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Formerly "Bulletin of Medical Sciences"

THE OFFICIAL JOURNAL OF THE UNIVERSITY OF MEDICINE, TIRANA

Citation Abbreviation: AJMHS (Formerly Bulletin of Medical Sciences)

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- Comparing the Real Outcome to the Probability for that Outcome by Generation of a Computer Model: a Minimum Standard of Burn Survival
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ALBANIAN JOURNAL OF MEDICAL AND HEALTH 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
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Postmortem Interval Estimation by Evaluating Saposin D Levels and Morphological Alterations in Hippocampal Neurons

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Abstract

Background: The correct estimation of the time of death is critical in the field of forensics and legal medicine, and often determines the outcomes of criminal investigations. Current postmortem interval (PMI) estimation methods involve the evaluation of physical, chemical, biological, and histological postmortem changes; however, their accuracy is limited.

Aims, Study design and Methods: In this study, we used rat brains to characterize postmortem alterations in the levels of prosaposin (PSAP) and its degradation product saposin D, as well as morphological changes in the hippocampus. Furthermore, we used fluorescent microscopy and observed profound morphological alterations in hippocampal pyramidal neurons after death.

Results: We found that PSAP levels decreased after death, whereas saposin D levels increased. Morphological alterations prolonged in hippocampal pyramidal neurons up to 7 days after death. Conclusion: These findings suggest that morphological alterations in the hippocampus and the PSAP and saposin D levels may improve the accuracy of PMI estimation.

Key words: Postmortem interval, MAP2, Prosaposin, Immunohistochemistry, Western blotting

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INTRODUCTION

The accurate estimation of the postmortem interval (PMI) is crucial in forensic sciences, given the importance of the PMI in determining the circumstances of death. Many PMI estimation approaches are based on physical, chemical, or biological cues. Algor mortis, livor mortis, rigor mortis, and supravital activity are among the most commonly used early PMI estimates, whereas physical changes (e.g., arthropod activity) and physiological changes (e.g., typical signs of decomposition) are used as late PMI estimates (1, 2). The decrease in body temperature is common PMI estimation method(3, 4). However, the temperature method can only be used up to 36 hours postmortem depending on the environmental conditions. Additionally, its usefulness is limited in certain death cases, such as burns and severe trauma. Similarly, livor mortis provides limited accuracy in cases of severe blood loss and severe trauma. Although forensic entomology can also provide viable information in later stages of death, this approach is restricted to local fauna and insect accessibility to the dead body. Each of these methods provides only a rough estimate of PMI, and often different methods yield contradicting results. Thus, novel, more accurate PMI estimation methods are required.

Postmortem decomposition of biomolecules has gained increasing attention over the last years. Several studies have investigated postmortem alterations of RNA, DNA, or proteins (5-10). We have used rat models to investigate postmortem changes in brain biomolecules, which are protected from environmental insults (e.g., bacterial infections) by the skull. Specifically, we have analyzed changes in protein levels and histological changes in rat brains at two different time points after death. Increasing evidence suggests that the levels of RNA, DNA, proteins, and other biomolecules decrease over time after death. Nevertheless, it remains unclear if the levels of some biomolecules increase after death. Prosaposin (PSAP) is a ~66-kDa glycoprotein precursor of four sphingolipid activator proteins, saposins A, B, C, and D. Saposins are abundantly found in the brain, where they act as neurotrophic factors (11-15). Importantly, the structure of PSAP is highly conserved between rats and humans. We hypothesized that due to decomposition, PSAP levels decrease and saposin levels increase after death.

Organs decay over time. In particular, the abdominal organs are strongly affected by autolysis and bacterial putrefaction, leading to specific alteration patterns (16). Tomita et al. (17) comprehensively analyzed the alterations in abdominal organs postmortem. However, the postmortem histological changes that occur in the brain remain unknown (18). In this study, we investigated the usefulness of the PSAP and saposin levels and histological changes in the brain to accurately estimate the PMI.

MATERIALS AND METHODS

Animals and tissue preparation

Nine Wistar rats (8 weeks old) were purchased from CLEA Japan (Kyoto). Rats were housed at a constant temperature (22°C) under a 12/12-h light/dark cycle and were given food and water ad libitum. All experiments were conducted in accordance with the ARRIVE guidelines and the Guide for Animal Experimentation of the Ehime University School of Medicine, Japan. Animal protocols were approved by the Animal Care Committee of Ehime University (permit number 05A261). Animals were euthanized by carbon dioxide inhalation and stored in a 21°C thermostatic chamber for 0, 3, 6, or 12 h, or 1, 2, 3, 5, or 7 d postmortem (n = 1 at each time point). Brains were collected and divided into left and right parts. Brain tissues were placed into Eppendorf tubes (1.5 mL) on ice and stored at −80°C until homogenization.

Anti-rat PSAP antibody

The anti-rat PSAP antibody (PSAP-Ab: IM-1) was prepared as previously described (19). Briefly, the amino acid sequence of rat PSAP was identified (20), and a synthetic oligopeptide corresponding to the proteolyzed portion of PSAP (409-PKEPAPPKQPEEPKQSALRAHVPPQK-434; Fig. 1) was used to generate a rabbit polyclonal antibody against rat PSAP. The generated antibody reacted with PSAP but not with saposins.

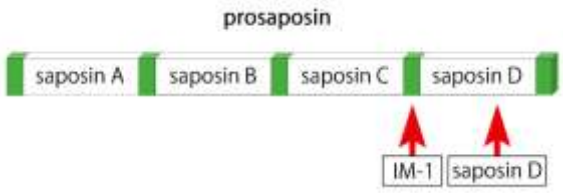

Figure 1. Structure of prosaposin (PSAP). PSAP is the precursor of four saposins. Anti-PSAP antibody (IM-1) was generated using a synthetic oligopeptide corresponding to the proteolysis site located between saposin C and saposin D. Because this sequence is absent from mature saposins, the antibody does not react with saposins; it only reacts with PSAP. The anti-saposin D antibody reacts with both PSAP and saposin D.

Immunohistochemical analysis

Rat brains were fixed in 4% paraformaldehyde (in 0.1 M phosphate buffer), embedded in paraffin, sectioned, and deparaffinized. The sections were washed for 15 min in PBS plus Tween 20 (PBS-T) and blocked overnight in PBS (0.1 M) containing 5% bovine serum albumin, 5% normal swine serum, and 0.1% NaN3. Subsequently, samples were probed with the primary antibodies rabbit polyclonal anti-PSAP IgG (1:5,000) and mouse monoclonal anti-MAP2 IgG (1:5,000). The sections were washed twice for 15 min in PBS and incubated with the secondary antibody Cy3 anti-rabbit IgG (1:250) or FITC-conjugated anti-mouse IgG or IgM (1:500). Cell nuclei were counterstained with DAPI for 2 h. Then, the sections were washed twice for 15 min in PBS, mounted in PermaFluor Aqueous Mounting medium (Thermo Shandon; Thermo Scientific, West Palm Beach, FL, USA), and examined under a Nikon A1 confocal microscope (Nikon, Tokyo, Japan). The

hippocampal CA1 regions were examined to assess pyramidal cell body alignment. Pyramidal cell bodies are aligned in rows, extending long apical dendrites in the same direction. Tissues were compared for these characteristic histological features.

Nomenclature of hippocampal CA1 neurons

The hippocampal CA1 region is divided into five layers: alveus, stratum (st.) oriens, st. pyramidale (pyramidal neuron layer), st. radiatum, and st. lacunosum-moleculare (Fig. 3)(21). Typically, one or two primary apical dendrites emerge from the neuronal cell body of CA1 pyramidal neurons, and then they are divided within the st. radiatum. Some basal dendrites originate from the CA1 cell body, bifurcating two or three times before terminating near the alveus (22-24).

Western blotting

Tissue samples were homogenized using a homogenizer (Tietech, Japan) in 50 mM Tris-HCl buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl) supplemented with 1× complete protease inhibitor cocktail (1%, Nacalai Tesque, Inc., Kyoto, Japan). Homogenates were centrifuged for 15 min at 12,000 × g at 4°C, and the supernatants were collected, aliquoted, frozen in liquid nitrogen, and stored at −80°C until further use.

Equal amounts (21 μg) of protein were loaded onto NuPAGE Bis-Tris gels following the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). After electrophoresis, proteins were transferred onto 0.45-μm polyvinyl difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% BSA in 1× Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated at 4°C overnight with one of the following primary antibodies: anti-saposin D (1:1,000), anti-PSAP (1:1,000), anti-α-tubulin (1:1,000), anti-β-catenin (1: 500), or anti-GAPDH (1:500). Subsequently, membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (1:5,000, Dako, Denmark). Protein signals were developed using ECL Prime Western blotting detection reagent (GE Healthcare, Buckinghamshire, UK) and imaged on an ImageQuant LAS 4000 imaging system (GE Healthcare, Marlborough, MA, USA).

RESULTS

Postmortem changes in PSAP and saposin levels

We extracted crude brain proteins at different time points (up to 7 d) after death to investigate changes in the levels of each proteins. The levels of α-tubulin and β-catenin, which are abundantly expressed in most tissues, decreased over time early postmortem (Fig. 2. a, b). No significant changes were observed in GAPDH levels until 5 d after death, when its levels began to decrease (data not shown). We also found that the PSAP levels (single band at ~65 kDa) decreased over time (Fig. 2c, d). In contrast, the saposin D levels (single band at ~25 kDa) gradually increased after death (Fig. 2e, f).

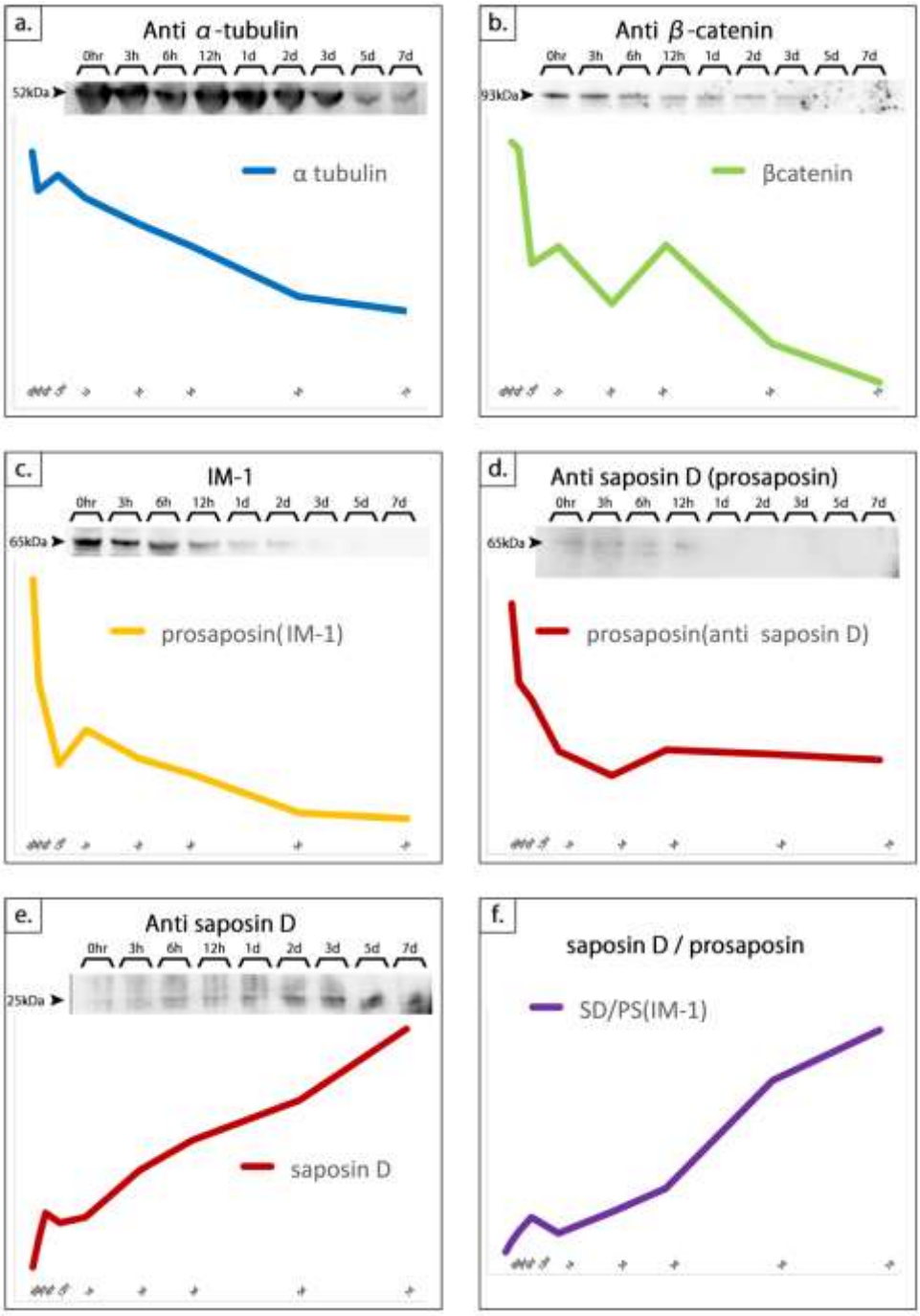

Figure 2. Crude hippocampal extracts obtained at different time points (from 0 h to 7 d) after death were stained with anti-α-tubulin (a), anti-β-catenin (b), anti-PSAP (c), or anti-saposin D (d, e). The intensity of the single band at 65 kDa (possibly corresponding to PSAP) decreased over time. (e, f) The intensity of the single band at 25 kDa (possibly corresponding to saposin D) increased over time. (f) The ratio of saposin D to PSAP gradually increased during the postmortem period (f).

Histological postmortem changes in CA1 pyramidal cells

We also compared the histological characteristics of CA1 pyramidal cells between the time of death (0 h; Fig. 3) and at different time points after death (Fig. 4). Pyramidal cells consist of the soma (containing the nucleus), axons, apical dendrites, and basal dendrites. The apical dendrite forms oblique branches terminating in a narrow tuft. PSAP is predominantly localized in the cytoplasm around the nuclei and large dendrites. The structures of the hippocampal CA1 region and pyramidal neurons were assessed by immunostaining with anti-MAP2, anti-PSAP, and DAPI (Fig. 3). The hippocampal CA1 region is divided into five layers. The dendrites of pyramidal neurons stained strongly with anti-MAP2. Primary apical dendrites were evident at the neuronal cell body and branched within the st. radiatum. Some basal dendrites originated from the CA1 cell body and bifurcated into secondary basal dendrites. PSAP was localized mainly in the cytoplasm of the CA1 cell body and in some dendrites (Fig. 3, 4).

We found almost no morphological changes in the CA1 pyramidal at cells 3 h after death (Fig. 4a1-4). The density of basal dendrites started to decrease by 6 h after death (Fig. 4b4). At this time point, the apical dendrite width was decreased but the length and number were maintained; some of the secondary branches had disappeared 6 h after death (Fig. 4b1, b2). The morphology of the cell soma, as well as the expression levels and distribution pattern of PSAP, were unchanged at 6 h after death (Fig. 4b3). The density of basal dendrites was markedly reduced by 12 h after death (Fig. 4c4). Additionally, the distal part of apical dendrites and the side branches had almost disappeared at 12 h after death (Fig. 4c2), and the cell nuclei had begun to shrink (Fig. 4c3). We observed no basal or apical primary dendrites at 1 d after death, and only the stems of pyramidal neurons were maintained (Fig. 4d1, d2). The nuclei had shrunk further, and the cell bodies became narrower (Fig. 4d3). The PSAP levels were further reduced extracellularly at 1 d after death, although PSAP levels were maintained intracellularly (Fig. 4d3, d4).

