kg), Ge (0.001<0.035mg/kg), Se (0,06<0,825mg/kg), B (0,44<0,9mg/kg), Fe (9,6<11,5mg/kg), Na (107<135mg/kg), K (26<41,5mg/kg), Rb (0,04<0,096mg/kg), Cd (0,005<0,05 mg/kg), Hg (0,091<0,4 mg/kg), Tl (0,0005<0,002 mg/kg), Pb (0,50<0,8 mg/kg), Be (0,005<0,02 mg/kg),Ag (0,022<0,15 mg/kg), Sb (0,002<0,066 mg/kg), Bi (0,14<0,6 mg/kg), U (0,03<0,06 mg/kg).

- high content of: Mg (215>77,5 mg/kg), Ca (2379>475 mg/kg), S (393225>47000 mg/kg), V (0,32>0,042 mg/kg), Cr (2,5>0,525 mg/kg), Mo (0,039>0,035mg/kg), Mn (1,33>0,34mg/kg), Co (6,2>0,0225mg/kg), Li (0,63>0,135mg/kg) Sr (16,3>4,1mg/kg), Al (13,6>7mg/kg), Ni

(2,7>0,2mg/kg), As (0,080>0,060mg/kg), Ba (5,3>2 mg/kg).

DISCUSSION

Quantitative analyses of chemical elements present in the hair can help determine certain physiological conditions in the body that may be associated with stress, unbalanced diet, altered homeostasis. ICP-MS method is especially used for biomonitoring the presence of heavy metals in the working or living environment (5, 11-14). Such analyses or screening tests have certain limitations and are therefore supplemented by other laboratory tests and medical examinations by a physician. Fu and Xi (1) observe that, occupational exposure to heavy metals occurs because of using these metals in a variety of industrial processes and/or a variety of materials, including color pigments and alloys. A series of adverse effects on human metabolism has resulted from exposure to heavy metal-contaminated drinking water, which has been recorded from around the world. The general mechanism of heavy metal toxicity is through the production of reactive oxygen species, the appearance of oxidative damage, and subsequent adverse effects on health (11-14). Conclusions of Singh et al. (5) are: presence of heavy metals in the body can result from consuming different foods and metabolic differences between individuals. Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. Vegetables are rich sources of vitamins, minerals, and fibers, and have beneficial anti oxidative effects. However, intake of heavy metal-contaminated vegetables may pose a risk to the human health. Heavy metal contamination of food is one of the most important aspects of food quality assurance. Heavy metals are no biodegradable and persistent environmental contaminants, which may be deposited on the surfaces and then absorbed into the tissues of vegetables (5). Gellein et al. (9) conclude that trace element analysis of human hair has the potential to reveal retrospective information about an individual's nutritional status and exposure. As trace elements are incorporated into the hair during the growth process, longitudinal segments of the hair may reflect the body burden during the growth period.

Therefore, the ICP-MS can deliver valuable information about our state of health, the application of certain drugs and diagnosis of some diseases (15). They can be used for further research and medical examinations of the respondent. The obtained results or quantitative analyses of chemical elements in the body, indicate or enable early diagnosis of certain diseases and facilitate their further treatment and therapy. The level of metals can also be affected by seasonal variations or synergistic and antagonistic effect, for instance (16). However, current techniques including plasma (ICP-AES or ICP-MS), are more and more commonly applied for multi-element studies (7, 17) especially in the field of human biomonitoring studies and occupational exposure to metals (18-27). But it is

necessary to elaborate a standardized methodology of human hair material treatment, sample preparation (including washing) and to evaluate reference values, which will take into consideration main parameters affecting elemental composition of hair. The proposed ICP-MS method for analysis of multiple chemical elements is a noninvasive method of investigation and it can be employed in routine analysis, which can extend the use of hair analysis for therapy, occupational, nutritional and toxicological controls. With this case report we want to emphasize the importance and significance of ICP-MS method as a powerful alternative to the usual bioanalytical methods in human biomonitoring studies.

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The authors declare that there are no conflicts of interest.

