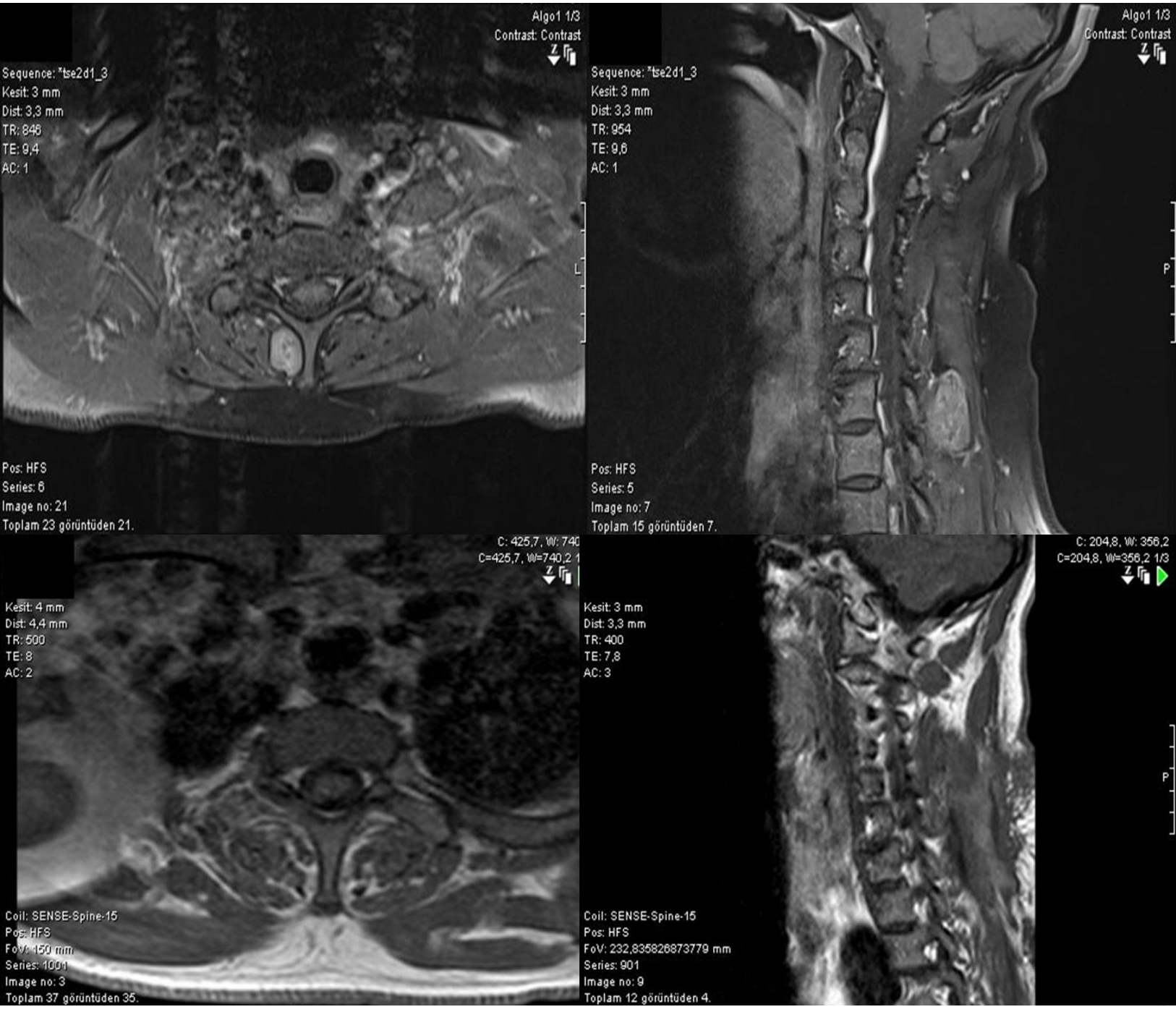
Dec. 2019, Vol 6, No 12

MSD

Medical Science & Discovery



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

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

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

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

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

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

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

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

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

Category: Multi Disciplinary Health Science Journal

Abbreviated key title: Med. Sci. Discov.

Frequency: Monthly

Review System: Double Blind Peer Review

Circulation: Globally, Online, Printed

Article Processing Charge (APC): Free

Licensing: CC-BY-NC 4.0 International License Environmental

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

Established: 30.04.2014

Web address: www.medscidiscovery.com

E-mail : editor [at] medscidiscovery.com

Phone : +44 020 3289 9294

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

Publisher: Lycia Press Inc.

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

Web address: www.lycians.com

Phone : +44 020 3289 9294

E-mail : office [at] lycians.com

E-mail : info [at] lycians.com

Instruction for Authors

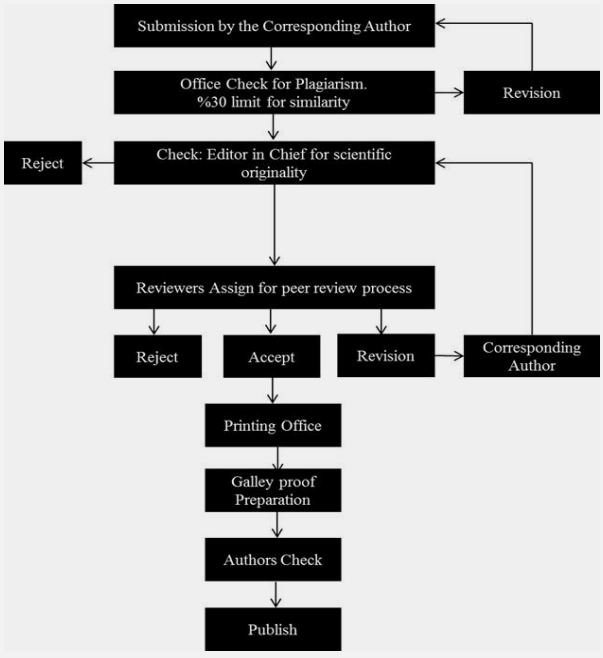
- **Important**
- MSD journal team, is committed to deterring plagiarism, including self-plagiarism. Your manuscripts will be screened for similarity detection with iThenticate, Similarity rate is expected under the %30 except for material and method section.
- For research studies using human or animal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

Instruction for Authors

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

Instruction for Authors

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



Instruction for Authors

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

Instruction for Authors

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

Instruction for Authors

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

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

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

- **References**

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

Medical Science and Discovery, December 2019, Vol.6, No.12

Contents

Research Article

Case Reports

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):305-9

Research Article Doi: 10.36472/msd.v6i12.320

A neurological approach to fear of falling in patients with stroke

Büşra Sümeyye Arıca Polat[1]*, Zuhal Yiğit[2], Kurtuluş Köklü[2], Nihal Köylü[2], Ali Rıza Sonkaya[1]

Abstract

Objective: The present study aimed to investigate the factors that affects the fear of falling, also known basophobia, in patients with stroke and the aspects of life quality.