Basal primary dendrites had disappeared completely at 2 d after death, and the stems of pyramidal neurons had started breaking down (Fig. 4e2). PSAP immunostaining was prominent in the nuclei of CA1 neurons at 2 d after death (Fig. 4e3). Almost no apical dendrites were observed at 3 d after death (Fig. 4f2), and nuclei shrinkage and deformation were even more evident. Furthermore, MAP2 was almost undetectable around the cell nuclei (Fig. 4f3). All dendrites and cytoplasmatic extensions had disappeared at 5 d after death (Fig. 4g1), and MAP2 immunoreactivity was observed only as fragments (Fig. 4g3). Seven days after death, the hippocampal CA1 region was fully deformed (Fig. 4h1) except for some remaining nuclei. The structural changes in hippocampal pyramidal neurons and the alterations in PSAP expression patterns are illustrated in Figure 5. Gradual changes were observed in dendrites up to 3 d after

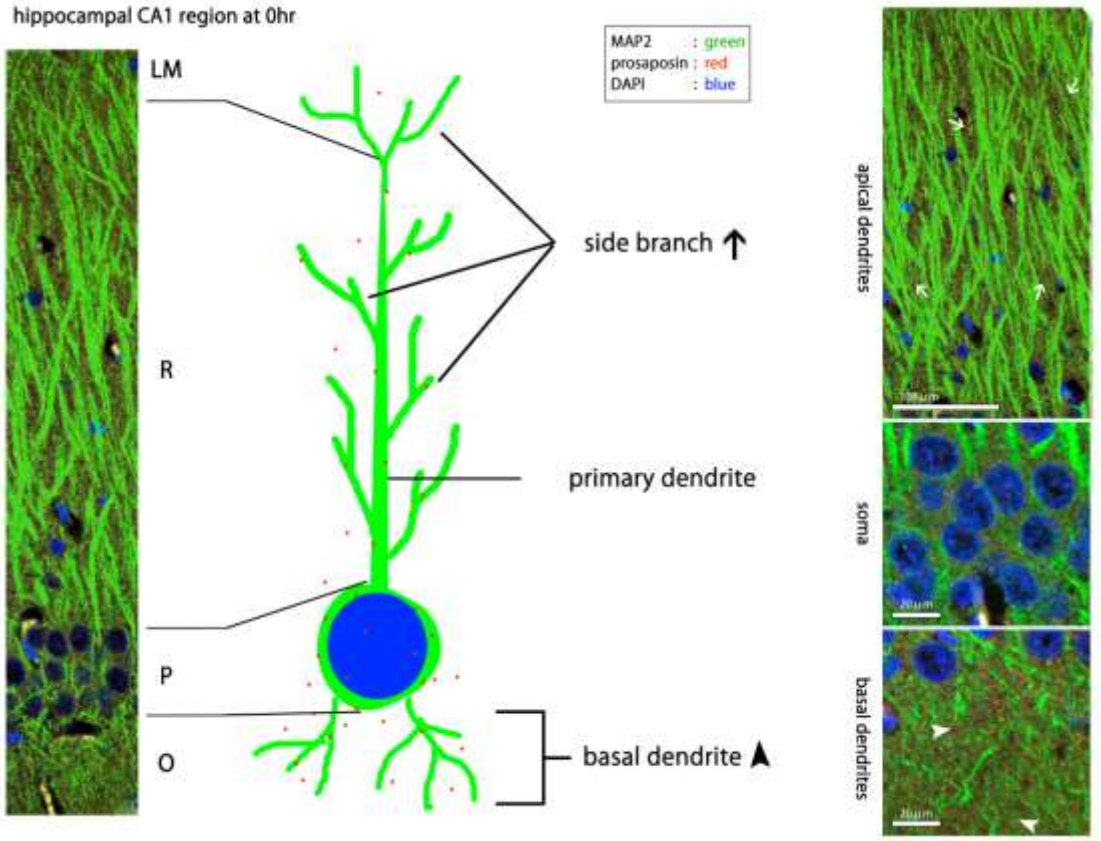

Figure 3. Immunofluorescence micrographs at 0hr. Structure of the hippocampal CA1 region and pyramidal neurons after immunostaining with anti-MAP2 (green) or anti-PSAP (red) antibodies or DAPI (blue). The hippocampal CA1 region is divided into five layers: alveus, st. oriens (O), st. pyramidale (P), st. radiatum (R), and st. lacunosum-moleculare (L-M). Primary apical dendrites emerge from the neuronal cell body and branch into the st. radiatum. Some basal dendrites emerge from CA1 cell bodies and bifurcate into secondary basal dendrites. PSAP was predominantly localized in the cytoplasm or dendrites of CA1 neurons.

death, and cell bodies were entirely fragmented at 7 d after death. Nuclear translocation of PSAP was observed mainly between 2 and 5 d after death. The structural changes in CA1 pyramidal neurons (apical and basal dendrites and neuronal somata), as well as the alterations in PSAP levels and its distribution during the postmortem period, are detailed in Fig. 5b.

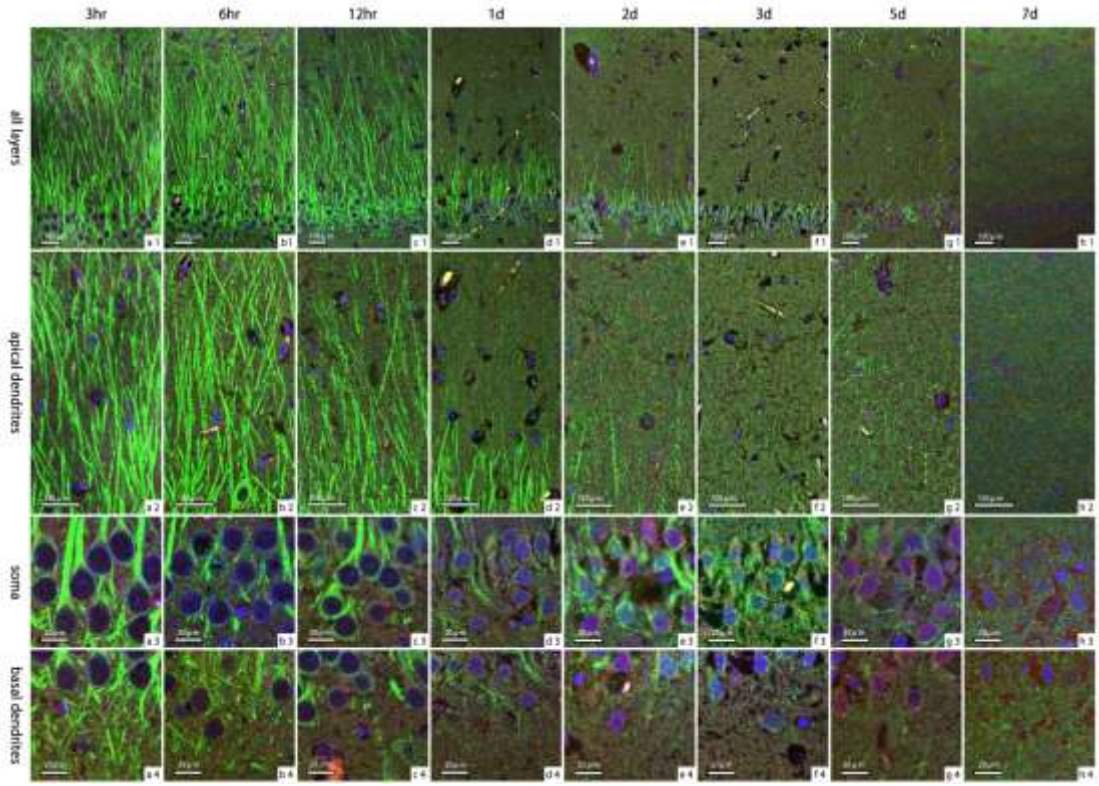

Figure 4. Representative immunofluorescence micrographs showing the characteristic structural changes in CA1 pyramidal neurons and the distribution of PSAP. Immunostaining was performed as in Fig. 3. At 3 h after death (a), almost no changes were observed. At 6 h after death, the density of basal dendrites was profoundly reduced (4b4). At the same time, the width of apical dendrites and the density of secondary branches were decreased, although the length and number of primary apical dendrites were unchanged (4b1, b2). Twelve hours after death (c), no basal primary dendrites, distal parts of apical dendrites, or secondary branches were evident. At 1 d after death, primary basal and apical dendrites had disappeared, and only the stems of pyramidal neurons were observed (d1, d2). The nuclei had shrunken further, and the cell bodies had become thinner (d3). The PSAP levels were further reduced extracellularly, but were maintained intracellularly (4d3, d4). Basal primary dendrites had completely disappeared by 2 days after death (e), and the stems of pyramidal neurons had begun breaking down. PSAP signals were prominent in the nuclei of CA1 neurons at 2 d after death. (f) Almost no apical dendrites were observed at 3 d after death, nuclear shrinkage and deformation were even more evident (f2, f3), and MAP2 signals were almost undetectable around the cell nuclei (f3). At 5 d after death (g), all dendrites and cytoplasmatic extensions had disappeared (g1), and MAP2 signals were only detected in cellular fragments. At 7 d after death (h), the hippocampal CA1 region was fully deformed (Fig. 4h1) except for some remaining nuclei.

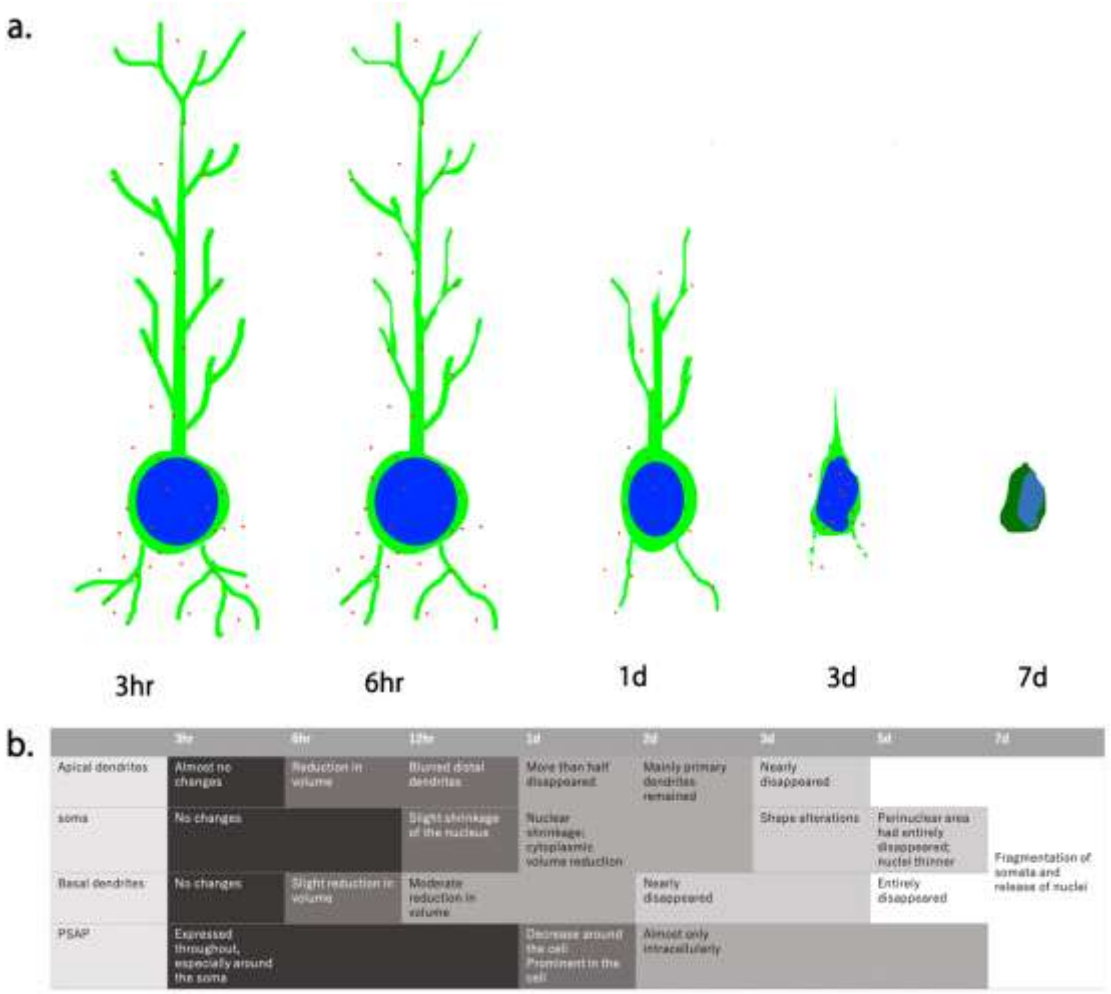

Figure 5.

a. Schematic presentation of the structural changes in CA1 pyramidal neurons and the distribution of PSAP during the postmortem period. At 3 h after death, no apparent changes were observed. At 6 h after death, secondary dendrites of both apical and basal neurons were fragmented. At 1 d after death, primary dendrites of basal and apical neurons had disappeared, only the stems of pyramidal neurons were observed, and PSAP immunostaining was observed in the cytoplasm around the nuclei of CA1 neurons. Neuronal bodies had disappeared at 3 d after death, and cell bodies were entirely fragmented by 7 d after death.

b. Detailed postmortem alterations in CA1 pyramidal cell morphology (apical and basal dendrites, neuronal somata) and PSAP levels and distribution.

DISCUSSION

In forensics, PMI estimation is predominantly based on corpse characteristics (e.g., temperature reduction, livid mortis, rigor mortis, and putrefaction) and forensic entomology. Nevertheless, these methods are strongly affected by premortem conditions and environmental factors, limiting their accuracy. Therefore, novel, more accurate, and objective PMI estimates are required. Current forensic research efforts are

focused on postmortem changes in the levels of different biomolecules (25-27). In this study, we characterized the postmortem alterations in rat brains. We identified characteristic postmortem changes in the levels and distribution of PSAP and saposin D (Fig. 2), as well as profound morphological changes in pyramidal neurons of the hippocampal CA1 region (Fig. 3, 4, 5).

Histological postmortem changes in the hippocampal CA1 region

Here, we focused on morphological alterations in the hippocampus, a structure located in the center of the brain. The hippocampal region has been extensively studied in the context of ischemia and memory. The morphology and structure of the hippocampal CA1 and CA3 regions have been comprehensively characterized (22, 23). In this study, we found that pyramidal cells and their long apical dendrites were aligned in rows (Fig. 3). Due to its well-defined structure, the hippocampus is the most suitable region to characterize morphological changes during the postmortem period. In this region, we performed immunohistochemistry for MAP2, PSAP, and DAPI, which allowed us to observe the overall hippocampal structure, as well as the morphology of neuronal somata, apical dendrites, and basal dendrites (Fig. 3-5). Density reduction of basal dendrites was the first morphological change in the hippocampal CA1 region postmortem, followed by characteristic changes in apical dendrites, which became narrower and shorter. Subsequently, cell nuclei shrunk, and the neuronal bodies eventually disappeared (Fig. 5).

Although numerous studies have assessed the usefulness of morphological changes to estimate PMI, the characterization of postmortem morphological alterations has proved challenging. Janssen et al. (28) failed to identify postmortem changes and antemortem degeneration by microscopical examination. Autolysis in a dead body can be distinguished from cell death in a living body by the fact that the former is diffuse rather than focal and does not invoke inflammatory responses (29). Additionally, postmortem structural changes do not involve the formation of cellular structures (e.g., autophagic vacuoles) or the induction of hydrolytic enzymes, and cell death does not coincide with organismal death. Excessive cell death frequently accompanies pathological processes and is a natural process occurring throughout life (30). Hence, cell death or tissue degeneration alone cannot be used as a PMI estimate, and comprehensive morphological characterization of relatively large tissue specimens is needed. To this end, we conducted fluorescence microscopy of the hippocampus; confocal microscopy may further increase imaging resolution. The morphological evaluation of the hippocampus after immunofluorescent staining enabled the identification of postmortem structural changes in pyramidal neurons at the tissue level that might not have been evident at the cellular level.

Currently, the most precise method to determine PMI is temperature measurements, as the body temperature decreases drastically after death.

However, this method is only useful in the early postmortem stage (0-36 h). Nunley et al. (30) and Tomita et al. (17) reported about morphological postmortem changes had mainly occurred by 24 h after death. In contrast to these findings, using fluorescence microscopy, we observed morphological changes in hippocampal pyramidal neurons up to 7 d after death, suggesting that morphological postmortem alterations can be used to estimate PMI for more extended periods.

Furthermore, immunostaining of the hippocampal CA1 region for PSAP revealed that PSAP gradually accumulated in the nucleus after death. Although PSAP has been previously reported to translocate into the nucleus in cell culture (31) and during development (32), this is the first study to show the nuclear accumulation of PSAP after death. However, the role of the potential neurotrophic effects of PSAP in dying pyramidal cells remains elusive. The clinical implementation of PSAP nuclear accumulation in estimations of PMI requires further investigation.

Postmortem changes in the levels of PSAP and saposin D

Numerous studies have demonstrated the postmortem degradation of proteins. In contrast to these previous approaches, we focused on saposin D, the levels of which increase after death. We found that although the PSAP levels were decreased after death, the levels of saposin D, a degradation product of PSAP, were increased postmortem. The levels of α-tubulin and β-catenin, which are abundantly expressed throughout the body, gradually decreased after death. The GAPDH levels only started decreasing by 3 d after death. Importantly, the use of two different antibodies (IM-1 and anti-saposin D antibodies) demonstrated that the PSAP levels gradually decrease after death. When the IM-1 antibody was used, the PSAP levels were already reduced by 50% at 3 h after death, and PSAP was almost undetectable at 3 d after death. In contrast, the saposin D levels increased after death due to the rapid degradation of PSAP. Therefore, the saposin D to PSAP ratio sharply increased after death, suggesting that it is a promising PMI estimate.

In conclusion, we identified characteristic postmortem alterations in the levels of PSAP and saposin D in the rat brain, in addition to extensive morphological postmortem changes in the hippocampal region. The implementation of these methods may improve the accuracy of PMI estimation when used up to 7 days after death. Considering that the rate of postmortem protein degradation is reduced at low temperatures or other unfavorable environments (7), future studies are warranted to further optimize the measurement of PSAP and saposin D levels to estimate the PMI.

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

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Comparing the Real Outcome to the Probability for that Outcome by Generation of a Computer Model: a Minimum Standard of Burn Survival

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Abstract

Background: Survival following burn injury has increased not only in developed countries over the past 20 years which is reflected with improvements in Lethal Area 50(LA50) or the burn size in which 50% of patients survive.

Aims: The aim of this study is to analyze mortality through LA50 and develop an objective predictive probability model for outcome in major burn patients based on age and BSA(%) which will help us to identify the patients with bad prognosis in order to help them during the course of the disease.

Study design: The study was retrospective clinical and analytical regarding outcome after severe burns. The data used are obtained by the analysis of the medical records of 5033 patients hospitalized with burns in the ICU of the service of burns and plastic surgery near UHC in Tirana, Albania during 1992-2019.

Methods: SPSS 23 software was used for the conduction of the statistical analysis. We have used Inferential Statistics through probability theory to draw conclusions. Concretely Simple Linear Regression for estimating Lethal Area 50 (LA 50), Binary logistic regression for creating the death probability chart. Statistical significance was defined as $p<0.05$.

Results: In the 28-year period, 5033 patients were admitted to Intensive Care Unit. Mean age (SD) was 20 (23.4) years old. Mean (SD) body surface area burn was 23.9 (16.9) %. Mortality was 12.3%. The mean LOS (Length of Hospital

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Stay) was 11.1±2.1 days while LOS in deaths was 8±10.7 days.LA 50 was improved in the last decade arriving 82.2%. From Logistic regression equation we calculate the death probability from 0-100% and present it as a surface contour chart.