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A Rare Case of Bullous Scabies in Children

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Abstract

Background: Scabies is a parasitic infection of the skin caused by Sarcoptes scabiei var. hominis. It affects mostly adults and is a worldwide disease with around 300 million cases reported per year. Bullous scabies is a rare subtype of the disease, with only 5 cases reported in children less than14 years old. Clinical findings appear several weeks after exposure, and are associated with pruritus. Burrows, excoriations, vesicles, papules and nodules are seen on physical examination. Differential diagnosis should be made with adverse cutaneous drug reactions, contact and atopic dermatitis, dyshidrotic eczema, pediculosis, other parasitosis, dermatitis herpetiformis and bullous pemphigoid. Infested individuals are at risk of secondary bacterial infection. Diagnosis is often made clinically, but can be confirmed by performing a scabies preparation and/or dermoscopy.

Case Report: We present the rare case of a 5 years old boy who came to our health center complaining of severe itchy rash for 4 weeks, getting worse during night time. On physical exam we found an erythematous papulonodular rash all over his body and bullous lesions on his genitals. He was clinically diagnosed with Bullous Scabies. Here we will discuss the differential diagnosis and treatment options.

Conclusion: Bullous scabies is a rare presentation in children. The diagnosis should be considered in all patients who present with bullous lesions accompanied by pruritus and

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maculopapular rash. These lesions do not resolve with topical steroids treatment. It is important to treat the patient and his family members with topical scabicides like permethrin 5% cream.

Keywords: child, bullous, scabies

INTRODUCTION

Scabies is a common skin disease in developing countries. According to WHO, it is estimated to affect more than 200 million people at any time worldwide. (1) Prevalence estimates in the recent scabies-related literature range from 0.2% to 71%. In the past, epidemics occurred in cycles every 15 years. The latest epidemic began in late 1960s but has continued to the present. (2) Scabies occurs all over the world and is a major public health problem in less- developed countries, with an estimated average prevalence of 5–10% in children. The most vulnerable groups are children and the elderly, especially in overcrowded and poor communities where there is limited access to treatment. Recurrent infestation are common.

Scabies is usually transmitted by skin-skin contact with an infested individual. Mites can remain alive for >2 days on clothing or in bedding, therefore scabies can also be acquired without skin-skin contact. The highest risk of transmission is in individuals with crusted scabies. Scabies mites of all developmental stages burrow into epidermis shortly after contact, no deeper than stratum granulosum where the adult female lays eggs. Females lay eggs in tunnels and burrow 2 to 3 mm daily. After 4–6 weeks the patient develops an allergic reaction to the presence of mite proteins and feces in the scabies burrow, causing intense pruritus and rash.

Patients, typically the immunocompetent hosts, present with severe intense widespread pruritus. Pruritus often interferes or prevents sleep and often presents in family members. Rash ranges from no rash to generalized erythroderma. The first sign of infestation consists of 1-2 mm read papules, some of which are excoriated, crusted or scaling. Other clinical findings are linear intraepidermal burrows, 0.5-1 cm gray or skin-colored ridges, either linear or wavy with vesicles or papule at the end of the tunnel. These burrows are present around the interdigital webs of hands, wrist flexors, anterior axillary folds, upper and lower extremities, umbilicus and belt line. Infants and small children will have a diffuse eczematous eruption that will typically involve scalp, neck, face, palms and soles that are generally spared in adults. In rare cases, bullae on the penis and scrotum of children and adult males are seen in the other rare variant called bullous scabies. Additional clues include facial sparing, affected family members, poor response to topical antibiotics and temporary response topical steroids (3)

Diagnosis of scabies can often be made clinically. Burrows are pathognomonic for human scabies. The diagnosis is confirmed by microscopic identification of mites, ova and scybala in epithelial debris. Scrapings most often test positive when obtained from burrows of fresh papules. (4, 5, 6)

CASE PRESENTATION

A 5 years old boy presented to ABC Health Centre, in December 2021 with a history of papular rash and nodules on his body for about

one month. These lesions were localized in arms, under the axilla, trunk and genital area. His biggest complaint was the appearance of a big vesicle on his penis in the last week and severe pruritus during the night time. The patient was treated with topical steroids for two weeks without improvement. Patient attended daycare where other children have shown similar symptoms. His family members manifested similar complaints and lesions.