Material and Methods: Stroke patients in the physical therapy were included in this study. Demographic data of the patients were recorded. Patients were asked whether they fell after the stroke and experienced fear of falling. Itaki Fall Risk Scale, Functional Ambulatory Categories, Functional Independence Measure, Beck Depression Inventory and Stroke Specific Quality of Life scale (SS-QoL) have been used. The data of the study were analyzed with SPSS 15 package program.

Results: This study was conducted on 102 patients with stroke. Of these, 36 (35.3%) patients were female with a mean age of 60.97. There was a fear of falling in 65 (63.7%) of the patients. 48 patients (47.1%) fell at least once after the stroke. Falls were significantly high with the ones who were experiencing fear of falling ($p = 0.006$). Fear of falling was significantly high in females, patients with ischemic stroke, elderly and the patients with coexistent systemic diseases ($p <0.05$). The total score and sub-units of SS-QoL was significantly low ($p <0.05$) in the group with the fear of falling. The depression score was significantly high in patients with fear of falling ($p = 0.00$).

Conclusion: Fear of falling in patients with stroke negatively affects many sub-units of the quality of life-related to movement, participation, and mood. Thus, the classic rehabilitation program for patients with stroke should include falling prevention training.

Keywords: Stroke, Quality of life, Fear of falling

Introduction

Fall is a common case in patients after stroke. It is seen 14-65% during hospitalization and 37-73% after discharge [1-2]. The localization of the stroke, cognitive deficits (such as anosognosia, somatognosia, and neglect), visual disturbances, sedative or psychotropic medications increase the risk of falls in patients with stroke [3-4]. In addition, balance and mobility problems, need for support for self-care, depression, and history of previous falls have also been found to be associated with falls in stroke patients [5]. Fear of falling is an important individual risk factor of falling for stroke patients apart from all other risk factors [6]. Fear of falling may be associated with falling concurrently with the onset of the stroke and post-stroke body change perceptions. Accompanying neglect and deep sensory impairment increase the fear of falling. Fear of falling in patients with stroke affects in a negative way the quality of life such as limitation of activity and functional independence. Besides, this situation increases the caregiver stress [7-10]. This study was aimed to investigate the factors affecting fear of falling that is one of the most important predictors of falling in patients with stroke and the aspects of the quality of life.

Material and Methods

Stroke patients under the physical therapy were included in the study. Demographic data (age, gender, marital status, the closeness of caregiver) of the patients were recorded. The time and the type (hemorrhagic/ischemic) of stroke and the affected side (left/right) were recorded. In addition, the orthosis which was used by the patients (no orthosis/ foot-ankle orthosis/ knee-foot-ankle orthosis etc.) and the support used for walking (canes, tripods, walkers, etc.) were recorded. This study was approved by the local ethical committee (Ankara Fizik Tedavi ve Rehabilitasyon Eğitim ve Araştırma Hastanesi Eğitim Planlama Kurulu protocol no: 838942379000-773.99-2638) and all participants were informed about the study then written consent form was taken.

Fall Risk Assessment

Patients were asked whether they fell after the stroke and answers were recorded. They were asked whether they experienced fear of falling and answers were recorded as yes or no. To assess the risk of falling, Itaki Fall Risk Scale was used. This scale comprises 19 risk factors which includes probability of the cause patient falls.

Received 15-10-2019 **Accepted** 10-12-2019 **Available Online** 25-12-2019 **Published** 30-12-2019
1 Health Sciences University Gülhane Education and Research Hospital Dept. of Neurology, Ankara, TR
2 Ankara Physical Therapy and Rehabilitation Training and Research Hospital, Ankara, TR
*** Corresponding Author:** Büşra Sümeyye Arıca Polat **E-mail:** busrarica@yahoo.com

Risk factors were categorized as major and minor which 1 point was given to minor risk factors and 5 points were given to major risk factors. Two risk levels were determined over the calculated total score after the evaluation of risk factors. The total score of less than 5 is low risk and total score 5 or above is the high risk [11].

Ambulatory and Functional Assessment

Functional Ambulatory Categories (FAC) was used for assessing the level of ambulation. According to this; FAC 0: No ambulation, FAC 1: While walking on a flat ground the patient does not need more support than manual support of one person so as not to fall yet the manual support is continuous, FAC 2: While walking on a flat ground the patient does not need more than one person's manual support so as not to fall yet, the form of support is continuous or intermittent touching, FAC 3: The patient can walk on flat ground without someone else's manual support, but for safety reasons, someone else is needed to lead the patient, FAC 4: The patient can walk independently on the flat ground, however, needs assistance with stairs and slopes, FAC 5: The patient is able to walk independently on flat ground as well as uneven surfaces [12].

Functional Independence Measure (FIM) was used for assessing the functional status of the patients. Turkish validity and reliability research of FIM had been performed previously [13]. FIM is a scale that measures independence in 18 different activities and evaluates each activity in seven levels. Section 13 is for assessment of motor functions and section 5 is for cognitive functions. Each category is scored from 1 to 7. The total score is between 18 and 126.

Assessment of Depression

Beck Depression Inventory (BDI) was used for assessing the depression status in patients. There are 21 questions in BDI. Each question is evaluated in four levels between 0 and 3. The total score is obtained by collecting scores from each question. The total score is between 0-63 points. High scores indicate that depression is more severe [14].

Quality of Life Assessment

Stroke Specific Quality of Life scale (SS-QoL) is used for assessing the quality of life in stroke patients [15]. Turkish validity and reliability study had been performed previously [16]. This scale includes a total of 49 items in 12 areas (self-care, mobility, upper extremity function, language, vision, work, thinking, family roles, social roles, personality, mood, and energy). Each field has at least 3 questions. Each item is evaluated with a Likert-type score ranging from 1 to 5, taking the last week into consideration. To calculate each field's average score on the scale, the points are obtained from all the sections associated with one particular field are summed up and then divided by the number of sections which belong to that field. The total score of the scale (49-245) is calculated by summing up the scores of all fields and subsequently dividing the total score by 12. Higher scores refer to better function.

Statistical Analysis

The data of the study were analyzed with SPSS 15 package program. Descriptive statistics were expressed as mean±standard deviation or median (minimum-maximum). Chi-square test was used to compare two independent groups of qualitative data (gender, stroke type, stroke side, orthosis use, assistive device use, ambulatory group). The Shapiro-Wilkons test was used to investigate whether the distribution of continuous quantitative variables was close to normal. Student-t test was used if normal distribution assumption of quantitative data was provided and non-parametric methods were used when normal distribution assumption was not provided. In this context, the Mann-Whitney U test was used to compare the variables which is obtained by assessment in two independent groups. $p < 0.05$ was considered as statistically significant.