Conclusion: There was a significant decrease in mortality in the last two decades which suggest major efforts have been made in burn care in Albania. We have developed a predictive model for mortality in major burn patients based only in age and burn size. Our opinion is that it is the responsibility of the burn team to continuously refresh and improve the probability chart in order to compile a chart after each year which should serve as a more accurate predictor for the patients of the following year. The probability for survival that the model assigns to the patients is the minimum standard because it is necessary to include in the model many other factors. The improvements in burn mortality should produce changes in the expectations of the burn care providers.

Key Words: Burns, Mortality, Death probability

INTRODUCTION

Burn is a severe traumatic injury with considerable morbidity and mortality. The clinical course of severely burned patients may be difficult and the outcome tends to be poor in patients with multiple comorbidities and especially in those with inhalational injury. Burns are a global public health problem, accounting for an estimated 180 000 deaths annually with the majority of these occurring in low- and middle-income countries (1). Many predictive models for mortality are developed in order to identify the most important factors which can influence the outcome and many prognostic scores are created, such as: Revised Baux score, Abbreviated Burn Severity Injury Score(ABSI), Ryan score, Belgium Outcome Burn Injury (BOBI) score, Fatality by Longevity, APACHE II score, Measured Extent of burn, and Sex score (FLAME). These scores are validated in many studies according to the characteristics of each country (2-6).

Survival following burn injury has increased not only in developed countries over the past 50 years which is reflected with improvements in Lethal Area 50 (LA50) or the burn size in which 50% of patients survive. This can be attributed to the advances in understanding of pathophysiology of burn injury, early nutrition, improved critical care, infection control and surgical interventions well-timed within a multidisciplinary burn care staff working as a team (7).

Objective estimates of the probability of death from burn injuries is difficult. The primary objective of this study is to examine data from a burn registry database in the Statistic's Department in the University Hospital Center in Tirana (UHC) and identify factors associated with increased mortality.

The aim of this study is to describe the characteristics of the patients admitted to our Intensive Care Unit (ICU) and to develop an objective predictive probability model for mortality in major burn patients based on Age and Burn Surface Area (%) (BSA). This model will help us quickly identify patients at risk and help them as much as possible to cope with this traumatic and devastating disease.

MATERIAL AND METHODS

Settings

The study is performed in the service of burns which consists of 35 hospital beds distributed between patients with severe acute burns, reconstructive burn patients, trauma patients, and plastic surgery patients. It consists of Emergency, the Operating theatre, the ICU with 10 beds and the Ward.

Study design

The study was retrospective, clinical and analytical regarding outcome after severe burns. Data are obtained by the analysis of the medical records of 5033 patients hospitalized with burns in the ICU of the service of burns and plastic surgery near UHC in Tirana, Albania during the period 1992-2019.

Patients with Steven-Johnson, Toxic Epidermal necrolysis as well as with degloving injuries were excluded from the study.

Information collected included:

- Year of admission
- Age, Group-Age (< 10 years; 10-19 years; 20-29 years; 30-39 years; 40-49 years; 50-59 years; 60-69 years; 70-79 years; > 80years)
- Gender (Male, Female)
- Etiology of burns (Scalds; Flame; Electrical; Chemical; Others)
- Body Surface Area (BSA) (%) burned: (0-10%; 11-20%; 21-30%; 31-40%; 41-50%; 51-60%; 61-70%; 71-80%; 81-90%; 91-100%)
- Degree (Partial-thickness; Full-thickness)
- Presence of Inhalation injury (Yes; No). Inhalation injury included cases when there was exposure to flame, steam or products of combustion together with laboratory findings and with positive bronchoscopy findings below the vocal cords.
- Length of Hospital Stay (LOS) (days)
- Outcome (Deaths; Survivors)

Statistical analysis

SPSS 23 software was used for the statistical analysis. Descriptive Statistics were conducted to summarize data for the central tendency (Mean) and variability (Standard Deviation). We used different graphs for the presentation of our data (Column graphs, surface contour graphs). We used Inferential Statistics through probability theory to draw conclusions. Concretely Simple Linear Regression for estimating Lethal Area 50 (LA 50) and Binary Logistic Regression for creating the death probability chart. Statistical significance was defined as $p<0.05$.

RESULTS

1. Patient demographics and burn injury characteristics

In table 1 is presented the demographic and clinical profile of our patients during the period 1992-2019. Of 5033 patients, 38.8% were female, 55.9% were of <10 years age and 61.5% have scalds as causative agent. The mean age of the patients was 20±23.4 years, the mean BSA (%) was 23.9±16.9, presence of full-thickness burn was in 20.5%, presence of inhalation burn was in 13.8% (n=694). The mean LOS was 11.1±2.1 days while LOS in deaths was 8±10.7 days.

Table 1. Demographic, clinical and burn injury characteristics 1992-2019 (n=5033)

Age, mean (SD)	20(23.4)
Gender, % female (n)	38.8(1955)
Group ages (years), % (n)	
<10	55.9(2815)
10-19	7(353)
20-29	6.4(323)
30-39	6.6(334)
40-49	8.7(437)
50-59	6.3(317)
60-69	4.5(224)
70-79	3(150)
>80	1.6(80)

Etiology of burns, %(n)	
Scalds	61.5(3095)
Flame	28(1407)
Electrical	4(202)
Chemical	5.5(4339)
Others	1(50)
BSA% burned, mean (SD)	23.9(16.9)
Full-thickness burn, %(n)	20.5(1032)
Inhalation injury, %yes (n)	13.8(694)
LOS, mean (SD)	11.3(13)
Mortality, %(n)	12.3(617)
Mortality in patients with inhalation injury, %(n)	47.2(328)
Mortality in patients without inhalation injury, %(n)	6.6(289)

2. Data regarding mortality

The overall mortality was 12.3% (617 deaths of 5033 patients). Of 4339 patients without inhalation burn there were 289 deaths, while of 694 patients with inhalation burn there were 328 deaths. Mortality in patients with inhalation injury was 47.2% vs. 6.6% in patients without it. In Figure 1 we have presented the mortality during years. It is evident that mortality has improved especially during the last decade.

Mortality is increased according to the burn size and the age as well as with the presence of inhalation injury (Figure 2). From Linear Regression for each unit increase of BSA (%) there is increasing odds of a bad outcome by 1.0 and for each unit increase of age (year) there is increasing odds of a bad outcome by 1.0.

3. Calculation of LA 50

LA50 is a well-established index suitable for the assessment of quality of care in burn patients taking in consideration only age and BSA (%) burned. We calculate this index for all the patients as well as for each of three periods with Linear regression. LA50 for all patients was 66.4%

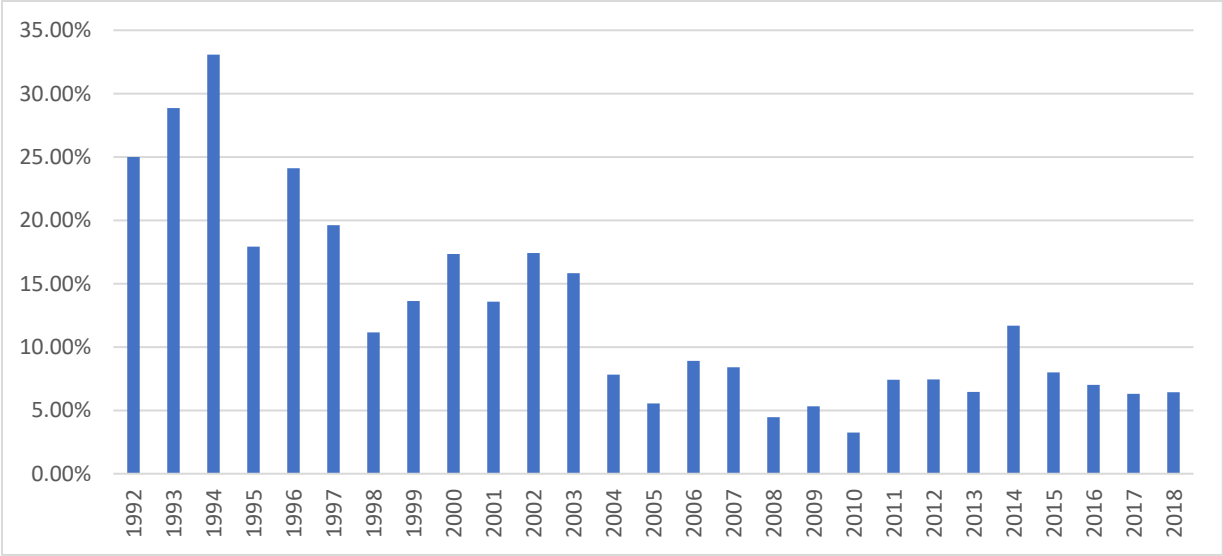

Figure 1. Mortality in years 1992-2019

while for the first decade 1992-2000 was 49.8%, for 2000-2009 was 73.3% and for the period 2010-2019 was 82.2% (Figure 3).

4. The death probability model

Logistic regression was used for the prediction of death probability by two risk variables, BSA (%)

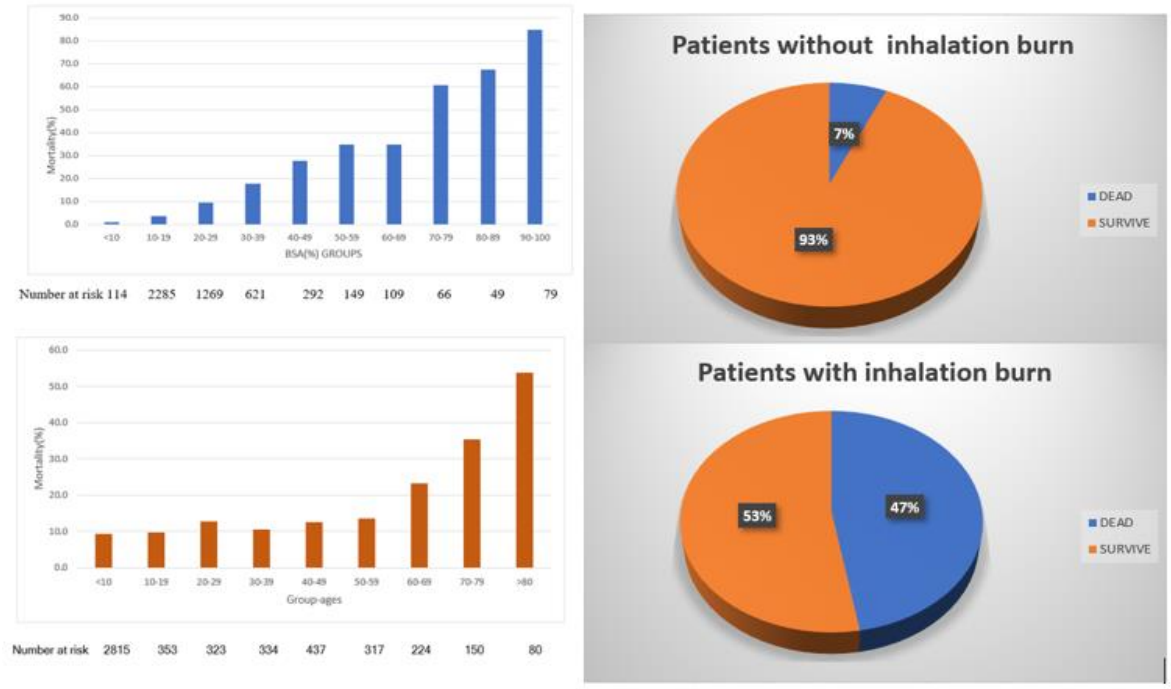

Figure 2. Mortality associated with percentage burn size, age and inhalation injury in 5033 patients (1992-2019)

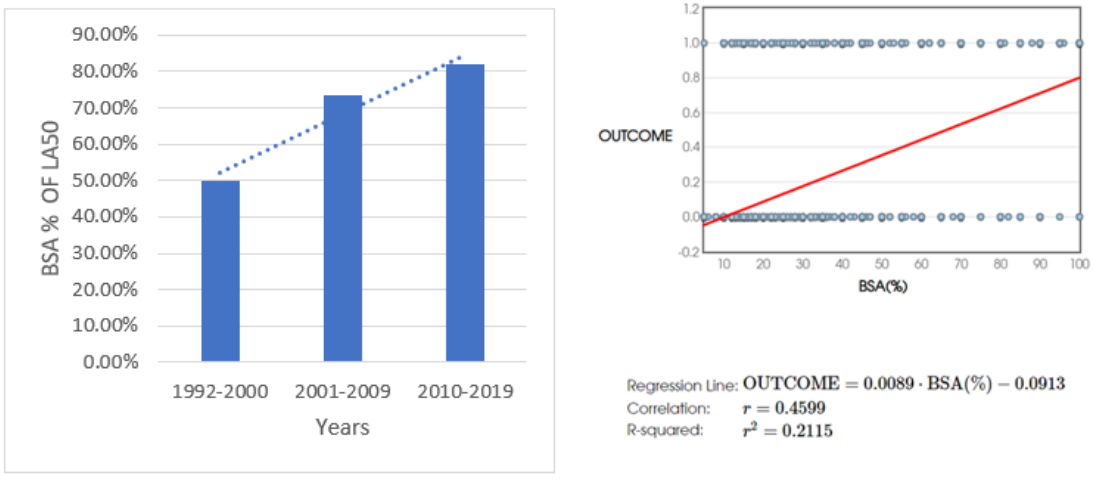

Figure 3. Improvement of LA 50 of patients in three periods of the study (From 50% to 82.2%) Mean LA50 for all the period was 66.4%. BSA (%) is responsible for 21% of the variance of outcome

Model: $$P = \frac{1}{1 + e^{-(-3.9495 + 0.0099x_1 + 0.0584x_2)}}$$

$$\text{Chi-Square} = 810.4530 \quad df = 2 \quad p\text{-value} = 0.0000$$

Variable	Coefficient	Standard Error	p-value	Odds Ratio	95% Confidence Interval
AGE	0.0099	0.0020	0.0000	1.0099	(1.0060, 1.0138)
BSA (%)	0.0584	0.0025	0.0000	1.0602	(1.0549, 1.0654)
Constant	-3.9495	0.1029	0.0000		

Figure 4. Logistic regression of age and BSA (%) for calculating death probability (%)

burned and age (years). According to the logistic regression methodology, both variables are transformed from continues to nominal and then logical. The equation of logistic regression for age and BSA (%) as continous variables without categorization has Odds more than 1.0 which indicates for positive correlation. Based on the weight of evidence the variables were grouped on strata by 10 units each. Then we performed binary logistic variable for both variables grouped in strata. In the figure 4 we present the logistic regression equation for age and BSA (%).

After calculating probability for each record, we have made respective grouping according mortality 0-100%. In the table 2 we have presented the mortality (%) in each corresponding pair (Age and BSA).

Table 2. Probabilities of death(%) according BSA(%) and Age(years)for 5033 patients

	BSA(%)									
Age(years)	**<10**	**10-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**80-89**	**90-100**
<10	0	2.6	10.1	23.2	28.2	34.6	37.0	70.6	66.7	75.0
10-19	0	1.3	7.4	9.8	20.0	33.3	20.0	50.0	80.0	83.3
20-29	0	4.7	2.4	4.9	17.6	25.0	30.0	81.8	75.0	83.3
30-39	0	0.0	0.0	1.8	17.9	15.4	19.0	14.3	88.9	93.3
40-49	0	0.8	3.3	6.2	25.0	28.6	27.3	58.3	40.0	80.0
50-59	10	2.7	6.6	9.8	26.7	30.0	42.9	40.0	33.3	81.8
60-69	0	9.9	12.5	15.0	26.1	70.0	61.5	50.0	75.0	100.0
70-79	0	16.2	29.0	55.0	55.6	100.0	100.0	100.0	100.0	100.0
>80	0	29.6	61.1	72.7	72.7	75.0	50.0	100.0	100.0	100.0

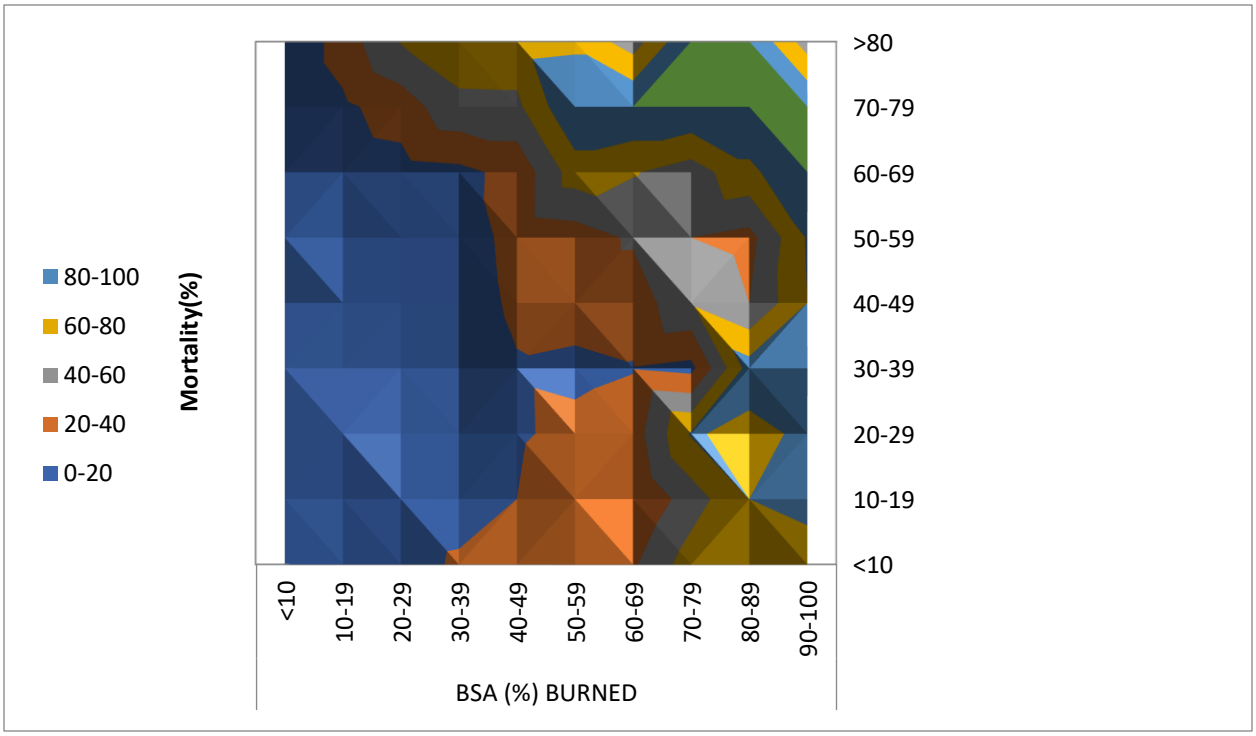

Figure 5. Model of death probability based on Age and BSA (%) for patients during 1992-2019