Skin examination revealed polymorphous lesions such as, erythematous papules all over his body, intraepidermal burrows of hands, wrist, anterior axillae, and around umbilicus. Excoriated maculopapular lesions and inflammatory nodules were localized more on the trunk. (Figure 1)

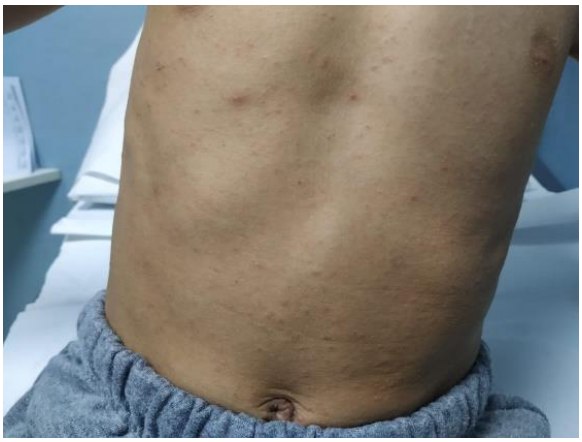

Figure 1. Excoriated maculopapular lesions, erythematous papules and intraepidermal burrows

During genital examination were noticed bullous lesions with diameter 1 to 3 cm, that were filled with fluid surrounded by papulo-nodular erythematous rash in the scrotal area. (Figure 2) There was no mucosal and facial involvement. No signs of dehydration. The Nikolsky sign over the lesions was negative.

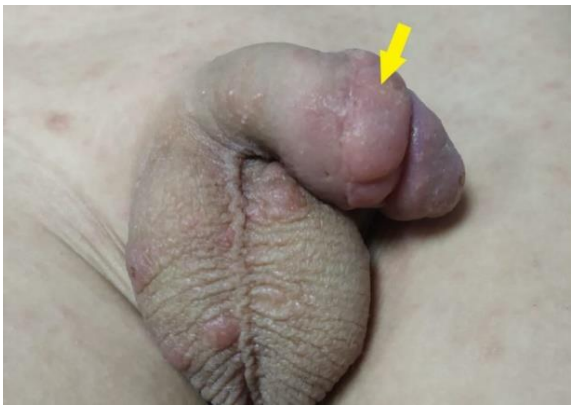

Figure 2. Penile bullous lesions with diameter 1 to 3 cm (demonstrated by the yellow arrow), filled with fluid surrounded by papulo-nodular erythematous rash in the scrotal area

Clinical diagnosis of bullous scabies was made based on 2018 IACS criteria considering borrows and penile bullous lesions as pathognomonic for bullous scabies. (10)

Other possible diagnosis such as, bullous pemphigoids, drug eruptions, urticaria, varicella, dermatitis herpetiformis, seborrheic dermatitis were ruled out based on history, clinical findings and no response to steroid treatment.

Patient was treated with Permethrin 5% cream. He was advised to apply the cream for 8-12 hours on his full body. Patient was prescribed antihistamine medication for pruritus. He showed improvement in 4 weeks. On skin examination was noticed disappearance of erythematous rash and flattening of the penile bullous lesions. (Figure 3) Family members were treated with Permethrin 5% cream and showed similar improvements. No recurrence occurred during a 6 months follow-up.