Results

Demographic data

The study was conducted on 102 patients with stoke, 36 patients were female (35.3%) and 66 (64.7%) were male, with a mean age of 60.97±10.81 (39-85 years). The demographic features of the patients are summarized in table 1. The elapsed time after the stroke is an average of 8 (2-36) months. The characteristics of the strokes are summarized in table 2. There was a fear of falling in 65 (63.7%) of the patients. 48 of the patients (47.1%) fell at least once after the stroke. Falls were significantly high with the ones who were experiencing fear of falling ($p=0.006$). In comparison of the group which had falling fear (10.7±4.24) and the group which did not have falling fear (7.1±4.08) in terms of falling risk, the risk of falling was significantly high ($p=0.001$) in the fear of falling positive group. Fear of falling was significantly high in females, ischemic stroke patients, elderly and the patients with coexistent systemic diseases ($p < 0.05$). No relationship was found to be between marital status, side of the stroke, or elapsed time after the stroke ($p>0.05$). FIM score ($p=0.012$) and ambulation level ($p = 0.000$) were significantly low in the group who had fear of falling. While the use of an orthosis did not affect the fear of falling ($p> 0.05$), the use of an assistive device to walk significantly decreased the fear of falling ($p = 0.012$). However, there was no relationship between risk of falls and gender, stroke type and side, FIM and FAC scores ($p> 0.05$). The score for SS-QoL was significantly low ($p < 0.05$) in the group with the fear of falling. Energy, family roles, mobility, mood, personality, social role, thinking, work sub-units of SS-QoL was significantly low ($p < 0,05$) (Table 3). A significant negative correlation was found between the total scores of qualities of life according to Itaki falling risk scale score ($p=0.008$, $r=-0.261$) and quality of life sub-units` scores which are summarized in Table 4. The depression score was significantly high in patients with fear of falling ($p = 0.00$) while, a significant weak positive correlation was found between the risk of falling and the depression score ($p = 0.024$ $r = 0.224$).

Table 1: Demographic features

		n (%)
Age		60.97 ±10.81 (mean: 61 / 39-85)
Gender	**Female**	36 (35.3)
	Male	66 (64.7)
Marital Status	**Married**	82 (80.4)
	Single	20 (19.6)
Primary caregiver	**None**	6 (5.9)
	Spouse	57 (55.9)
	Daughter/Son	33 (32.4)
	Parent	6 (5.9)
Number of people living at home		3.33±1.47 (mean: 3 / 1-9)

Table 2: Features of Stroke

		n (%)
Side	**Right**	55 (53.9)
	Left	47 (46.1)
Type	**Ischemic**	76 (74.5)
	Hemorrhagic	26 (25.5)
Ambulation Level (FAS)	**1**	15 (14.7)
	2	17 (16.7)
	3	29 (28.4)
	4	24 (23.5)
	5	17 (16.7)
Orthesis	**Yes**	38 (37.3)
	No	64 (62.7)
Assisting Device	**Yes**	68 (66.7)
	No	34 (33.3)

Table 3: The relation between fear of falling and quality of life

	Total patient (mean ±SD)	Fear of falling No	Fear of falling Yes	p
Total Score	3.40±0.96	3.84±0.74	3.15±0.99	0.001
Energy	3.50±1.24	4.11±0.91	3.15±1.27	0.001
Family	3.72±1.19	4.19±0.84	3.46±1.28	0.02
Language	3.60±1.50	3.71±1.41	3.54±1.55	0.582
Mobility	2.92±1.14	3.47±1.02	2.61±1.10	0.001
Mood	3.36±1.45	4±1.24	3±1.45	0.01
Personality	3.44±1.42	4.05±1.17	3.10±1.44	0.01
Self care	2.87±1.29	3.19±1.26	2.69±1.29	0.63
Social role	3.22±1.42	3.81±1.16	2.89±1.46	0.01
Thinking	3.59±1.32	4.20±1.01	3.25±1.35	0.001
Upper Limb Function	2.72±1.39	2.95±1.30	2.59±1.42	0.22
Sight	4.71±0.65	4.81±0.51	4.66±0.72	0.27
Productivity	3.14±1.27	3.65±0.97	2.86±1.34	0.02

Table 4: The relation between risk of falling and quality of life

	p	r
Total score	0.008	-0.261
Energy	0.002	-0.307
Family	0.01	-0.313
Language	0.144	0.144
Mobility	0.006	-0.270
Mood	0.006	-0.268
Personality	0.78	-0.175
Self-care	-0.195	0.50
Social role	0.015	-0.241
Thinking	0.003	-0.293
Upper limb function	0.132	-0.150
Sight	0.55	0.059
Productivity	0.014	-0.242

Discussion

Falling is an important problem after stroke, which affects both functionality and quality of life [1]. Also fear of falling is an important independent risk factor for falls, apart from all other coexistent reasons for falls [6]. In previous studies, the effect of fear of falling on the quality of life was investigated [7]. In this study, it was aimed to use a scale that is developed specifically for stroke patients, to evaluate the affecting part of the quality of life. Our findings demonstrated that fear of falling has a negative impact on many sub-units of quality of life like movement, participation and mood. 63.7% of the patients had fear of falling which were included in the study. In line with the the literature, it was observed that the fear of falling is 32-54% in stroke patients [8, 17-19]. In one study which was conducted by Schmid and Ritmann (2007), evaluated the patients with stroke in the first 6 months, fear of falling and post-stroke falls and post-stroke body shift perception were found to be associated [8]. Likewise, in another study evaluating patients with subacute stroke, fear of falling was found to be associated with muscle strength in the lower extremities of patients [18]. In a study conducted in patients with chronic stroke, it is stated that half of the patients who fell at least once after discharge will experience fear of falling and it was emphasized that this condition will persist in the following years [19]. It was found to be a significant relationship between fears of falling and falls in this study. In addition, it was observed that the fear of falling is increased in females, ischemic strokes, advanced age, and coexisting systemic disease. Likewise, low ambulation level and low functional independence level were found to be associated with fear of falling. However, it did not detect any association with marital status, stroke side, and elapsed time after the stroke. Furthermore, it was found that the use of a hand support that helped to walk reduced the fear of falling while the use of the orthosis had no effect. Fear of falling affects the confidence of the individual's daily life activities negatively thus leads to a less active lifestyle and additional health problems such as muscle atrophy and muscle weakness, especially in the lower extremities [20,21]. There are many studies have showed that fear of falling affects the quality of life negatively for elderly population as well as for stroke patients [17, 20-25]. However, many systems (upper and/or lower extremity functions, language, cognitive functions, etc.) are affected together in stroke patients. For this reason, the use of special constructed scales to evaluate the quality of life in patients with stroke can help to better assessment of the quality of life and identify the problem comprehensively. In one research which was investigated the quality of life in stroke patients via scale, scale scores were found to be significantly low especially in the areas of energy, personality, thinking, personal care, work/productivity and social roles in patients who had a fear of falling, comparing to the healthy volunteers who did not have a fear of falling, furthermore, these patients experienced decreased performance satisfaction and increased anxiety [17]. In our study, it was clearly showed that the patients who had fear of falling have a loss in quality of life in fields of energy, mobility, work/productivity which are related with the loss of movement. Along with these, it was observed that fear of falling has an influence on sub-units such as family roles, mood, personality, social role, thinking. It was also detected that patients with fear of falling had higher depression scores.