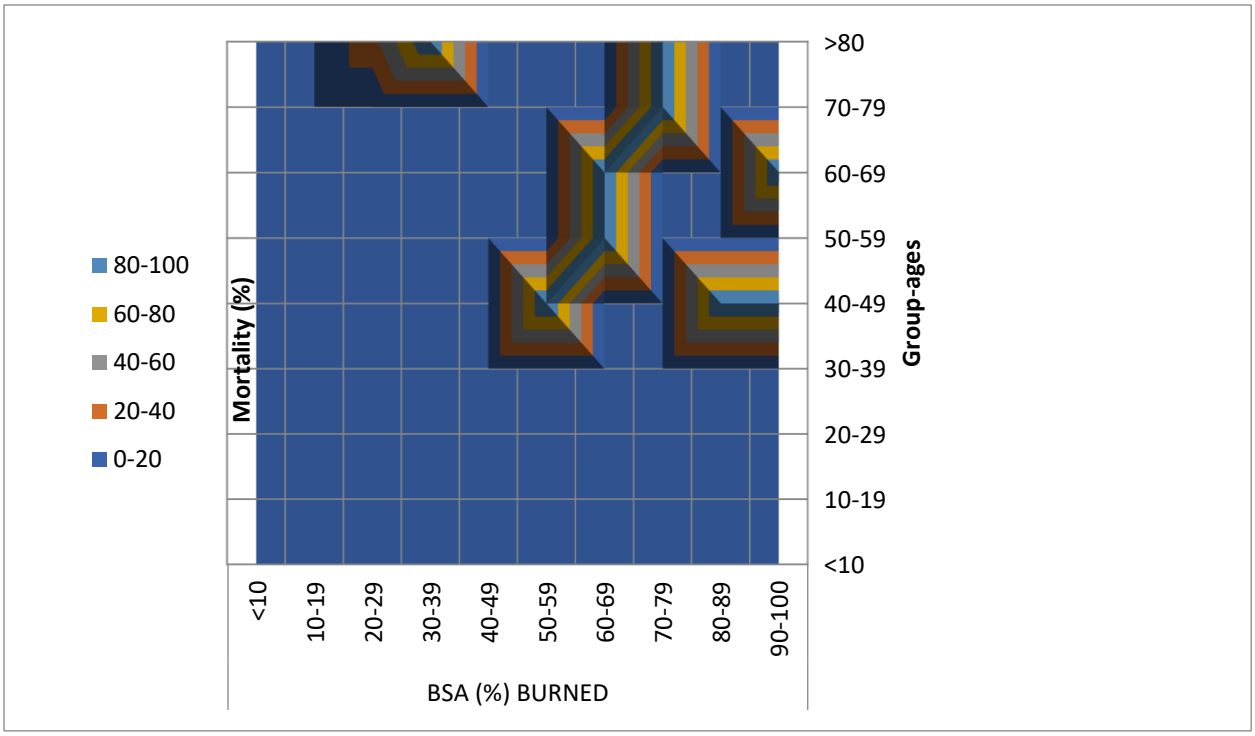

Figure 6. Death probability for patients hospitalized during 2019

We have used surface charts (type contour) to present the computer model of death probability. In the figure 5 we have presented the probability of death chart for patients 1992-2019.

During 2020, patients admitted to the burn center with a determined burn injury are plotted in the graphic and are assigned a probability of death. Because the model has used data from 1992, we decided to perform a new chart only with patients hospitalized in ICU during 2019 because the first chart did not represent the actual outcome. The new patients plotted in the new chart till now had a similar outcome with the chart prediction. We have presented it in the figure 6.

DISCUSSION

This study characterized the trends of mortality after burn injuries in Albania over a 28-year period, taking in consideration only burn characteristics on admission. Older age, a larger burn TBSA and inhalation injury are well-known predictors of burn mortality but in mortality had greater impact infection and sepsis as well as the concomitant illness and the immunity of the burned patients.

Burn mortality is still one of the major outcome measurements in burn centers. From our data we have an important improvement in mortality from 25% in 1992 to 7% in 2019. The mortality rate of the last decade is comparable with rates of other European countries like in Belgium (7.1%), Turkey (6.3%), France (9%) and Hong Kong (8.7%) (7,8,9,10). Different studies show better outcomes in Sweden (3%), Netherlands (4.1%), Spain (3.4%) and Portugal (3.7%) (11,12,13,14,). Although every burn center has its own particular limitations, it is clear that exists a minimum standard of survival after burn injury which is LA50. In the 1940s, LA50 in the United States was 40% (15). With the development of broad-spectrum antibiotics and specialized burn units, also with standardization of a multidisciplinary approach instituted at tertiary health care centers, LA50 increased to approximately 60% in the 1970s (16). Currently, most burn centers in the United States report LA50 over 90% (16). Europe experienced a similar improvement in LA50 over time. Wasserman showed an overall mortality of 11.8% and LA50 of 60% in 1985 in France (17). In 1999, Barrett et al. demonstrated an overall mortality of 3.5% and LA50 of 90% in Spain (18). Our LA50 is improved from 49.5% in 1992-2000 to 82.2% in the last period 2010-2019 which speaks for a better work of the staff in the service of burns.

Comparing burn centers, since many geographic and social parameters differ, the generation of computer probability models has proven useful in surveying the outcome having the benefit of comparability (19,20,21,22).

It is important to explain the model mechanism. In the model are presented the age, BSA (%) and the probability of death. When a patient is hospitalized in the service of Burns, we plot the age and BSA (%) burned and see the corresponding probability for survival. Afterwards, the real outcome of the patient is compared to the probability for that outcome and disparities are analyzed on a case per case basis.

We built the first model with a big number of patients (n=5033), taking in consideration the fact that the more cases, the more accurate the

predictive model, but we did not take into account that especially the first 10 years were accompanied with higher mortality. Because our model has included all cases with different prognosis for a long period it was not reliable for the recent situation of improved mortality values. So, we created a chart of the last year (2019) and we are analyzing the correlation of prediction and observed mortality with the aim of validating the predictive chart. We are looking forward for the decreasing of mortality in the future which is going to lead into the adaptation of new models.

CONCLUSION

This study evidenced that the overall registered mortality was 12.3% and survival following severe burns has improved over the past 28 years and LA50 for all patients was 82.2%. Our opinion is that it is the responsibility of the burn team to continuously refresh and improve the probability chart in order to compile a chart after each year which should serve as a more accurate predictor for the patients of the following year. Improvement in the treatment of severe burns has been accomplished due to a combination of preventive health care, appropriate treatment protocols and improvements in equipment and infrastructure. The probability for survival that the model assigns to the patients is the minimum standard because it is necessary to include in the model many other factors. The improvements in burn mortality should produce changes in the expectations of the burn care providers.

The strengths and limitations of the study. This study being at the same time descriptive retrospective and analytical provides the basic requirements for further epidemiological studies as the prerequisite for better planning and implementing of prevention programs. The advantage of the probability model is that it excludes all the local geographic and social differences between the burn centers and the results are comparable. The disadvantage is that the prediction is based only in BSA (%) and age and did not take in consideration other burn and clinical characteristics.

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

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Research and Development Steps for an Effective Vaccine, during the COVID-19 Pandemic Situation

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Abstract

Introduction: The COVID-19 vaccine, considered by many as a "game-changer" and therefore the most effective & prophylactic strategy tool for pandemic control and prevention, is being developed by many research, pharmaceutical and scientific institutions. Vaccine development has always been a really long and expensive process, but modern innovative technologies, along with incredible and specific economic and scientific efforts, immensely helped to narrow the development time and progress, in order to have in less than 12 months from official start of the pandemic, more than one approved vaccines and actually make possible the beginning of a massive vaccine worldwide distribution and administration campaign, in record time.

Methodology and objectives: Through the scientific literature review, this article aims to supply for interested healthcare professionals and public, a summarized and detailed picture of the vaccines under development or approved ones, implemented platforms, clinical study trials phases, institutions, developing countries and funders, to explain vaccine options and candidates, against SARS-CoV -2.

Results: There are currently 261 vaccines in development, 78% of which are in the preclinical phase. 56 candidates (22%) from different developers have been admitted to clinical trials,

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of which 44% are in Phase I trials; 30% are in Phase I/II; 7% in Phase II; 21% in Phase II/III and 21% in clinical trials of Phase III. Also, based on the platform used for development, actual data shows that there are around: RNA-based technology 13% of vaccines, DNA 8%, non-replicating viral vector 14%, viral replicating vector 8%, virus like particles 6%, inactivated virus 6%, live virus attenuated 1%, protein fraction 31%, unknown platform 13%. 45% of studies are conducted by the pharmaceutical industry, 28% by public and academic institutions. Among candidates, 11% of them received funding support from public and private grants. The U.S.A. includes around 26.8% of global research, followed by Asia 29% (China, leader with 19.5% globally and 43% of clinical trials). Europe also 29% of developing vaccines with 15 countries involved.

Conclusions: At the end of 2020, first vaccine candidates received important regulatory approvals and began their global use. This is a fantastic news but we are just in the middle of our campaign to fight back against the pandemic. Ongoing & future studies, together with global real-time data, will focus on the determination of SARS COV-2 antibodies and T lymphocyte-mediated immunity, optimizing pharmaceutical product development and vaccine efficacy protection against COVID-19.

Keywords: COVID-19, vaccine, research and development, pandemic

INTRODUCTION

The COVID-19 pandemic is caused by Severe Acute Respiratory Sindrome Coronavirus 2 (SARS-CoV-2), with reports of more than 75 million cases of COVID-19 (January 2021) (1). The daily changing statistics data show over 215 countries and territories affected, resulting in more than 1.9 million deaths and 64.71 million people recovered from the virus, with a global death-case ratio of 2.15 %.

The virus is spread mainly among people during close contact, most often through droplets produced by coughing, sneezing and talking. Virus particles usually fall to the ground or spread through the air. People can also become infected by touching a contaminated surface and then touching their face, introducing the virus particles to their organism (2). The virus is more contagious after infection, about three days before the onset of symptoms, or even through asymptomatic individuals.

Common symptoms include fever, dry cough, fatigue, shortness of breathing, and loss of taste or smell (ageusia/anosmia). Complications may include, among others, pneumonia and acute respiratory distress syndrome (3). The time from exposure to the onset of symptoms is usually about five days but can range from two to fourteen days. There is not yet a specific antiviral treatment; primary treatment is symptomatic and supportive therapy, so the approval of one/more effective vaccine(s) can be a "game-changing" strategy (2).

A. Vaccine development for COVID-19

A pandemic can involve multiple waves of COVID-19 over 1-3 years, and SARS-CoV-2 can become an endemic virus globally. We need to prepare for a worst-case scenario, in which the rapid development and high rates of COVID-19 vaccination are essential to the morbidity, mortality reduction and limitation of economic damages that accompanies a pandemic, from day one of onset till the slow return to the "old normality" (4).

Vaccines are the most effective strategy available to prevent and reduce the impact of a flu outbreak. But although vaccination is the most effective means of prevention, a specific vaccine may not be available during the initial wave(s) of a pandemic.

If SARS-CoV-2 resembles other coronaviruses that infect humans, those who become infected will be immune for months and years (still unknown..), but not their entire lives (5). When most of a population is immune to an infectious disease, this ensures indirect protection or "herd immunity" to those still "susceptible" to the disease.

"Herd immunity", also known as "population immunity" is a form of indirect protection against infectious disease (4). This occurs when a large percentage of a population has become immune to an infection, either through vaccination or previous infections, providing a resultant level of protection for the rest, individuals who are still not immune. The greater the number of immune individuals in a community, the less likely it is

that the rest of non-immune individuals will contact an infectious individual, helping to protect non-immune individuals from infection (4). According to estimations on the COVID-19 infectivity rates, we should need at least 70% of the population to be immune to achieve the "herd immunity" objective.

Supposing that the herd immunity epidemiological state is present and maintained in a population, for an adequate period of time, the disease is going toward elimination, since endemic transmissions no longer occurs. If "this local" elimination is achieved and spread worldwide, with a following number of cases permanently reduced to zero, then disease can be declared officially as "eradicated" (4,5).

The immune system does not distinguish between natural infections and vaccines induction effect, forming an active response to both, so the immunity resulted through vaccination is similar to what would have occurred in a case of recovering from the disease (6).

- ***Introduction to vaccine immunology***

Immunity to COVID-19 remains a quest with many unknown topics, an "enigma" that science and scientists must solve.

The ideal vaccine candidate should be a "safe product, able to produce a strong, consistent, protective immune response, seek as few doses as possible, and be affordable and accessible to all globally"(6).

There are two main types of "memory" immune responses. The first is driven by B lymphocyte cells, which produce antibodies. The second type of cell capable of recalling an infection is T lymphocyte cells. T cells may be sufficient to control infection in the absence of antibodies and act by organizing immune defenses (T-helpers) or by directly killing infected cells, to limit new virus production (cytotoxic T cells) (6,7). It is possible that the Sars-CoV-2 T cells' memory may last longer than antibodies, similar to other coronaviruses cases.

We currently do not have a test to assess the T cell response, but also even the presence of antibodies does not give us a full and clear overview of protection level in patients with COVID-19 (7).

B. How long does a vaccine take from formulation to global distribution?

Developing a new vaccine has always been a long, complicated, challenging process, that requires a great deal of professionalism to analyze, study and resolve possible problems, encountered from the start, up to the moment of approval, before widespread human use (7). Vaccine development requires many stages; under normal conditions the preclinical and clinical phase can last 5-15 years until completion. Following the development of vaccines, an ongoing commitment to post-licensing safety analysis is required. Taking into account post-licensing safety studies, the whole process can take approximately 10-30 years (8).

- ***Challenges of a clinical trial performed during an ongoing pandemic situation***

Recruitment of a sufficient number of patients: In emergency conditions (like a pandemic), it

becomes more challenging to recruit an adequate and required number of patients.

Policymakers face many decisions to make, including:

(a) the selection of the "right" candidates for a study process (limited number of patients and resources, make the candidate selection critical),

(b) choosing the right study model (some study models work best for different situations) (8).

- ***Vaccine approval process in pandemic conditions***

Many countries have implemented alternative authorization procedures that can help, to speed up the availability of vaccines (8). For pandemic vaccines, two main approaches are being used:

- *"Mock-up" procedure*

This procedure allows a vaccine to be developed and authorized before a pandemic occurs, based on the presumption that some viruses have the "potential" to cause a pandemic (9). Once the current type of virus causing the pandemic has been identified, the manufacturer can replace this mutant material in the pre-prepared vaccine (for which regulatory approval has been given previously) and request it to be authorized as a 'final' pandemic vaccine (8,9).

The vaccine will be given a *"Conditional Approval"*, which means that the benefits outweigh its risks, but full data to support its authorization are not yet available, and further studies should provide these after licensing.

- *Emergency Procedure*

Authorization of these pandemic vaccines is faster; the information (M.A. dossier) applied by the manufacturer is evaluated in an accelerated time frame (about 70 days rather than 210 days) (10). Vaccines should submit a complete dossier of information reporting data on developing vaccines as soon as they become available, rather than waiting for a complete set of data to be collected. Once sufficient data has been collected, focused on assessing the risk-benefit ratio, the manufacturer applies to the regulatory authorities (FDA/EMA or other local institutions) for a formal marketing authorization approval (MAA – Marketing Authorization Approval). Regulatory Authorities have to evaluate the submitted dossier and reach a final decision within approximately 25-40 days (10).

METHODS

Type of study: The study methodology is based on the conceptual and critical literature review, conducting detailed scientific analysis, to compare and evaluate a range of perspectives on COVID-19 vaccines development key moments. Grouping articles by concepts, identifying current "understanding" and a photographic overview of "where things are" in the area of research and development processes, for an effective vaccine in the COVID-19 pandemic.

Timeframe: This review was performed periodically from April 2020 - November 2020, focusing on the latest researches and publications.

Inclusion criteria: During publications research have been used the following keywords: "COVID-19; COVID-19 vaccine, COVID-19

vaccine research, COVID-19 vaccine development, COVID-19 vaccine pipeline, COVID-19 vaccine safety" in Pubmed, Google scholar, medical journals with high impact factor such as *New England Journal of Medicine*, *Lancet, Nature*, paper position of international associations of microbiology and infectious diseases (ECCMID), of immunology, clinical immunology (EAAACI),
respiratory (ERS), etc., sites of pharmaceutical companies.

Exclusion criteria: Media articles with no appropiate certified references or any scientific certification.

Data analysis: The data were analyzed and processed, yielding graphical presented results.

Limitations of the study:

- The information is new, recently published, which may lead to lack of proper scientific formatted, even by publishers.
- As there are numerous publications in a short amount of time, few articles can be missed, but all the efforts have been made toward comprehensiveness of the revised literature.