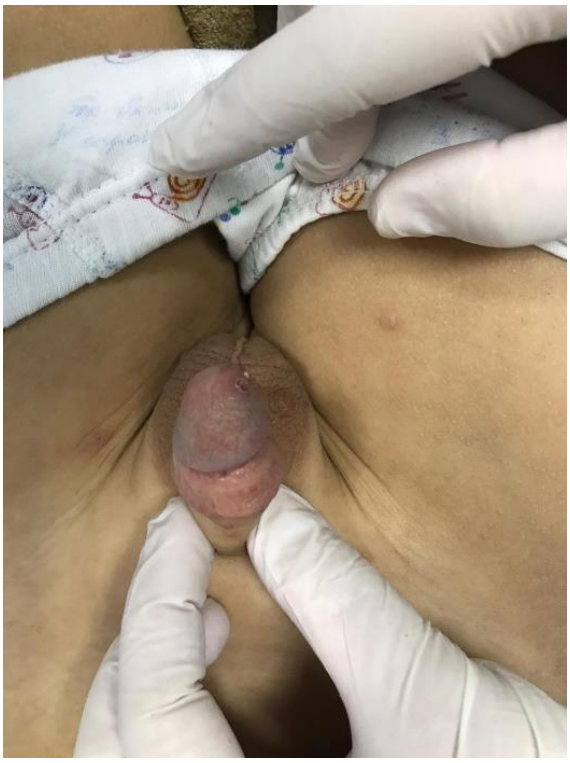

Figure 3. Flattening of the penile bullous lesions and disappearance of erythematous rash

DISCUSSION

Bullous Scabies is a rare diagnosis in children. In our case, bullous lesions on genital organs, scabies burrows, nocturnal itching and response to antiscabies treatment confirmed the diagnosis of bullous scabies based on 2018 IACS criteria (Table 1). (10)

The diagnosis of "confirmed scabies" needs direct visualization of mites or mite products like eggs and faeces by methods including direct microscopy or dermatoscopy. (6) However, there were some cases in the literature, like in our case, when these methods could not be used and diagnosis was made based on clinical findings of tense bullous lesions accompanied by pruritus and a maculopapular rash. (8) The "clinical scabies" 2018 IACS criteria help health workers to diagnose scabies and is being used in literature to confirm the diagnosis.

The differential diagnosis depends on the type of lesions. Papulovesicular lesions are misidentified as urticaria, varicella, drug eruptions, dermatitis herpetiformis and folliculitis. Eczematous lesions may look like atopic dermatitis and seborrheic dermatitis. Both clinical and pathological features of bullous scabies are quite similar to those of bullous pemphigoid (7). Even when

Table 1. Summary of 2018 IACS criteria for the diagnosis of Scabies

Criterion	Diagnostic features
A: Confirmed scabies	*At least one of:* A1: Mites, eggs, or faeces on light microscopy of skin samples A2: Mites, eggs, faeces visualized on individual using high-powered imaging device A3: Mites visualized on individual using dermoscopy
B: Clinical scabies	*At least one of:* B1: Scabies burrows B2: Typical lesions affecting male genitalia B3: Typical lesions in a typical distribution and two history features
C: Suspected scabies	*One of:* C1: Typical lesion in a typical distribution and one history feature C2: Atypical lesion or atypical distribution and two history features
History features	H1: Itchy rash H2: Close contact with individuals who has itch or typical lesions in a typical distribution

histopathology and scraping findings are positive for scabies, it is still difficult to differentiate and to exclude the possibility of scabies and concomitant bullous pemphigoid. It is also reported that patients diagnosed with scabies have an increased risk for bullous pemphigoid. Detection of scabies mites and/or its eggs from scraping, good response to scabicides treatment, and no response to topical or oral steroids are important clues for the diagnosis of bullous scabies. (11)

The treatment of bullous scabies is done with topical scabicides like permethrin 5% cream which is applied to the full body from the neck down. In infant's scabies is usually found above the neck, requiring treatment of the scalp. Additional therapies may include lindane 1% lotion or cream, sulfur ointment 5-10 %, malathion 0.5% in aqueous base, and benzyl benzoate emulsion 10-25 %. (4, 6) Topical steroids, however, do not resolve bullous scabies lesions in contrast with other bullous cutaneous diseases. (11) For severe infestations and or in immunocompromised patients oral ivermectin 200 µg/kg per dose should be given orally for 2 doses, taking it 2 weeks apart which is highly effective and is approved in several countries. (9) Clothing, bed linens and towels should be washed in hot water and dried using high heat. All close contacts of the infested child which includes family members and care takers should be treated.

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None declared.

Conflict of interest:
None declared.

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