Conclusion

In stroke rehabilitation; not only the improvement of loss on its own but also the improvement of the quality of life and increasing participation of the individual should be aimed. Fear of falling in stroke patients negatively affects many sub-units of the quality of life-related to movement, participation, and mood. Thus, the classic rehabilitation program for stroke patients should include falling prevention training. In addition, cognitive and emotional support to reduce the fear of falling should be placed into the program in order to increase the quality of life of individuals in the short and long term.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: BSAP; Research concept and design, Research the literature, preparation of the article, BSAP; Revision of the article.

References

1. Nyberg L, Gustafson Y. Patient falls in stroke rehabilitation a challenge to rehabilitation strategies. Stroke 1995;26:838-842.

2. Teasell R, McRae M, Foley N, Bhardwaj A. The incidence and consequences of falls in stroke patients during in patient rehabilitation: factors associated with high risk. Arch Phys Med Rehabil. 2002;83:329-333.

3. Batchelor FA, Mackintosh SF, Said CM, Hill KD. Falls after stroke. Int J Stroke 2012;7:482-490.

4. Ng MM, Hill KD, Batchelor F, BurtonE. Factors predicting falls and mobility outcomes in patients with stroke returning home after rehabilitation. Who are at risk of falling. Arch Phys Med Rehabil. 2017;98(12):2433-2441.

5. T Xu,Clemson L, O'Loughlin K, Lannin NA, Dean C, Koh G. Risk factors for falls in community stroke survivors: A systematic review and metaanalysis. Arch Phys Med Rehabil. 2018;99(3):563-573

6. Jalayondeja C, Sullivan PE, Pichaiyongwongdee S. Six-month prospective study of fall risk factors identification in patients post-stroke. Geriatr Gerontol Int. 2014;14:778-785.

7. Monteiro RB, Laurentino GE, Melo PG, Cabral DL, Correa JC, Teixeira-Salmela LF. Fear of falling and the relationship with the measure of functional independence and quality of life in post cerebral vascular accident (Stroke) victims. Cien Saude Colet. 2013;18:2017-2027.

8. Schmid AA, Rittman M. Fear of falling: an emerging issue after stroke. Top Stroke Rehabil. 2007;14:46-55.

9. Batchelor F, Hill K, Mackintosh S,Said C. What works in falls prevention after stroke? A systematic review and meta-analysis . Stroke 2010;41:1715-1722.

10. Eunkyoung Hong..Health-related quality of life of community-dwelling stroke survivors: a comparison of fallers and non-fallers. J Phys Ther Sci. 2015;27:3045-3047.

11. T.C. Sağlık Bakanlığı. İtaki Düşme Riski Ölçeği. http://www.kalite.saglik.gov.tr/TR,13486/itaki-dusme-riski-olcegi.html Erişim Tarihi: 15.06.2017

12. Holden MK, Gill KM, Magliozzi MR. Gait assessment for neurologically impaired patients. Standards for outcome assessment. PhysTher. 1986;66:1530-1539.

13. Küçükdeveci AA, Yavuzer G, Elhan AH, Sonel B, Tennant A. Adaptation of the functional independence measure for use in Turkey. Clin Rehabil. 2001;15:311-319.

14. Beck AT, Ward CH, Mendelson M, Mock N, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-571.

15. Williams LS, Weinberger M, Haris LE, Clark DO, Biler J. Development of a Stroke- Specific Quality of Life, Stroke 1999;30:1362-1369.

16. Gülendam Hakverdioğlu Yönt. İnmeye Özgü Yaşam Kalitesi Ölçeğinin Türk Toplumu İçin Geçerlik Ve Güvenirliğinin İncelenmesi, Hemşirelik Esasları Anabilim Dalı Programı Doktora Tezi. Ege Üniversitesi Sağlık Bilimleri Enstitüsü,2009.

17. Schmid AA, Acuff M, Doster K, Gwaltney-Duiser A, Whitaker A, Damush T et al. Poststroke fear of falling in the hospital setting. Topics in StrokeRehabilitation 2009;16:357-366.

18. Kim EJ, Kim DY, Kim WH, Lee KL, Yoon YH, Park JM, et al. Fear of falling in subacute hemiplegic stroke patients: associating factors and correlations with quality of life. Ann Rehabil Med. 2012;36:797-803.

19. Da Silva PC, Carlegis M, Suchma K, Ostwald SK. Falling, balance confidence and fear of falling after chronic stroke. Stroke Physical and Occupational Therapy in Geriatrics 2014;32:353-367.

20. Chang HT, Chen HC, Chou P. Factors associated with fear of falling among community-dwelling older adults in the Shih-Pai study in Taiwan. PLoSOne. 2016, 11(3):e0150612. doi: 10.1371

21. Hawk C, Hyland JK, Rupert R, Colonvega M,HallS. Assessment of balance and risk for falls in a sample of community-dwelling adults aged 65 and older.Chiropr Osteopat. 2006;14:3.

22. Çınarlı T , Koç Z .65 yaş ve üzeri yaşlılarda düşme risk ve korkusunun günlük yaşam aktiviteleri ve yaşam kalitesi üzerine etkisi. Gümüşhane University Journal of Health Sciences 2015;4:660-678.

23. Yıldırım KY, Karadakovan A. Yaşlı bireylerde düşme korkusu ile günlük yaşam aktiviteleri ve yaşam kalitesi arasındaki ilişki. Türk Geriatri Dergisi 2004;7:78-83.

24. Schmid AA, Van Puymbroeck M, Altenburger PA, Miller KK, Combs SA, Page SJ. Balance is associated with quality of life in chronic stroke.Top StrokeRehabil. 2013;20:340-346.

25. Jung Y, Lee K, Shin S, Lee W. Effects of a multifactorial fall prevention program on balance, gait and fear of falling in post-stroke in patients. J Phys Ther Sci. 2015;27:1865-1868.