RESULTS

In the conditions of the COVID-19 pandemic, during April-November 2020, there have been a high substantial number of publications describing the unprecedented rapid path taken in the research and development of vaccines candidates for COVID-19. Following the review of more than 38 articles, with respective data analysis, there are the following summarised results:

1. Pharmaceutical companies, medical staff and academic institutions worldwide have been working intensively to discover effective treatments and prevention for COVID-19. In July, there were around 464 studies in development, while in December 2020, this number reached 580 studies. It should be noticed that vaccines in the development process constitute the most significant interest of researchers, with around 44% of researches (11). Other main topic of interest, with respectively 16% of studies (July) and 14% (November) continue to be for monoclonal antibodies and 5% for antiviral drugs. There is an increase in the number of studies for cell-based treatments from 4% to 6%, meanwhile studies for gene therapies (RNA) remain the same and there is a decrease in studies aimed to discover new indications for existing drugs from 5% to 4%. Increased interest has been shown in studies on medical devices. During this period, 9 clinical studies were discontinued. (Figure 1.a) (12).

2. Actually, there are 261 vaccines in different stages of development, 56 more than in July, most of them are in the preclinical phase, but 58 candidates have already entered the clinical phase, 35 more than in July.(Figure 1.b) (12).

3. Among the vaccines that have entered the clinical phase till November 2020, 43% are in the first clinical phase, compared to 48% in July, but there is an increased number of vaccines in the third clinical phase, 20% of all vaccines in the

clinical phase, while in July there were no vaccines in clinical phase III.(Figure 2.a) (11,12,13). Also there are 3 vaccines that have finished their phase III studies, publishing their results and received first approvals.

4. It turns out that from the beginning of the pandemic until November 2020, vaccines with nine different types of platforms are under development, the most used platform continues to be the one with Protein subunit (30%), followed by vaccines with a non-replicating viral vector (12%) in July and (14%) in November, with an RNA-based technology (13%) of vaccines, with DNA about 7%. The less-used platform continues to be vaccines with a live attenuated virus (1%) (12,13,14).

5. Not all developers have commented on how vaccines are administered. From this study results that the most common method of administration is the intramuscular route in 27% of cases, followed by the intranasal route (21%), the oral route (16%), the intradermal route (11%) and other routes such as intravenous, microneedle arrays, oral/nasal, subcutaneous, (Figure 2.b) (15,16,17).

6. Most vaccine studies are being conducted by the pharmaceutical/private sector and industries (45%), followed by 22% from unknown entities, 16% from public institutions, 12% of academic institutions/universities and 5% non-profit organizations. (Figure 2.c) (18).

7. The development of new drugs and vaccines is a very expensive process, which for many companies and institutions is unaffordable, the most expensive stages are related to the clinical testing phases, in large number of individuals. For emerging vaccines with promising results and features, special funds (grants) have been guaranteed from the private or public sector. These go for approximately 11% of vaccines, to reach clinical trials, while the rest of 89% of vaccines are not externally funded (Figure 2.d) (11,12).

8. Since discovering the new coronavirus complete viral genome, research teams globally have been mobilized in an unprecedented race time to seek and develop an effective vaccine. This race is against time and against rivals (rival companies or different countries), which the objective of becoming the new "field leader". The largest number of vaccines is being developed in North American countries, accounting for 33% of researchers. It is followed by regions such as Asia and Europe, each with about 29% of emerging vaccines. In Asia, China is the leading country with 19.5% globally, followed by Japan (2.93%) and other countries. Europe has the largest number of countries involved (15 countries). The U.K. has the highest number of researches, 6.34% of global researches. Russia is the second country with the largest number of researches in Europe, 4.88% of global researches, followed by other European countries each with a percentage of 1-4%. It is worth mentioning the contribution of India, where about 3.9% of research is being conducted. Australia and South America have lower numbers of research studies, than to other

regions, in Australia 2.4% and in South America only 0.98% (Figure 3) (11,12,13,14,15,19).

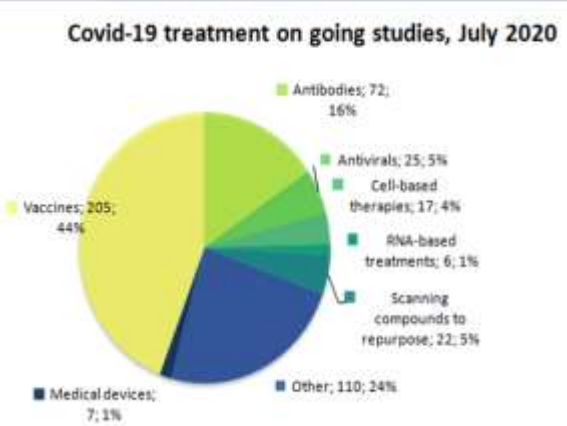

Figure 1a. COVID-19 treatment studies - July 2020

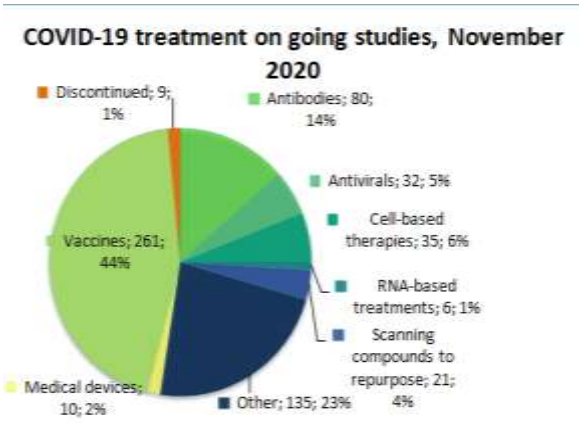

Figure 1.b. COVID-19 treatment studies, November - 2020

Vaccines with virus

- *Live attenuated virus vaccines*

Live attenuated virus vaccines are live viruses passed on to animals or tissue cultures so that the virus loses its virulence.

As a natural infection, the attenuated virus elicits strong immune response, B cells and T cells, longer-term immune responses, but may not be suitable for individuals with compromised immunity. A small possibility of a virus mutation could reverse its virulence and lead to the onset of the disease like the case with SARS 1 vaccines, where a reversal of the virus has been observed. Moreover, such vaccines need a cold chain for community distribution (20).

- *Inactivated virus vaccines*

In an inactivated vaccine, the virus is treated with various methods, such as chemicals (formaldehyde), solar radiation or heat treatment, to inactivate the virus. Such vaccines are safe and cannot cause the disease, but do not cause a high immune response and may need repeated dosing and adjuvants to boost immunization (21).

The advantage of complete virus vaccines is that they include all the natural ingredients of a virus, as proteins, lipids, and nucleic acids, facilitating broad and potent immune responses, unlike other platforms that tend to exceed natural potency.

Protein subunit vaccines

Protein subunit vaccines are based on inducing an immune response against the virus spike protein (protein S), thus preventing them from binding to the ACE-2 receptor.

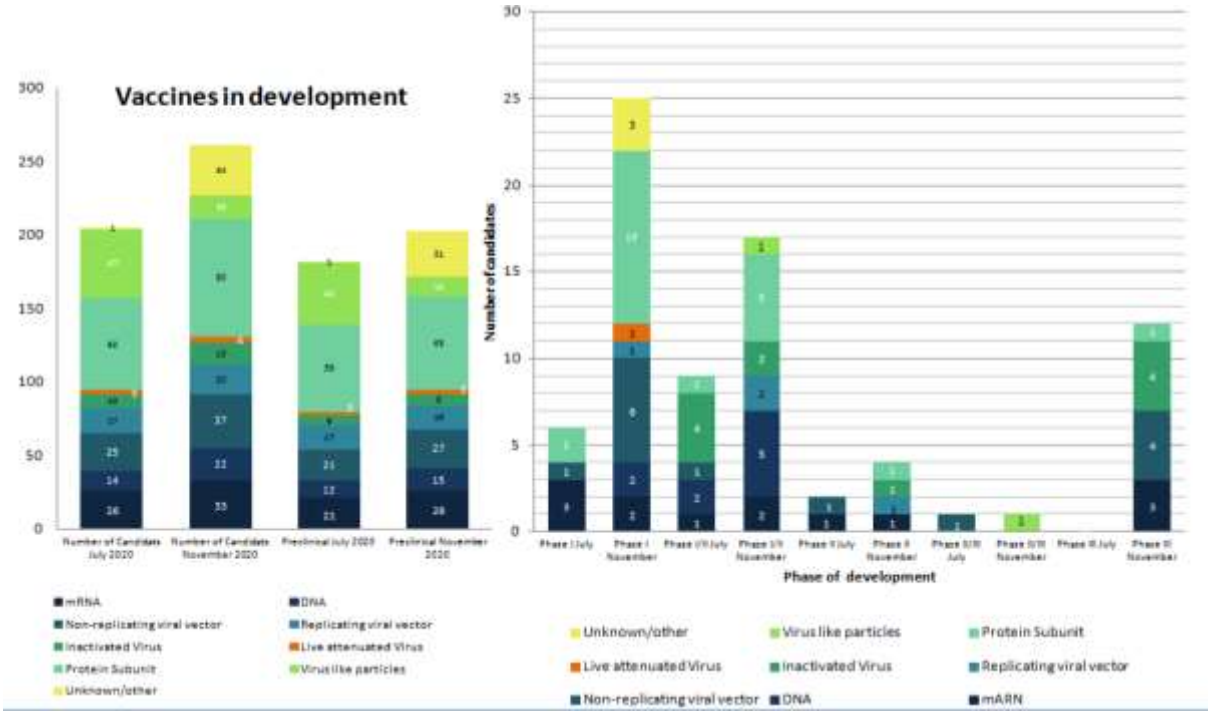

Figure 2.a. COVID-19 vaccines in different stages of development, July vs. November 2020

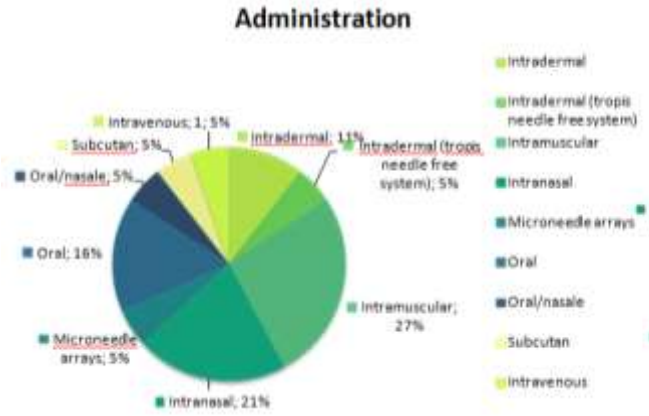

Figure 2.b. Administration methods used in COVID-19 vaccines

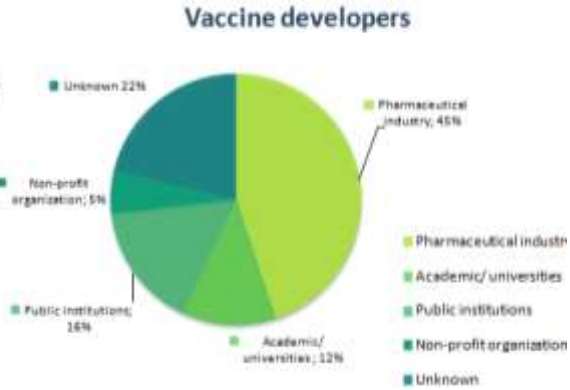

Figure 2.c. COVID-19 Vaccine developers

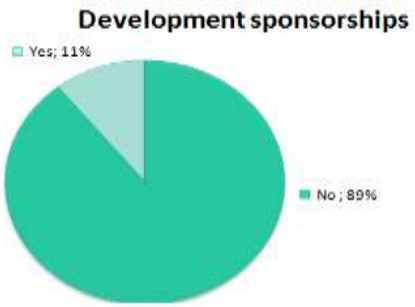

Figure 2.d. Vaccine development – funding

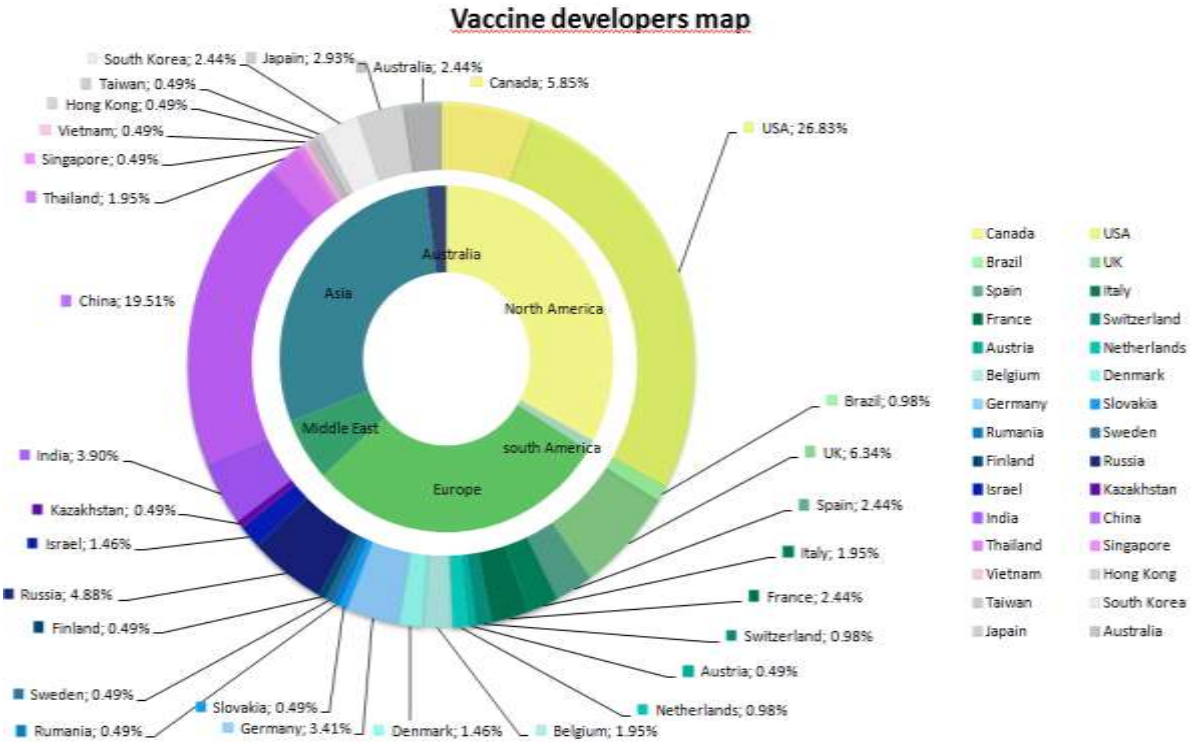

Figure 3. Map of countries - vaccine developers

Spike proteins cause the creation of higher neutralizing antibody titers than any coronavirus antigen. Studies for the development of subunit-based vaccines have reported an increased T cell immune response and the generation of high-titer neutralizing antibodies in vivo (22). These vaccines are very safe and have fewer side effects by boosting the immune system, without introducing infectious viruses.

Virus-like particle vaccine (VLP)

In this case, vaccines are made up of virus particles without genetic material. Such vaccines are safe and provide an adequate immune response, however they are challenging to produce (23). Virus-like particles (VLP) are formed from viral structural proteins, which have an inherent self-structuring property and mimic pathogen's morphology. Unlike "live" viruses, VLPs are non-infectious and non-replicating, as they have no infectious genetic material (24).

Nucleic acid vaccines

- *DNA vaccines*

Nucleic acid vaccines work by inserting DNA or RNA sequences, which encodes a specific disease related antigen in the organism. Once the sequence is translated into the corresponding peptides, an immune response is elicited. Nucleic acid vaccines generally stimulate both humoral and cellular immune responses, unlike conventional vaccines that stimulate only one antibody response (25).

DNA vaccines contain a plasmid with the necessary DNA sequence, which must be inserted into the host cell DNA, to direct the production of antigenic proteins. The production of DNA vaccines is much easier than the one with conventional vaccines. Synthetic DNA is temperature resistant and distribution can be accomplished without cold chain conditions.

The concern is also raised about the possible side effects, from the plasmid interaction with human DNA, disrupting normal transcription. In vitro studies suggest that the rate of mutagenicity is lower than the rate of spontaneous mutations in human cells (26).

- *mRNA vaccines*

Once injected, RNA can be processed by immune cells and directly produce the targeted protein through translation. When the newly produced protein is released from the host cell, the presenting antigen cells will rapidly capture and present to MHC I and MHC II (Major histocompatibility complex) the antigen, located on the surface of the presenting antigen cells. This step is important for the subsequent activation of B cells and T cells, which is the key to the humoral and cytotoxic response (27).

An mRNA vaccine is considered the most promising candidate because it also can be produced very rapidly and save essential time. Meanwhile, the main restrictions and difficulties of development regard distribution and stability issues for RNA degradation, together with safety concerns regarding immunogenicity (28).

Viral vector vaccines

The genetic material from the pathogen SARS-CoV-2, is used to create viral vectors, containing the virus' genetic material. Some vectors used in vaccines are capable of reproducing in the body, meanwhile, other candidates use a non-reproducible vector.

The most commonly used viral vector are adenoviruses. The only reason why these vaccines can be ineffective is if the recipient already has some form of immunity to the generated vector, making it impossible for the virus to enter our cells (27).

C. Comparison of the most promising vaccines for COVID-19

The following table has included summarized information and comparisons; between the most promising vaccine products. These candidates during December 2020 and January 2021 have received the first marketing authorization approvals from FDA and EMA, followed by massive vaccination campaigns in specific countries.