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):310-5

Research Article Doi: 10.36472/msd.v6i12.327

Is there any association between antibiotic resistance and virulence genes in *Enterococcus* isolates?

Nergis Aşgın[1]*, Emre Taşkın[2]

Abstract

Objective: In this study, we aim to determine the frequency of antibiotic resistance and five virulence genes in *Enterococcus* species and the relationship between antibiotic resistance and virulence genes.

Material and Methods: A total of 86 *Enterococcus* strains isolated from inpatients between 2015 and 2016 were included. Identification and antibiotic susceptibilities of strains were determined using a BD Phoenix fully automated system. The presence of virulence-associated genes (*esp, gel E, asa1, hyl, and cyl*) were investigated by using PCR method.

Results: Of the 86 *Enterococcus* strains, 53 (61.6%) and 33 (38.4%) were *Enterococcus faecium* and *Enterococcus faecalis*, respectively. Vancomycin and high-level gentamicin resistance (HLGR) in *E. faecalis* strains were 0.6% and 60.6%, respectively. Furthermore, 52 of the 53 *E. faecium* strains were both vancomycin-resistant and HLGR. The frequency of *esp, gel E, asa1, cyl, and hyl* was 91.9%, 60.5%, 54.7%, 43%, and 26.7%, respectively. The *asa 1, cyl, and gel E* genes were detected at high frequencies in vancomycin-susceptible and non-HLGR strains, whereas hyl gene was detected at high frequencies in vancomycin-resistant and HLGR strains.

Conclusion: Virulence genes were more frequent in vancomycin-susceptible and non-HLGR *Enterococcus* strains than in the resistant strains. Although infections caused by multidrug-resistant strains are difficult to treat, it should be considered that susceptible strains have more virulence genes. This may reduce the in vivo efficacy of drugs and lead to treatment failures. Therefore, in addition to the in vitro susceptibilities of drugs, clinical efficacy should be monitored.

Key words: Antibiotic resistance, *Enterococcus faecalis*, *Enterococcus faecium*, virulence gene.

Introduction

Enterococci are Gram–positive, catalase-negative, no spore-forming facultative anaerobic bacteria. They can survive in a wide range of temperature (5°C–65°C) and pH (4.5–10) as well as in the presence of 6.5% NaCl (1,2). Enterococci are part of the human gut microbiota. After gastrointestinal colonization, they can cause blood-stream infections, endocarditis, and intra-abdominal and pelvic infections in critically-ill patients (2,3) In recently, enterococci are the second most common cause of nosocomial wound and urinary tract infections and the third most common cause of nosocomial bacteremia. (1,4,5) The *Enterococcus faecalis* and *E. faecium* are most prevalent species in human diseases (1-4).

Enterococci are intrinsically resistant to cephalosporins, sulfonamides, and low concentrations of aminoglycosides (6-8). In addition, acquired vancomycin and high-level aminoglycoside resistance limit the treatment options.

However, enterococci have various virulence factors such as enterococcus surface protein and aggregation substance that enable adhesion to host, colonization, and biofilm formation (1-3,9). Furthermore, hyaluronidase, cytolysin, and gelatinase enzymes secreted by enterococci contribute to invasion by causing damage to the host tissue (4,8,9).

Some of the genes encoding virulence factors are chromosomal origin, whereas others are subsequently acquired via plasmids (3,9). In this study, we aim to determine the frequency of antibiotic resistance and virulence genes in *Enterococcus* species and the relationship between antibiotic resistance and virulence factors.

Received 16-11-2019 **Accepted** 22-12-2019 **Available Online** 25-12-2019 **Published** 30-12-2019
1 Karabuk University School of Medicine, Dept of Medical Microbiology, Karabuk, TR
2 Karabuk University School of Medicine, Dept of Medical Biology and Genetics, Karabuk, TR
*** Corresponding Author:** Nergis Aşgın **E-mail:** drnasgin@gmail.com

Material and Methods

We included 86 *Enterococcus* strains isolated from rectal swabs and clinical samples of inpatients at Karabuk Training and Research Hospital between January 2015 and December 2016. The strains were stored in tryptic soy broth containing 10% glycerol at −25°C until molecular tests were performed. Only one strain from each patient was included. The ethical approval was obtained from the Non-interventional Clinical Research Ethics Board of Karabuk University (2016 13/3).

Identification and antimicrobial susceptibility testing: Clinical samples sent to the microbiology laboratory were inoculated on Columbia agar supplemented with 5% sheep blood, eosin-methylene blue (EMB) agar, and chocolate agar, whereas rectal swabs were cultured only on Enterococcosel agar. [all, Becton Dickinson (BD) and Company Franklin Lakes, NJ, USA)]. Blood samples were collected in BD BACTEC Plus vials and incubated in Bactec FX 40 (BD, MD, USA) fully automated blood culture system for seven days. The vials that were positive for bacterial growth were cultured on blood agar, EMB agar, and chocolate agar. After incubation for 24–48 h, Gram staining was performed on catalase−negative, pyrrolidinyl aminopeptidase−positive colonies. Colonies that were found to be Gram−positive cocci on microscopic examination were further analyzed for identification and antibiotic susceptibility using a BD Phoenix™ (Becton Dickinson and Company BD, Sparks, MD, USA) fully automated system. Vancomycin resistance was also confirmed using gradient minimum inhibitory concentration (MIC) method with E-test strips (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility test results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (10). *Enterococcus faecalis* ATCC 29212 was used as the quality control strain.

PCR detection of virulence genes: The stock cultures of *Enterococcus* strains were inoculated on 5% sheep blood agar. The pure bacterial culture was obtained after 24 hours of incubation. PCR analysis of each virulence gene was separately performed. The genomic DNA was isolated using the GF-1 Bacterial DNA extraction kit (Vivantis, Malaysia). Previously defined primers were used for the detection of virulence genes and shown in Table 1 (11-13).

The Bioer TC 96 thermal cycler (Hangzhou Bioer, Zhejiang, China) was used for amplification. The amplification conditions were as follows: Initial denaturation for 5 min at 95°C; followed by 40 cycles of denaturation for 1 min at 95°C, annealing for 20 sec at 56°C, extension for 20 sec at 72°C, and final extension for 10 min at 72°C.

The amplified products were separated by electrophoresis for1 h at 100 V in a 1.5 % agarose gel, on 1.5% agarose gel, using an EV231 (Consort, Belgium) device.

Ethidium bromide-stained products were imaged using Syngene™ IG3 (Syngene, Cambridge, UK) gel DOC instrument under UV light. A 100-bp DNA ladder (New England Biolab, Ipswich, MA, USA) was used to compare the band size of each gene. *Enterococcus faecalis* MMH 594, and *Enterococcus faecium* C68 were used as positive control strains [*E. faecalis* MMH 594 *(esp, cyl, asa1* and *gel E* (+) ,*E. faecium C68 hyl* (+)].

Statistical analysis: The data were analyzed using the Minitab 17 (Minitab, Inc., PA, USA) statistical software program. The Anderson Darling test was performed to determine whether the data were normally distributed. Descriptive statistics were shown as numbers and percentages. The correlation between virulence genes and antibiotic resistance of strains was calculated using Chi-square test. A *P*- value ≤ .05 was considered statistically significant.

Results

The 86 *Enterococcus* species were obtained from rectal swab (n=41), blood (n=22), urine(n=19) and wound (n=4) samples. The 53 (61.6%) strains were *E. faecium* and, 33 (38.4%) strains were *E. faecalis.* Moreover, 54 (62.8%) and 72 (83.7%) strains were resistant to vancomycin and high-levels of gentamicin, respectively.

The frequency of vancomycin resistance and HLGR in *E. faecalis* were 0.6% (2/33) and 60.6% (20/33), respectively; 52 (98.1%) of *53 E. faecium* were vancomycin-resistant and HLGR. Of the 86 strains, 91.9 % (n=79) carried *esp* gene. This was followed by 60.5% *gel E* (n=52), 54.7% *asa1* (n=47), 43% *cyl* (n=37) , and 26.7% *hyl* (n= 23).

The distribution of virulence genes in *Enterococcus* species has been shown in Table 2. The *esp* gene was detected in >90% strains in both species (*P* = .88).

However, *asa1*, *cyl*, and *gel E* virulence genes were significantly more frequent in *E. faecalis* strains than in *E. faecium* strains (*P* < .001). Furthermore, 90.9% of *E. faecalis* strains carried *asa*1, whereas this rate was 32% for *E. faecium* strains (*P* < .001). Similarly, *gel E* was more frequent in *E. faecalis* strains, and the rates were 84.8% and 45.3% in *E. faecalis* and *E. faecium*, respectively. In addition, *cyl* gene was detected in 78.8% of *E. faecalis* strains and 20.8% of *E. faecium* strains (*P* < .001). In contrast, *hyl* gene was significantly more frequent in *E. faecium* strains than in *E. faecalis* strains (39.6% vs. 6%, *P* < .001).

The distribution of virulence genes in HLGR and non-HLGR strains has been shown in Table 4. The hyl gene was significantly more frequent in HLGR strains, whereas *asa1*, *cyl*, and *gel E* genes were significantly more frequent in non-HLGR strains.

Table 1. Primer sequences used for the amplification of the target genes.

Virulence factor	Target gene	Primer sequences (5'-3')	Amplicon size (bp)	Reference
Aggregation substance	***asa1***	GCACGCTATTACGAACTATGA	375	[11]
		TAAGAAAGAACATCACCACGA		
Gelatinase	***gel E***	CGA AGT TGG AAA AGG AGG C	372	[13]
		GGT GAA GAA GTT ACT CTG A		
Enterococcal surface protein	***esp***	AGATTTCATCTTTGATTCTTGG	510	[11]
		AATTGATTCTTTAGCATCTGG		
Hyaluronidase	***hyl***	CCCTGGACACATGAAATGCG	605	[12]
		AGCATCGGCCGTTGATAGAC		
Cytolysin	***cyl***	ACTCGGGGATTGATAGGC	688	[11]
		GCTGCTAAAGCTGCGCTT		

Table 2: Distribution of virulence genes among *Enterococcus* species (n %)

Virulence gene	*E. faecalis* (n=33)	*E. faecium* (n=53)	*P*-value
asa1	30 (90.9)	17 (32.1)	< .001
cyl	26 (78.8)	11 (20.8)	< .001
esp	30 (90.9)	49 (92.5)	.88
gel E	28 (84.8)	24 (45.3)	< .001
hyl	2(6.0)	21 (39.6)	< .001

Table 3: Distribution of virulence genes among *VRE and **VSE enteroccal strains (n %)

Virulence gene	VRE (n=54)	VSE (n=32)	*P*-value
	n %	n %	
asa1	17 (32.1)	30 (90.9)	< .001
cyl	11 (20.8)	26 (78.8)	< .001
esp	50 (94.3)	29 (87.8)	.74
gel E	24 (45.3)	28 (84.8)	< .001
hyl	21 (39.6)	2 (6.0)	< .001

*VRE: Vancomycin-resistant enterococcus. **VSE:Vancomycin-sensitive enterococcus.

Table 4: Distribution of virulence genes among *HLGR and non HLGR enterococcal strains (n %)

Virulence gene	HLGR (n=72)	non HLGR (n=14)	*P*-value
	n %	n %	
asa1	34 (47.2)	13 (91.6)	.002
cyl	24 (33.3)	13 (91.6)	<.001
esp	66 (91.7)	13 (91.6)	.88
gel E	40 (55.5)	12 (85.7)	.02
hyl	22 (30.6)	1 (7.1)	.04

*HLGR: High level gentamicin resistant

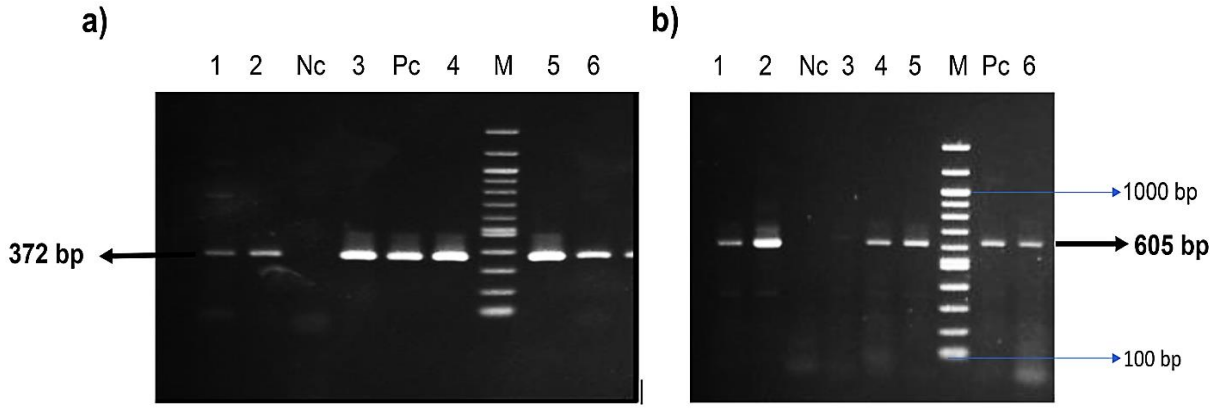


Figure 1. a) Agarose gel electrophoresis images of ***gel E*** (372 bp) in *Enterococcus* strains. Note: Nc: Negative control (ultrapure H_2O). Pc: Positive control (*E. faecalis* MMH 594) M:100 bp DNA Ladder (New England Biolabs, USA) **b)** Agarose gel electrophoresis images of ***hyl*** (605 bp) in *Enterococcus* strains. Note: Nc: Negative control (ultrapure H_2O). Pc: Positive control (*E. faecium* C68) M:100 bp DNA Ladder (Product code :N3231S, New England Biolabs, USA)

http://dx.doi.org/10.36472/msd.v6i12.327

Discussion

Although enterococci are members of the normal human intestinal flora, they cause severe infections such as sepsis, endocarditis, and intra-abdominal abscess following gastrointestinal colonization in patients hospitalized for a long time, exposed to invasive intervention, and those using broad-spectrum antibiotics (2,3,6,7). Enterococcus faecalis is the most frequently isolated species in clinical specimens, and a high level of antibiotic resistance is common in E. faecium strains (2,7,14)