Table 1. The most promising vaccines for COVID-19, November 2020 (16,17,26,28,37,38)

	Pfizer and BioNTech (*BNT162b2*)	**Moderna (mRNA-1273)**	**AstraZeneca & University of Oxford (AZD1222)**	**J&J (Ad26.COV2.S)**
Platform used:	LNP-mRNA	LNP-encapsulated mRNA	Non-Replicating Viral Vector	Non-Replicating Viral Vector
Clinical study starting date	April 29, 2020	March 16, 2020	April 23, 2020	August 10, 2020
Participants:	43,998 participants, 12-85 years.	30 000 US participants, 18+ years	40051 participants, 18+ years	A target of 60,000 adult participants 18+ years
Study design:	Multiple locations in different countries (150 clinical trials sites with racially and ethnically diverse backgrounds) Participants have a 50% chance of receiving either the vaccine candidate or a placebo (randomized, placebo-controlled). The study is also "observer-blinded" . Masking is Triple (Participant, Care Provider, Investigator)	Randomized, Stratified, Observer-Blind, Placebo-Controlled Study. The randomization is in a blinded manner using a centralized Interactive Response Technology (IRT), according to pre-generated randomization schedules. Randomization was stratified based on age and, if they are < 65 years of age, based on the presence or absence of risk factors for severe illness from COVID-19 based on CDC.	Randomized. Participants are assigned to one of two or more groups in parallel for the duration of the study. Masking is Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Double-Blind, placebo controlled. (Two or more parties are unaware of the intervention assignment.)	Randomized, double-blind, placebo-controlled clinical trial. Participants will be randomized in parallel in a 1:1 ratio to receive intramuscular (IM) injections of Ad26.COV2.S or placebo . The study will consist of: a screening phase (up to 28 days), and a long-term follow-up period (1 additional year)
Dosage and administration:	Two doses are required (separated by 21 days) IM administration	2 doses of mRNA-1273 on Day 1 and on Day 29. IM administration	2 doses AZD1222 4 weeks apart IM administration	Single dose IM administration

Study completion date:	January 29, 2023 Participants will be monitored for two years after they receiving second dose of the vaccine candidate or placebo.	October 27, 2022	October 25, 2022	March 10, 2023 The duration of individual participation, including screening, will be maximum 2 years and 1 month. The end-of-study is considered as the completion of the last visit for the last participant in the study.
Phase III Study Results:	Efficacy was consistent across age, gender, race and ethnicity demographics. Based on an analysis of 170 cases of COVID-19 in trial participants, the vaccine efficacy rate was 95%, 162 cases of COVID-19 were observed in the placebo group versus 8 cases in the vaccine group. No serious safety concerns observed; the only Grade 3 adverse event more significan than 2% in frequency was fatigue at 3.8% and headache at 2.0%	Vaccine promotes the creation of neutralizing antibodies in older adults at comparable rates to younger adults It is 94.5% effective. The analysis was based on 95 covid-19 cases, of which 90 were observed in the placebo group and five were reported in the vaccine grup The majority of adverse events were mild or moderate in severity. Grade 3 (severe) events more significan than or equal to 2% in frequency after the first dose included injection site pain (2.7%), and after the second dose included fatigue (9.7%), myalgia (8.9%), arthralgia (5.2%), headache (4.5%), pain (4.1%) and erythema/redness at the injection site (2.0%)	The vaccine induces potent antibody and T cell immune responses across all age groups, including older adults. Phase 3 interim analysis including 131 Covid-19 cases indicates that the vaccine is 70.4% effective when combining data from two dosing regimens In the two different dose regimens vaccine efficacy was 90% in one and 62% in the other Higher efficacy regime used a halved first dose and standard second dose, half dose regime may be more effective because it “better mimics” a real infection. There were no hospitalized or severe cases in anyone who received the vaccine The vaccine was on hold after two UK participants developed a severe and unexplained illness. The participants developed a serious neurological condition and after investigation, clinical trials continued.	Phase III results are not published yet. Johnson & Johnson's COVID-19 vaccine trial was on hold after an unexplained illness in a volunteer, but after investigation, the unidentified illness was not related to the vaccine.

Authorization application:	On **November 20**, a submitted request to the U.S. Food and Drug Administration for an Emergency Use Authorisation. **FDA approval on 11 December 2020** **EMA approval on 21 December 2020**	**On November 16** Moderna announced that the trial had met the statistical criteria pre-specified in the study protocol for efficacy. **FDA approval on 18 December 2020** **EMA approval on 6 January 2021**	**On 12 January 2021**, EMA announced the submission for conditional marketing authorisation At end of January 2021 is expected an opinion on marketing authorization Approved in UK on **3 December 2020**	In January- February 2021
Manufacturing and distribution:	Vaccine will have to be chilled to -70°C. The vaccine is estimated to cost $19.50 a dose. The companies expect to produce globally up to 50 million vaccine doses in 2020 and up to 2 billion doses by the end of 2021	Vaccine will have to be chilled -20°C for 6 months, 2°-8°C/ for up to 30 days, Room temperature: 12 hours post thaw The vaccine is estimated to cost $35 a dose It is expected to produce 20 million doses by the end of the year and up to three billion doses of the vaccine in 2021.	The vaccine is estimated to cost around $4 per dose The vaccine can be stored at fridge temperature (2-8°C). It is expected more than 300 million doses will be available by the end of the first quarter of 2021. At peak manufacturing capacity, 100 to 200 million doses of the vaccine can be produced in a month. Large scale manufacturing ongoing in over 10 countries to support equitable global access	The vaccine can be stored at fridge temperature (2-8°C). The vaccine is estimated to cost $10 a dose The pharmaceutical company can supply 100 million doses of its candidate, in a month.

DISCUSSION

❖ ***Vaccine efficacy and mechanism of action***

Although many companies announced previously to approval that the COVID-19 vaccine was almost ready, this process has many complications and even after approval requires further evaluations. The vaccine should be effective, stimulating the synthesis of specific antibodies, at a certain concentration (titer) and providing protection for a reasonable time, together with appropiate availability to widely globally distribution.

However, vaccines do not create immunity in all vaccinated people. The causes are complicated, extending from genetic and immunological components to the quality of the vaccines or methods of administration (8).

Assuming that the vaccine elicits an effective immune response in a significant number of vaccinated individuals, the timing of vaccine protection for them is debatable. It takes a long time to check the viability after vaccination of anti-COVID antibodies. Efficacy also depends significantly on the type of vaccine, technology or platform used.

Another important aspect to be considered, is the internal ability and presence of infrastructures, which will enable companies to produce vaccines on a large scale, to achieve rapid global distribution. The proposed new platforms can generate billions of doses in a few months and optimistically, this capabilities will increase in coming months.

There are still many unknown patterns about coronavirus immunity, which makes immunization with a vaccine difficult. Present and future mutations in SARS-COV-2 (U.K./South Africa mutations, etc) can occur at any time, which automatically implies a moderate/high-risk level of impact in efficacy, for any vaccine that is being developed and/or approved (29).

Another crucial aspect of impact is age. Several studies on influenza vaccines have shown that aging of the immune system dramatically reduces vaccination effectiveness, wich impacts vaccine efficacy in eldery as a high risk category. Therefore, for all anti-SARS-COV-2 vaccine, all of the above aspects should be evaluated, and critical immunization failures minimized through dose adjustment or the number of administrations (8).

❖ ***Vaccine safety profile.***

Vaccination safety includes various elements such as: production, product quality control, evaluation of its effectiveness and guarantee of safety, transport and distribution, implementation of good practices for the use and administration, and a vigilant surveillance system, after distribution and use (29).

Most of vaccines under development are using the same technology, as in the production of seasonal flu vaccine, but also are present new technologies or new adjuvants. This leads to the need of the implementation of additional multiple control steps, to ensure their safety and

effectiveness, preventing unknown side/adverse effects (8).

❖ ***Antibody-dependent enhancement, ADE***

The immunological objective of candidate vaccines is to induce neutralizing antibodies that inhibit the CoV entry into cells. Antibodies play a crucial role in controlling viral infections, including blocking virus binding to cells and activating complement system cascades.

A serious concern for vaccine developers is the risk of "antibody-dependent enhancement" (ADE), which is a phenomenon in which antibodies generated against an infection or from a previous vaccination may worsen the pathogenesis of a subsequent infection, by the same virus or similar (8).

ADE occurs when non-neutralizing antibodies facilitate the entry of the virus into the host cells. Antibodies bind to antibody receptors on the plasma membrane of cells, and the virus binds to the other end of the antibody where the antigen binds.

The ADE's mechanism is not yet fully understood and is thought to vary according to pathogen and patient. This *"Trojan Horse"* strategy allows the virus to infect a cell by escaping from its endosome and trigger damage to the immune system, causing a more aggressive pathogenesis (30).

❖ ***The risk for ADE from vaccine in COVID-19 disease***

It is still unknown whether antibodies generated by the vaccine may increase the severity of disease caused by SARS-CoV-2 infection or mediate harmful immune responses. However, this potential risk needs to be considered, during future possible waves, after massive vaccitation campains.

In the current COVID-19 pandemic state, there are several pending questions about the nature of SARS-CoV-2. Suspicions have been raised that patients with COVID-19 suffering from severe symptoms may have been preceded by one or more previous coronavirus exposures and are experiencing ADE's effects (30). However, this cannot be confirmed because although current clinical evidence suggests that this is just as a possibility, the host molecular and immune response to SARS-CoV-2 infection has not yet been fully elucidated to prove that ADE is occurring.

ADE can occur in COVID-19 infection, but at this moment, it is not known and proved that ADE is occurring in COVID-19 cases, so both antigen treatment and vaccine developments will need to consider this phenomenon and monitor possible presentations (31).

❖ ***Specificity selection***

Most likely, SARS-CoV-2 infection results in a combination of neutralizing and strengthening antibodies, the amount and concentration of which form the patient's clinical response. Therefore, attention must be paid to the nature of the immune response that a vaccine is intended to elicit.

The primary determinant of whether an antibody is protective or strengthening is the epitope for which it was created (30). Given the key role of

the "spike" protein in mediating virus entry into the cell and the immune responses, it induces, almost all SARS-CoV-2 vaccines aim to target it. However, spike protein has also been shown to be a major mediator of antibody-dependent stimulation (ADE).

❖ ***Avoidance of side effects from ADE.***

Some examples of "in vitro" studies have shown that several spike protein epitopes tend to be more "dangerous" than others, for the types of antibodies that are allocated to them. The most "neutralizing" region of the spike protein is the binding site (RBD-receptor binding domain) within the S1 subunit, making it a target of choice for specific and effective vaccines.

An alternative target may be the nucleocapsid (N) protein since protein N is not a virus surface protein, consequently, vaccine-induced antibodies that target protein N will not facilitate virus entry, generating a humoral and cellular immune response specific for this protein (31).

Another major barrier to vaccine development is the higher mutation rate of RNA viruses compared to DNA viruses, resulting in higher genetic diversity. Moreover, RBD of protein S is the most variable region in the coronavirus genome. Therefore, ADE and higher genetic diversity should be considered as essential factors for the current development of vaccines, future modifications and antibody-based drugs against SARS-CoV-2 (30).

❖ ***Vaccine that induces cytotoxic T cells (CTL), without antibodies correctly***

The immunological goal of an ideal vaccine for SARS-COV-2 is to elicit CoV-specific cytotoxic T responses, to kill infected cells in the absence of antibody generation. CoV-infected patients with mild or asymptomatic symptoms develop less antibody responses, CoV-specific CTL responses are mainly controlling their disease.

The more severe the disease, the greater the antibody response is detected in patients in the convulsive phase (32). Vaccine-induced CTL can protect against CoV transmission if CTL kills CoV-infected cells before the virus begins to multiply.

❖ ***Cells immunopathology***

During early testing of experimental vaccines for SARS-CoV, some experimental animals developed after immunization lung or liver histopathology events, characterized by significant infiltration of lymphocytes, monocytes, and eosinophils (33).

In-depth literature analysis suggests that TH17 responses (T-helper 17 cells) may direct these cellular responses after immunization with inactivated viruses and attenuated vaccines, virus vectors, and other vaccine elements. In part, this demonstrates the link between the development of TH17 cells and IL-6 (interleukins 6), an increased cytokine in patients with COVID-19, who experience cytokine storm reactions. Aluminum, an adjuvant that promotes TH2-type immunity, reduces immunopathology and highlights the importance of selecting vaccine with appropriate adjuvant delivery platforms (31).

❖ ***Difficulties in developing vaccines during a pandemic.***

The costs of developing one or more vaccines, including the clinical development and distribution process are approximately of US $ 2 billion. These cost estimations presume a successful development process, reaching final marketing authorization approval or license under emergency use guidelines. An important amount of funds are needed during all stages of COVID-19 vaccine development, preclinical and clinical phases.

There is a major disruption to the candidates' journey from Phase II to III, which partially reflects the very high costs of Phase III trials (34). Approximately development of phase III trials study is responsible for about 70% of the total development costs.

❖ ***Glycolysis of viral proteins***

Glycosylation is one of the most critical forms of modification after protein translation and it is an important way to regulate the localization of proteins and their role. However, high protein glycosylation levels can work as a sort of "invisible camouflage", which may help the virus escape successfully from recognition by the human immune system and increase his survival rates. Therefore, the higher the rate of glycosylation, the greater the probability that the virus will escape the immune response and lower the vaccine development's success rate (35).

However, the use of mRNA-based vaccine technology, with a focus target only to the S protein, and not the whole virus, can lead to the production of S protein antibodies from the human immune system, without viral glycosylation effect.

CONCLUSION

✓ ***The role of pharmaceuticals and clinical trials.***

Pharmaceutical companies are contributing to the fight against COVID-19 on numerous fronts. COVID-19 has driven the pharmaceutical sector and industry to be the center of global attention, playing a key role, through their scientific and economic resources and by devoting itself to the development of diagnostics tools, effective treatment and safe vaccines.

As a science-driven industry that aims to address the world's greatest healthcare challenges, the biopharmaceutical industry is positioned in the best way possible to respond quickly to the COVID-19 pandemic.

More than ever before, the onset of COVID-19 pandemic pushed towards effective coordinated international cooperation, between private and public sectors and institutions, producing incredible results on the research, development and production of diagnostics, drugs and vaccines.

The pharmaceutical industry is fully committed to bringing its unique expertise to:

- ✓ Discovery of new indications for existing drugs in the treatment of COVID-19
- ✓ Accelerate research and development of safe and effective vaccines and new specific drugs.

- ✓ Develop diagnostic tests and provide them to the market continuously.
- ✓ Provide the necessary tools, medicines and vaccines on an ongoing basis.
- ✓ Support to the global health system.
- ✓ ***Vaccines, finally near us…***

Numerous platforms and products have been under development during months of efforts and studies.

Among those with the greatest potential are genetic vaccines (DNA and RNA-based platforms, with already approved representants), followed by those with recombinant subunits.

RNA and DNA vaccines can be produced rapidly because they do not require culture or fermentation, but rather use synthetic processes. To date, there are ongoing approval processes worldwide for RNA vaccines (Pfizer/BioNTech and Moderna, approved from FDA, EMA and other local RA), Regulators worldwide are working intenstively, to evaluate data submitted and approve global use of as many as possible vaccine representants.

The approval of COVID-19 vaccines is just one step (a huge one) but it is fundamental to have virtuous global administration and distribution, in order to reduce to the minimum delays and monitor closely any possible efficacy or safety issues. Further studies and real-world data will tell us, if COVID-19 vaccines will be remembered as a "history of unprecedented medical and scientific success," changing the course of the pandemic and helping the return to the "old boring and not so bad normality..."

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None declared

Conflict of interest

None declared.

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The Importance of Qualitative and Quantitative Biological Methods for Evaluation and Screening of Mycotoxins

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Abstract

Mycotoxins are toxic compounds and the secondary products of the metabolism of various fungal species that grow and develop on substrate of animal or plant origin. The chemical nature (of most mycotoxins) makes them highly liposoluble compounds, that can be absorbed by the gastrointestinal and respiratory tracts and through the blood stream, where they can be passed throughout the body and accumulated in different organs such as the liver and kidneys. The degree of intoxication varies between individuals, mostly depending of the amount of food contaminated with mycotoxins, ages of the subjects, sex, their general health, physiology and immunity. Having in mind the confirmed hepatotoxic and carcinogenic effect of the mycotoxins on the one hand and the conclusions arising from the latest research on the other hand, it is very important to emphasis the need for constant monitoring, the importance of evaluation and screening of the mycotoxins in food, especially in cereals and dairy products, with only one intention to prevent mycotoxicosis. The intention of biomedicine and food biotechnology is to find a faster, much simplified and exact methods for rapid evaluation and screening of mycotoxins, which can be very useful for permanent human biomonitoring and a healthy population.

Keywords: mycotoxins, intoxication, biological methods, evaluation, screening

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INTRODUCTION

Mycotoxins are toxic compounds and the secondary products of the metabolism of various fungal species that grow and develop on substrate of animal or plant origin. Their name comes from a greek "mykes", which means fungus and lat. "toxicum", which means poison. The most common foods and compounds that can be contaminated with mycotoxins are: cereals (rye, wheat, corn, barley, rice), oilseeds (peanuts, soy, sunflower), nuts (walnuts, hazelnuts, almonds) , dried fruits (figs, grapes), beer, wine, spices, cocoa and especially milk and dairy products (1-4). The chemical nature (of most mycotoxins) makes them highly liposoluble compounds, that can be absorbed by the gastrointestinal and respiratory tracts and through the blood stream, where they can be passed throughout the body and accumulated in different organs such as the liver and kidneys (5,6). There are 400 different types of mycotoxins that differ by the type of fungus that they synthesize, their chemical structure, the mechanism of action and the degree of toxicity, but there are a few mycotoxins that are a huge threat to the human health, they are: aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol (7-9).

Mycotoxicosis

Diseases caused by mycotoxins are called mycotoxicosis and from aflatoxins, they are called aflatoxicosis, respectively. The typical and most common symptoms of mycotoxin intoxication are fever, gastrointestinal pain, and immunosuppression (Figure 1).