In the present study, 53 (61.6%) and 33 (38.4%) of the 86 Enterococcus strains were identified as E. faecium and E. faecalis, respectively. The majority of clinical isolates of E. faecalis and E. faecium strains were vancomycin-resistant that were isolated from the rectal swabs (n = 41). Vancomycin resistance and HLGR were 62.7% and 83.7% in E. faecium strains and 0.6% and 60.6% in E. faecalis strains, respectively. Consistent with our results, several studies from Turkey have also reported higher rates of vancomycin resistance and HLGR in E. faecium strains (15-17).

The esp was the most frequently virulence gene encoding the enterococcal surface protein. In 1999, the enterococcal surface protein was first identified in gentamicin resistant E. faecalis species (18). The esp gene secreted from the pathogenicity islands of both E. faecalis and E. faecium (1,3,18). It is associated with adhesion, invasion, and evasion (9,19). In addition, it contributes to biofilm formation. Biofilm formation is closely-associated with the development of endocarditis and urinary tract infection (1,9,19).

In the present study, esp was detected in 90.9% and 92.5% of E. faecalis and E. faecium strains, respectively (P = 0.8). Studies conducted in Turkey have reported esp frequencies of 25.6%–87% (15-17,20-22). The high frequency of esp, might be due to regional differences and the collection date of the strains. In enterococci isolates, the acquisition of virulence genes increases in time parallel to the increase in antibiotic resistance. The prevalence of esp gene reported in other countries were 46.6% in Iran, 76% in Brazil, 81.5% in Australia, and 100% in Trinidad (23-26). Vankerckoven et al. reported that the frequency of esp gene was 65% in 271 E. faecium isolates collected from European countries (11). Udo et al. reported a lower rate (31.5%) in 466 E. faecalis isolates collected from eight hospitals in Kuwait (19). Oancea et al. reported that esp gene is transferred among the enterococcal species via the conjugative plasmid in vitro (27). We detected esp gene in >90% strains in both species, supporting this finding.

In the present study, gel E gene was the second most common virulence gene (60.5%). The gel E is of chromosomal origin and encodes gelatinase, also known as a metalloprotease that hydrolyze small peptides such as collagen, elastin, and casein. Thus, it provides nutrients to the bacteria and contributes to invasion (1-3). In this study, the frequency of gel E gene was 84.8% in E. faecalis and 45.3% in E. faecium strains (P: 0.000). Similarly, Al Taib et al. detected gel E in 76.5% and 66.7% of E. faecalis and E. faecium strains, respectively, isolated in Malaysia (13). The frequency of gel E gene is between 0% and 22.8% in studies conducted in Turkey (15-17,21). However, a higher frequency (0%–78%) is reported in studies around the world (13,19,24,25). The *gel E* gene among representative strains is shown in Figure 1.

In the current study, asa1 gene, encoding the aggregation substance, was the third most common virulence gene with a frequency of 54.7%. Aggregation substance is a glycoprotein expressed on the surface of *E. faecalis*. It provides contact between cells during adherence to eukaryotic cells and conjugation. It increases the adhesion of *enterococci* to the surfaces of neutrophils, endocardial and, renal epithelial cells (2,3,9). Besides, it increases the hydrophobicity of the enterococcal surface and enables escape from the immune system by preventing the fusion of lysosomal vesicle and phagosome (3,11,19).

The asa1 gene was found to be significantly more frequent in *E. faecalis* strains than in *E. faecium* strains (90.9% vs. 32.1%, respectively, P = 0.000). Similarly, Baylan et al. (20) and Coşkun (16) from Turkey also detected asa1 gene in *E. faecalis* strains at significantly higher rates. In a study conducted in Iran, Hasani et al. reported the prevalence of asa1 as 92.4% in *E. faecalis* and 7.6% in *E. faecium* (28). In the present study, *asa1* was significantly more frequent in vancomycin-susceptible strains than vancomycin-resistant strains (90.9% vs. 32%, respectively, P = 0.00). This can be attributed to the fact that vancomycin-susceptible strains are mostly *E. faecalis* and vancomycin-resistant strains are mostly *E. faecium*. Mete et al. did not detect asa1 in vancomycin-resistant strains (15). The *asa1* was significantly more frequent in non-HLGR strains than in HLGR strains (91.6% vs. 47.2%, respectively, P = 0.002). In contrast, Al Hasan et al. found *asa1* at similar rates in HLGR (54.1%) and non-HLGR strains (52.8%) in 220 enterococcal isolates in Iran (28). The frequency of *asa1* in enterococcal strains of human origin is between 11.2% and 45% in Turkey (15-17,20). In other studies, *asa1* frequency was reported to be 38% in Brazil, 54.3% in Iran, 79% in Sweden, and 100% in China (4,24,29,30). In a study conducted in Australia, Worth et al. did not detect asa1 gene in vancomycin-resistant *E. faecium* strains (25).

Cytolysin is a toxin whose expression is under the control of eight genes defined as cyl (15,19). Cytolysin is hemolysis in the human, rabbit, and horse erythrocytes. However, it is not active against to sheep erythrocytes (1,3,15). Moreover, it is a bacteriocin that shows bactericidal effect against many Gram-positive bacteria. *Cyl* has a lytic effect in retinal and intestinal cells as well as macrophages and neutrophils (1,3). The *cyl* genes are transmitted via plasmids (3). However, cyl genes can also be found in the pathogenicity islands integrated into the bacterial chromosome (3,15). In the present study, the frequency of *cyl* gene was 78.8% in *E. faecalis* strains and only 20.8% in E. faecium strains (P = 0.000). Additionally, the frequency of *cyl* gene was extremely high in vancomycin-susceptible and non-HLGR strains. The frequency of *cyl* gene was reported to be 6.9%–33.2% in Turkey (15-17), 13% in Sweden (29), and 30.4% in Iran (4).