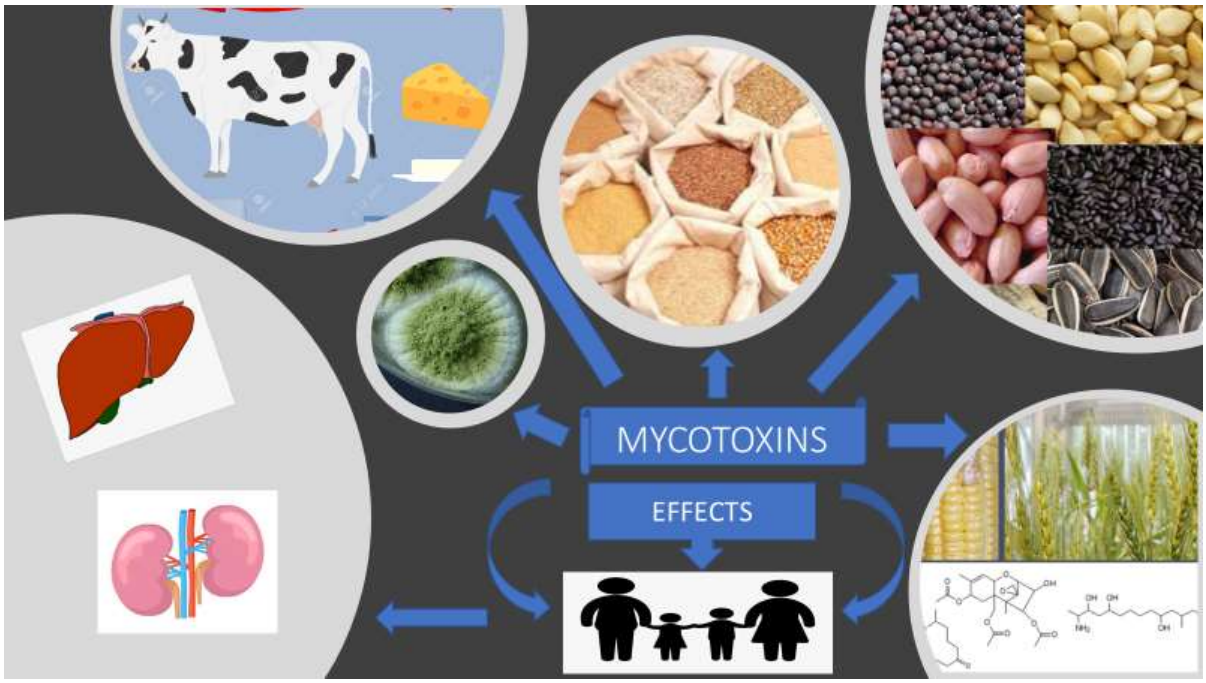

Figure 1. Common effects of Mycotoxins

Mycotoxicoses can be defined as acute or chronic. Acute toxicity generally has a rapid obvious toxic response, while chronic toxicity is characterized by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects. The adverse effects of mycotoxins in humans can induce liver cancer, reduction of immunity, alterations in the protein metabolism, gangrene, convulsions, and respiratory problems, among others (10, 11). Radovanovic et al., (12) linked mycotoxins as one of the risk factors for tumor of urinary organs in a focus of Balkan's endemic nephropathy. The last studies (13-15) observe that mycotoxicosis may eventually result in death. The most common and most toxic mycotoxins are aflatoxins, which can be produced by several species of fungi: *Aspergillus flavus, Aspergillus parasiticus or Aspergillus nomius*. Aflatoxins are confirmed carcinogens that belong to the first group of carcinogens according to the classification of the International Agency for Research on Cancer (IARC) (16,17). Aflatoxins (AF), as mycotoxins, have a hepatotoxic, immunosuppressive, teratogenic and mutagenic effects. There are different types of AF. AF-B1 is more mutagenic and carcinogenic than AF-G1, reflecting the fact that the AF-B1 8,9- exo -epoxide intercalates more readily into DNA, yielding higher levels of adducts for a given dose. AF-B2 and AF-G2 are generally considered to be far less biologically active due to the absence of an 8,9 double bond and consequently 8,9-epoxide formation (18,19). The most favorable conditions for growth and development of these fungi that increase concentration of mycotoxins are: high temperature and humidity, especially during transport and storage of crops or inadequate hygiene or storage of food products of plant or animal origin. Also, the degree of intoxication between individuals mostly depends on the amount of food contaminated with mycotoxins, ages of the subjects, sex, their general health, physiology and immunity. Previous research and studies indicate that every human can be exposed to intoxication of mycotoxins, but the most vulnerable group are children that have a daily intake of dairy milk products, that are contaminated with mycotoxins (20).

DESCRIPTION OF TECHNOLOGY / METHODOLOGY

As a modern and very rapid methods for screening of aflatoxins and other mycotoxins are:

- high performance (pressure) liquid chromatography (HPLC) with fluorescence detector
- enzyme linked immunosorbent assay (ELISA)
- liquid chromatography-mass spectrometry (LC-MS)

In the latest decade, the mostly used method for screening of mycotoxins was thin layer chromatography (TLC), but according to its limitations for quantification and impossibility to repeat the procedure, it is neglected as a method in the process of evaluation of mycotoxins (21-24). Enzyme-linked immunosorbent assay (ELISA) approaches are typically less expensive, but an additional issue is a lack of commercially

available kits or antibodies. While LC-MS provides robust data, the analytical costs are prohibitive for most laboratories (25). The choice of the methods is determined by the aim of the screening procedure, need of qualification or quantification and the types of the food for analysis.

DISCUSSION

The stability of mycotoxins to various preservatives and emulsifiers used in the process of preparation and packaging of the food, also affects the degree of their toxicity and the process of evaluation and screening (26-28). World health Organization (WHO) and Food and Agriculture Organization of the United States", FAO underline that daily intake of aflatoxin of 2 μg/kg present a potential health risk for humans. Medina et al.2015 (29) in their study conclude that in recent years, certain mycotoxins have appeared in atypical agricultural commodities and associated food products and/or unusual regions/climates and global warming is likely to play a very important role in this.

Due to the growing awareness of consumers for their health, the food contamination with mycotoxins has become an important topic for both consumers and subjects in the food business and academic and professional public. Apart from the adverse effects to the health of people and animals, it can also have a significant implications and negative economic effect.

According to these, it is essential and necessary to keep the public informed about presence of mycotoxins in food and feed and negative effects of mycotoxins on human health, it is essential to perform organized food control and take preventive measures in production and warehousing of plant and animal products.

CONCLUSION

Having in mind the confirmed hepatotoxic and carcinogenic effect of mycotoxins on the one hand and the conclusions arising from the latest research on the other hand, it is very important to emphasis the need for consistent monitoring, importance of evaluation and screening of mycotoxins in the food, especially in cereals and dairy products, with only one intention to prevent mycotoxicosis. The intention of biomedicine and food biotechnology is to find faster, much simplified and exact methods with high sensitivity for rapid evaluation and screening of mycotoxins, which can be very useful for permanent human biomonitoring and healthy human population.

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Conflict of interest:

None declared.

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The Status of and Response to COVID-19 in the Republic of Kazakhstan: Multidisciplinary Cooperation in the Republic of Kazakhstan and Cooperation from Japan and Other Countries Around the World is Needed to Respond to and Contain SARS-CoV-2

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Abstract

Background: A year after its emergence in 2020, COVID-19 has continued to spread around the world. People are constantly worried and concerned. Countries are threatened by COVID-19, but effective responses and measures have yet to be identified. The situation is the same in the Republic of Kazakhstan.

Aims: The aim of this study is grasped the progress and status of COVID-19 in the Republic of Kazakhstan, and necessary correspondence and collaboration system are considered about the disease in the country based on the results.

Study Design: This study is summary of progress and epidemiological trends on COVID-19. Therefore, this is descriptive design based on these items.

Methods: We have previously examined studies on and responses to COVID-19 and COVID-19 trends. In light of those findings, the current report discusses what measures the Republic of Kazakhstan should take to deal with COVID-19.

Results: The response to COVID-19 in the Republic of Kazakhstan is currently in dire straits. Detailed epidemiological trends on COVID-19 need to be verified in the Republic of Kazakhstan as well as elsewhere around the world, and responses that appear effective need to be identified so that they can be adapted to the Republic of Kazakhstan.

Conclusion: Multidisciplinary cooperation in the

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Republic of Kazakhstan and collaboration with Japan and other countries around the world and implementation of a wide range of responses is crucial to containing COVID-19.

Keywords: COVID-19, Republic of Kazakhstan, measures, care

INTRODUCTION

On December 31, 2019, the WHO China Country Office officially announced an initial outbreak of pneumonia of unknown etiology in the City of Wuhan, the capital of Hubei Province, China (1). Since January 10, 2020, the WHO has published a number of guidelines for all countries and technical documents on how to prepare for cases of COVID-19 in their territories, including methods of treating the infected (2,3). Since January 21, the WHO has published daily reports of pneumonia caused by SARS-CoV-2 from the Emergency Committee under international health regulations; these reports contain information on the number of confirmed cases, deaths, risk levels, as well as recommendations for infection control and other relevant information (4).

MATERIALS AND METHODS

This report mainly examined the trends of and response to 'COVID-19' in the Republic of Kazakhstan so far. Based on those findings, this work cites the need for a specific response in the future.

RESULTS AND DISCUSSION

On January 27, 2020, the Ministry of Healthcare of the Republic of Kazakhstan adopted a set of measures to prevent the import and spread of COVID-19 in Kazakhstan (5). The Ministry of Healthcare of the Republic of Kazakhstan posts information about respiratory hygiene, proper hand washing, and other measures to prevent COVID-19. On February 25, the Ministry of Healthcare of the Republic of Kazakhstan published a Resolution of the Chief State Sanitary Doctor 'on further enhancing measures to prevent COVID-19 in Kazakhstan' (6).

On March 11, the WHO declared COVID-19 a pandemic (7). In Kazakhstan, the first cases of COVID-19 were recorded on March 13 in two Kazakhs who arrived from Germany. The pandemic has attracted the attention of health professionals and people around the world, as previous coronavirus infections in humans did not exceed the acceptable level of biological risk. However, mutations in these viruses can lead to a public emergency (8).

The total population of Kazakhstan as of January 1 was 18,632,169 (9). At the beginning of April, there were 380 confirmed cases of COVID-19 in Kazakhstan , including 184 cases in Nur-Sultan, 86 cases in Almaty, 15 cases in the Karaganda region, 16 cases in the Atyrau region, 19 cases in the Akmola region, 3 cases in the Zhambyl region, 3 cases in Shymkent, 2 cases in the East Kazakhstan region, 9 cases in the Almaty region, 4 cases in the Aktobe region, 5 cases in the North Kazakhstan region, 1 case in the Pavlodar region, 1 case in the Mangistau region, 25 cases in the Kyzylorda region, 2 cases in the West Kazakhstan region, and 5 cases in the Turkestan region. With the support of the United Nations Children's Fund (UNICEF), the Republican Scientific and Practical Center for Mental Health of the Ministry of Healthcare of the Republic of Kazakhstan created a website on April 4 to provide psychological assistance and support to

the population. This site provides answers to questions and, when requested, an online consultation with a psychologist or psychotherapist. On April 6, Kazakhstan launched a service for online self-diagnosis of COVID-19 in three languages (Kazakh, Russian, and English) (10).

A new type of infection, COVID-19 is a serious disease. The disease is caused by SARS-CoV-2 in the coronavirus family. It can vary in severity, from mild to severe. People of retirement age are at high risk, they are highly susceptible to the virus, and in most cases they have a severe form of the disease. Serious chronic diseases such as diabetes, problems with the heart and lungs, and hypertension also play a key role in the development of the disease. An infection can develop in conjunction with these conditions and eventually lead to death. The most susceptible to the virus are all people who do not have acquired immunity. The infection is transmitted by airborne droplets. For this reason, the main protection for people is considered to be protective masks, as well as personal hygiene rules and social distancing (11).

Figure 1 shows the number of confirmed cases of COVID-19 in Kazakhstan by days since the collection of official statistics started. As of November 17, 121,653 cases of COVID-19 were recorded in Kazakhstan. The day prior, the number of infected increased by 602. There have been 1,899 total deaths from COVID-19 in

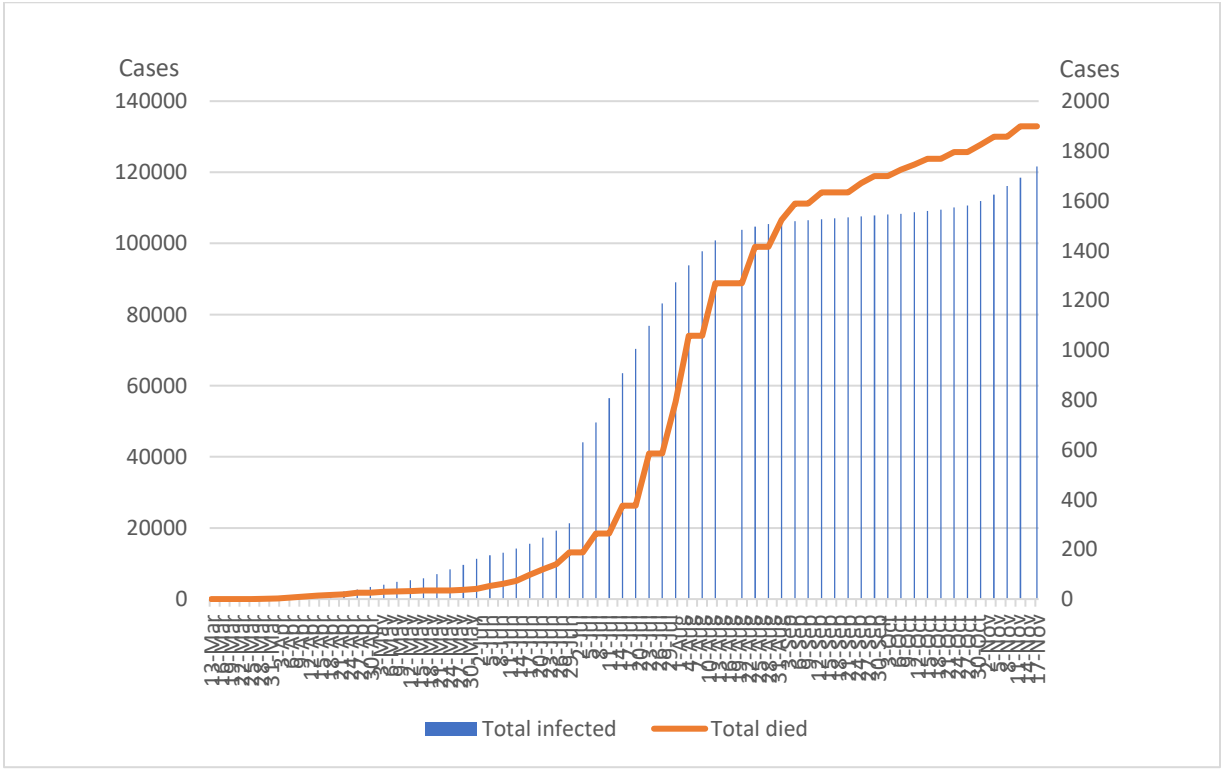

Figure 1. General epidemiology of COVID-19 in the Republic of Kazakhstan from 13 March 2020-17 November 2020

Kazakhstan. Nine thousand three hundred and seventy-two people have active disease, and 221 of them are in critical condition. The fatality rate is 1.56%. As of November 17, 2020, there were 110,382 confirmed cases of a complete cure of the virus in Kazakhstan (10).

Like all other negative processes on a global scale, the current pandemic increases anxiety, distress, and other reactive states in people. In society, there are and have been negative reactions that were generally expected: denial, avoidance, aggression, and suspicion. The main problem is that these negative reactions can contribute to the spread of infection and lead to certain neurotic disorders. The first is the denial of the disease itself, that this disease does not exist, and that no precautions should be taken. The current pandemic is accompanied by the spread of an 'infodemic', where the spread of false news and rumors is no less harmful than the virus itself. The second is avoidance. This is when a person starts avoiding contact with the outside world, which includes even watching TV. A person tunes out from everything and is in a form of information void, which leads to asthenia, sleep disorders, etc. The third reaction is aggression, where the threshold of excitability is very low. In this state, the reaction to any remark is aggression, accusations, and blame-seeking. Fourth, suspicion is also common: 'someone was responsible for this' or 'we are victims.' These 4 negative reactions are always present everywhere, whether it be in Kazakhstan or some other country (12). All of these reactions lead to a denial of the epidemiological norms that need to be followed. Experts have also noted economic problems in a number of countries, as well as rising unemployment. Government support during a pandemic is very important to all citizens. The Ministry of Industry and Infrastructure Development of the Republic of Kazakhstan has developed a mechanism to help citizens in regions under lock-down. During the state of emergency, the government makes monthly payments to people who lost their income due to the state of emergency, and various benefits have been provided to small- and medium-sized businesses (13). Kazakhstan was one of the first countries to take drastic measures in response to the pandemic. The number of infected would be many times higher if prompt measures were not implemented.

CONCLUSIONS

The response to COVID-19 in the Republic of Kazakhstan is an uphill battle. As COVID-19 trends are being meticulously studied in the Republic of Kazakhstan, the situation in Japan and elsewhere around the world is being carefully considered, and COVID-19 measures that appear to be effective should be tailored to the Republic of Kazakhstan. In addition, various disciplines in the Republic of Kazakhstan need to work together to implement COVID-19 measures as needed. In medicine, clinical medicine, social medicine, and basic medicine must coordinate their response to COVID-19. The Republic of Kazakhstan should work with Japan and other

countries around the world to respond to and control COVID-19.

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Conflict of Interest Disclosure: These authors have no conflict of interest.

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Tinea Incognito due to Local Steroids Use

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Abstract

Tinea incognita is an uncommon dermatophytic infection of the skin resulting from corticosteroid abuse. Due to its tricky clinical feature it is often misdiagnosed with other dermatosis such as contact dermatitis, seborrheic dermatitis or rosacea.

A 48 years old woman presented for dermatologic consultation with a one-and-a-half-month history of some disturbing lesions on her face. Patient was diagnosed and treated as contact dermatitis with local corticosteroids without any improvement for 3 weeks. After the clinical consultation at the dermatological unit and fungal culture confirmation; the diagnosis of tinea incognito was confirmed. The patient was treated for four weeks with oral and local antifungals and the lesions were completely healed. The aim of the case is to raise awareness among primary care physicians on the possible existence of mycotic infections and to be careful with the use of topical steroids.

Keywords: Tinea, tinea incognito, topical antifungal, topical steroids

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INTRODUCTION

Tinea incognito (TI) is a fungal infection of the skin, masked and often exacerbated by application of a topical corticosteroid. Atypical, corticosteroid modified, clinical features are often a challenge for physician's diagnosing (1). Tinea incognito indeed is a mycotic skin infection but it appears by incorrect, sometimes also prolonged steroid treatment. Moreover, for skin changes steroids are often recommended, even without prescriptions. This might be also case due to incorrect diagnosis. Steroids, in particular topical, are often used due to irritating dermatological changes, they suppress the immune response and this favors mycotic agents to grow, which obscure clinical feature and can lead to misdiagnosis. For these reasons Tinea incognito remains challenging for right diagnosis (2). Often, particularly due to changes in the immune response, the clinical feature may look like other diseases such as: eczema, lupus erythematosus, contact dermatitis, erythema migrans (1). The lesions are more extensive, without clear borders, which makes the clinical appearance quite different from typical fungal infection, being a key diagnostic element. Diffuse blanching erythema with telangiectasis, sometimes associated by scattered papules, pustules and hyperpigmentation are present in Tinea incognita (3).

The aim of the case is to raise awareness of primary care physicians on the possible existence of mycotic infections and in uncertain cases to consult dermatologist in order to clarify the case and to improve the treatment.

CASE REPORT

A 48 years old woman was admitted in outpatient dermatology clinic with a history of some facial lesions for about one and a half month, without previous medical history. She was referred by her family physician that had primarily treated the lesions with topical steroid (bethametasone 0.05% cream) and a second-generation antihistamine (Bilastine 40mg/day orally for 3 weeks) suspecting allergic dermatitis. Nevertheless, patient did not feel any improvement, she repeatedly asked for medical help, then she was referred for further dermatologic opinion. At our Department, she presented with some annular erythematous patches extended all over her both cheeks. The lesions were of different size from 1-2 cm and confluent in between. Also, in periphery of the lesions we noticed some scaling (Figure1 and Figure 2). The panthenol cream was prescribed during this evaluation period. Direct microscopic examination revealed negative for dermatophytes. While we were waiting for fungal culture result, all laboratory blood tests revealed within normal ranges. Therefore, we also excluded the lupus erythematodes discoides. Fungal culture examination identified trichophyton rubrum. During four weeks the patient was treated with oral terbinafine 250mg daily, while topical clotrimazole cream was applied twice daily for six weeks. Skin changes

were getting better, they were smaller in size, scaling has disappeared, skin color was reestablished. The patient was also feeling better and the treatment was terminated.

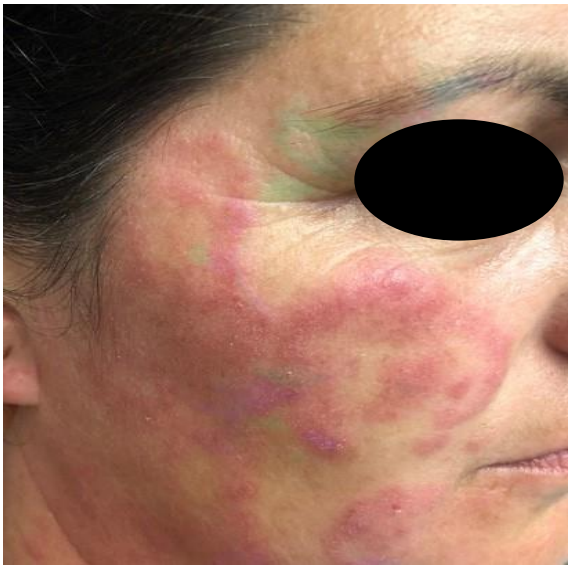

Figure 1. The erythematous patches extending on all face with some scaling

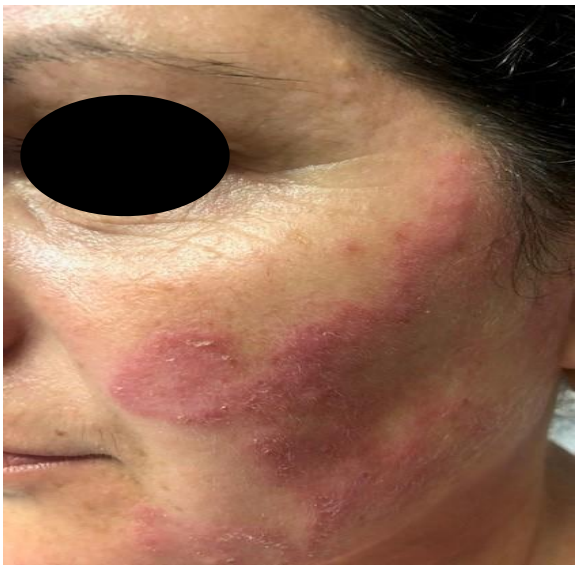

Figure 2. The erythematous patches extending on all face with some scaling

DISCUSSION

There are some moments which need deep, critical thinking and analyzing, making proper treatment choices, insisting on right diagnosis after considering the differential diagnosis (4). The easy, quick and cheap method of direct microscopy (according to existing institutional protocols) should be a must in cases presented with erythema and scales, insisting on proper sampling form the untreated lesion (5). This primarily leads to correct doubt, proper diagnosis and treatment. Immediate interruption of steroid agents is recommended along with initiation of antifungal treatment. In mild to moderate cases local antifungals have resulted in proper management, while severe forms demand oral antifungal in addition (6). Treatment by allylamine and the azole group drugs (itraconazole, fluconazole), remain the first choice in compare to griseofulvin because of the first ones affinity to the stratum corneum of the skin (1). Authors present the similar case as ours with erythematous and scaling patches but also papules and pustules treated with steroid and the skin changes got worse. Furthermore, they present negative direct microscopy and isolated Microsporum canis in culture. Tinea faciei incognito is often difficult to diagnose because of the clinical modification from the use of the topical steroids. The differential diagnoses at this site include contact dermatitis, rosacea like dermatitis and folliculitis, that's why misdiagnosis maybe an issue (7). There are cases of tinea incognita where direct microscopy is

negative and culture or polymerase chain reaction specific are positive for dermatophytic infection. This is seen in few cases with the same lesions (8). Since tinea incognito sometimes can be very difficult to diagnose as the authors in this case describe, where only after a biopsy the authors had seen inflammatory changes and suspected for tinea incognito. Therefore, authors suggest suspecting in tinea incognito when we have scaly erythematous changes on the face (9). There are cases of using dermatoscopy as a simple noninvasive in vivo diagnostic tool for tinea incognito, as it is in the case presented from Sonthalia et al. With dermatoscopy they saw perifollicular scaling, black dots, broken, comma and cork-screw hair (10). Kim et al published a 9-year multi-center study of Tinea incognita in Korea, which demonstrated that 82% were misdiagnosed as eczema-like manifestation and 0.7% as folliculitis-like presentation that was exceptionally rare (11).

CONCLUSION

Mycotic infections can sometimes be challenging due to misdiagnosis and inappropriate treatment. Sometimes, because of steroid treatment the skin changes can be modified, the disease masked, along with the patients ongoing disturbances. Physicians must consider tinea (incognito) infection, in particular when there is not seen any improvement, or the changes are worse besides the treatment. Diagnosed based treatment is required for appropriate case resolution. Novel diagnostic tools as dermatoscopic in vivo examination could be a promising option for fast and accurate diagnoses of tinea incognito.

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A Simple and Effective Appliance for Class II Malocclusion Treatment

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Abstract

Background: Class II malocclusion dental or even skeletal is common in patients seeking orthodontic treatment. Maxillary protrusion, mandibular retrusion or the combination may contribute to develop a skeletal class II and affect facial appearance. Cases of mild skeletal discrepancy with an overall good profile can benefit from distalizing maxillary molars when there is no mandibular retrusion. Among several devices used is also Carriere Distalizer.

Aims: The aim of this short report was to evaluate treatment of class II malocclusion with Carriere Distalizer.

Methods: All patients in this study had moderate skeletal and class II dental malocclusion and a good profile. Diagnosis was performed by means of cephalometric analysis, study models. They were all treated with Carriere Distalizer. Before starting the treatment, which consisted in applying class II intraoral elastics the anchorage in lower arch was achieved by means of lingual arch.

Results and Conclusion: CarriereDistalizer provides maxillary molars distalization, derotation and correction of class II relationship in a short time period with an average of 6 months. The standard edgewise treatment mechanics for class II correction uses intraoral class II elastics after initial alignment and leveling. For the levelling to be complete it may require, depending in the severity of crowding more than 12 months. Carriere Distalizer shortens

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this time since intraoral class II elastics are used before leveling and alignment. Thus, the required collaboration of the patient is complete since it is the beginning of treatment and they are strongly motivated to see results.

Keywords: orthodoncy, dental malocclusion treatment

INTRODUCTION

According to Angle (1) class II malocclusion is the distal relation of the lower dentition to the upper to the extent of more than one-half the width of one cusp. The maxillary dentition may sometime protrude. If skeletal anteroposterior relationship between maxilla and mandible is altered, the profile becomes less esthetic.

McNamara (2) performed a study with 277 Class II patients 8-10 years old. He found that are four main elements causing the Class II characteristics: the maxillary skeletal position, the maxillary dental position, the mandibular skeletal position and the mandibular dental position. In 60% of the cases, mandibular retrusion was the cause of class II malocclusion.

There are many methods that can be used for Class II correction. In selecting the most accurate treatment protocol several factors including etiology, skeletal and dental components of the malocclusion are evaluated. Age and skeletal discrepancy determine treatment alternatives of skeletal class II that include functional treatment, compensatory and combined orthodontic treatment and orthognatic surgery (3).

Young age patients may be treated with various functional appliances (Herbst, Twin Bock). These appliances are effective in correcting skeletal class II malocclusion and perhaps also increasing length of mandible (4, 5).

Extractions are indicated in cases that cannot benefit from functional treatment but have still and acceptable profile. Extractions pattern is determined from the skeletal discrepancy in sagittal and vertical dimensions. Where an increased overjet is present and a regular mandibular arch only extraction of maxillary first bicupids is indicated (6).

Distalization is often required in skeletal and dental class II with harmonious profile. There are many appliances used to distalize maxillary molars. Classification can be depending on appliance location intra or extra orally. The intra oral appliances can also be classified based on the placement site palatally or buccally. Appliances used to distalize maxillary molars may require strong collaboration from the patients. Since not always young patients do not offer collaboration by following the recommended wearing time, most of the available distalization appliances are fixed intraoral. Limitations in use of extra oral distalizing appliance related to the missing collaboration and with the clinician's preference to have a better control of the distalization with a fixed intra oral appliance, lead to development of many intra oral fixed appliances.

Paul (7) in his study aimed to compare the efficacy between fixed and removable intra oral appliances, showed that there was no statistically significant difference between the two methods. Bondenmarkand Karlsson (8) performed a study comparing the treatment effects between head gear and Nance with NiTi coil springs and found that intra oral appliance was significantly more effective that head gear.

Carriere distalizer was introduced by Luis Carriere in 2004 (9). The appliance consists in a rigid arm bonded to the cuspid and the first molar.

The cuspid pad has a hook for class II elastics. The molar pad contains a ball that moves inside the socket. This movement provides crown up righting and bodily distalization. Another effect is distal rotation around the palatal root.

The aim of this study was to evaluate class II correction by means of Carriere distalizer appliance.

MATERIALS AND METHODS

We first used Carriere Distalizer in 2014. This is a retrospective study showing results taken from some of among 20 patients we decided to treat with this protocol. The treatment was not performed simultaneously.

Patient selection to be treated with Carriere Distalizer was done based on these criteria:

- No previous orthodontic treatment
- Dental class II malocclusion uni or bi laterally
- Mild skeletal class II with good profile.

Before starting the treatment, all patients were informed about the need to collaborate by wearing the intraoral class II elastic following prescription. All patients were treated by one of the authors of this study.

After appliance selections done by measuring each patient's model, bonding was performed following manufacturer's instructions.

In the lower arch bonded lingual arch was used to obtain anchorage for class II elastics.

After bonding the appliance, patients were given instructions regarding recommended wear time 24 h and change every 24 hours. They were also instructed how to wear and remove, as well as to wear new elastics in case of any broke before 24 hours of wearing. Elastics used were 3.5oz ¼ inch and 3/8 inch.

Patients were clinically monitored every 4 weeks. Instruction were to notify for possible appliance decementation and anticipate next visit.

RESULTS

Class I cuspid and molar relationship was achieved in all patient in an average period of 4-6 months depending on the severity of class II cuspid and molar relationship (Fig 1-4). Molar derotation was also achieved. Spacing and better alignment was observed in the anterior region indicating that spaced was gained in the posterior area. After correction of class II relationships treatment continued with bonding of both arches with 022 slot brackets and sequencing archwire to finish the case.

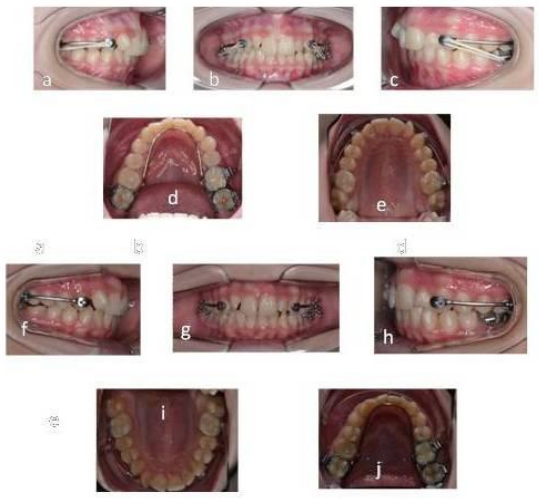

Figure 1. a-j. 4 months of treatment with CarriereDistalizer

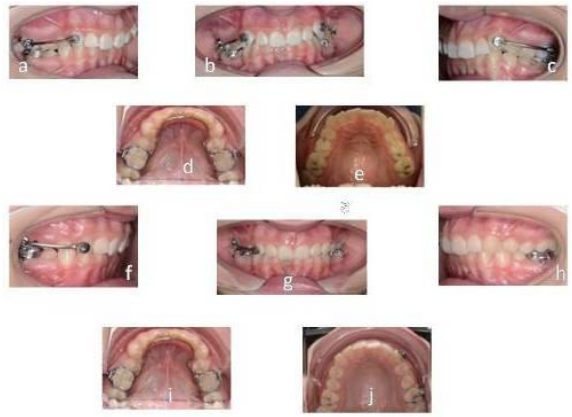

Figure 2. a-j. 6 months of treatment non very collaborative patient

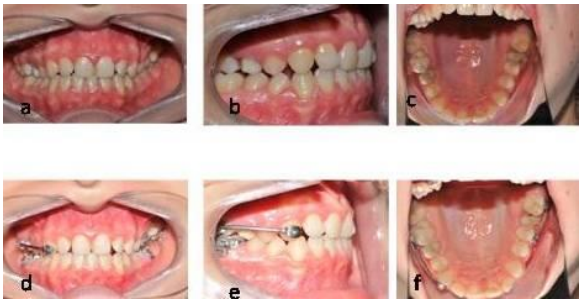

Figure 3. a-f. Unilateral class II. 3 months' result

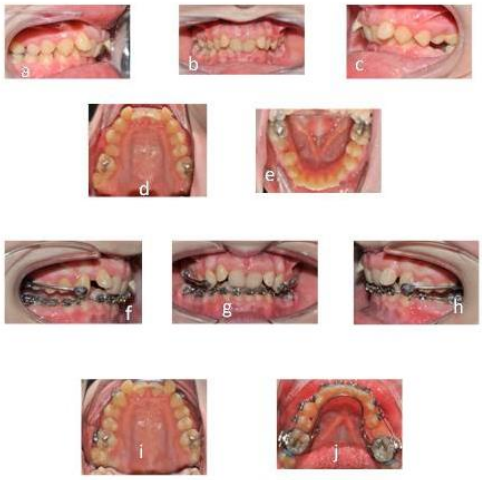

Figure 4. a-. j Class II division II. Initial to progress in 5 months

DISCUSSION

Distalization of maxillary first molars is the preferred non-extraction treatment method in class II malocclusion (10). The developing and use of many distalization appliances provides clinicians the opportunity to choose according to the severity of malocclusion and patients specific needs. Carriere Distalizer is one of the appliances available to distalize molars in young and adult patients as well. The fast and initial correction of class II molar relationship is facilitated by absence of any wire and bracket thus avoiding friction (11). Besides, significant changes in the length of correction time between class II elastics used with fixed appliance and class II elastics used with Carriere Distalizer were observed (12). Minor alignment changes occur during the distalization of molars facilitated by spaces created in the posterior area. The overall treatment duration can be shortened since after the correction of dental class II alignment and levelling with the straight wire appliance does not require a long treatment time.

CONCLUSION

Although it is an appliance that requires patient collaboration, good results can be achieved in treating class II malocclusion when distalization of molars is required. Advantages of use consist not only in the design but also in using class II elastics in the beginning of the treatment with the patient that is motivated to have a better smile not with crowded and protruded teeth. Mild skeletal class II and molar class II relationship

hypodivergent cases have the best indications for Carriere Distalizer treatment.

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