The *hyl* gene encoding the hyaluronidase enzyme is found especially in E. faecium species and causes tissue damage by degrading the hyaluronic acid (3). Furthermore, disaccharides formed as a result of hyaluronic acid degradation may be a source of food in bacteria. (3,12,19) In the present study, we detected hyl gene at a frequency of 26.7%. The *hyl* gene among representative strains has been shown in Figure 1. This frequency was 39.6% in *E. faecium* strains and 6% in E. faecalis strains (P = 0.000). Coşkun (16) and Çopur et al. (17) reported the frequency of *hyl* gene as 8.7% and 12.9% in Turkey. On the other hand, Gozalan et al. did not detect *hyl* gene in any of the 55 vancomycin-resistant *E. faecium* isolates (21). In other studies, *hyl* gene frequency was reported to be 2.4% in Australia (25), 27.5% in China (30), and 35.4% in Iran (12). Bilström et al. reported a frequency of 4% in 267 *E. faecium* isolates in Sweden (8).

There are some limitations of the study. The study was conducted at a single center and had a small sample size. Furthermore, vancomycin-resistance genes were not investigated using molecular methods.

Conclusion

We detected *esp* gene in >90% *Enterococcus* species. The *asa1, cyl, and gel E* genes were significantly more frequent in *E. faecalis* strains, whereas *hyl* gene was significantly more frequent in *E. faecium* strains. Vancomycin resistance and HLGR were lower in *E. faecalis* strains, but virulence genes (except *hyl*) were more common. All virulence genes (except *hyl*) studied in vancomycin-susceptible and non-HLGR strains were more frequent than those in vancomycin-resistant and HLGR strains. The limited of treatment options for multidrug-resistant enterococci infections is a great concern. Vancomycin -susceptible and non-HLGR strains have multiple virulence factors, which may reduce the *in vivo* efficacy of antibiotics, leading to treatment failures. Therefore, clinical efficacy as well as *in vitro* efficacy of antibiotics used in the treatment of enterococcal infections should be monitored.

Acknowledgements: The authors would like to thank Professor Baris Otlu for providing positive control strains (*E. faecalis* MMH 594 *and E. faecium* C68).

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: NA, ET; Research concept and design, Research the literature, ET; Genetic analyses NA; preparation of the article, Revision of the article.

References

1. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology. 2009;155(6):1749–57.

2. Cesar AA, Murray BE. Enterococcus species, Streptococcus gallolyticus Group, and Leuconostoc Species. In: Bennet JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia (PA): Elsevier Inc.; 2015.p. 2328–39.

3. Baylan O.Virulence Factors And Immunopathogenesis In Enterococcal Infections. Nobel Med. 2019; 15(2):5–16.

4. Heidari H, Emaneini M, Dabiri H, Jabalameli F. Virulence factors, antimicrobial resistance pattern and molecular analysis of enterococcal strains isolated from burn patients. Microb Pathog. 2016; 90:93–97

5. Guzman Prieto AM, van Schaik W, Rogers MRC, et al. Global emergence and dissemination of enterococci as nosocomial pathogens: Attack of the clones? Front Microbiol. 2016;7(5):1–15.

6. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. Virulence. 2012;3(5):421–33.

7. Cetinkaya Y, Falk P, Mayhall C. Vancomycin-Resistant Enterococci. Clin Microbiol Rev.2000;13(4):86–707.

8. Billström H,Lunda B, Sullivana A, Nord CE.Virulence and antimicrobial resistance in clinical Enterococcus faecium. Int J Antimicrob Agents. 2008; 32(5):374–77.

9. Fiore E, Van Tyne D, Gilmore M. Pathogenicity of Enterococci. Microbiol Spectr. 2019. 7(4):GPP3-0053-2018.

10. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.1, 2015. http://www.eucast.org. Accession date: 10 October 2019.

11. Vankerckhoven, V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, et al. Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium. J. Clin. Microbiol. 2004. 42(10): 4473–79.

12. Soheili S, Ghafourian S, Sekawi Z, Neela V, Sadeghifard N, Ramli R et al. Wide distribution of virulence genes among Enterococcus faecium and Enterococcus faecalis clinical isolates. The Scientific World Journal, 2014. http://dx.doi.org/10.1155/2014/623174

13. Al-Talib, Zuraina N, Kamarudin B,Yean CY. Genotypic variations of virulent genes in Enterococcus faecium and Enterococcus faecalis isolated from three hospitals in Malaysia. Adv Clin Exp Med. 2015; 24(1):121–27.

14. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015; 8:217–230.

15. Mete E, Kaleli I, Cevahir N, Demir M, Akkaya Y, Kiris Satilmis O. Evaluation of Virulence Factors in Enterococcus Species. Microbiol Bul. 2017; 51(2):101–14.

16. Say Coskun US. Investigation of the relationship between virulence factors and antibiotic resistance of Enterococci isolates. Cell Mol Biol. 2019, 65(2):114–17.

17. Baylan O, Nazik H, Bektöre B, Çitil BE, Turan D, Öngen B et al. The relationship between antibiotic resistance and virulence factors in urinary Enterococcus isolates. Mikrobiyol Bul. 2011; 45(3): 430–45.

18. Shankar V, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS. Infection-derived Enterococcus faecalis strains are enriched in esp, a gene encoding a novel surface protein. Infect. Immun. 1999;67(1): 93–200.

19. Udo EE, Al-Sweih N. Frequency of virulence-associated genes in Enterococcus faecalis isolated in Kuwait hospitals. Med Princ Pract. 2011; 20(3): 259–64.

20. Copur SS, Sahin F, Gocmen JS. Determination of virulence and multidrug resistance genes with polymerase chain reaction method in vancomycin-sensitive and -resistant enterococci isolated from clinical samples. Turk J Med Sci. 2016: 46(3): 877–91.

21. Gozalan A, Coskun-Ari FF, Ozdem B, Unaldi O, Celikbilek N, Kirca F et al. Molecular characterization of vancomycin-resistant Enterococcus faecium strains isolated from carriage and clinical samples in a tertiary hospital, Turkey. J Med Microbiol. 2015;64(7):759–66.

22. Cakirlar FK, Samasti M, Baris I, Kavakli H, Karakullukcu A, Sirekbasan S. et al. The epidemiological and molecular characterization of vancomycin-resistant enterococci isolated from rectal swab samples of hospitalized patient in Turkey. Clin Lab. 2014; 60(11):1807–12.

23. Arshadi M, Mahmoudi M, Motahar MS, Saber Soltani S, Pourmand MR. Virulence Determinants and Antimicrobial Resistance Patterns of Vancomycin-resistant Enterococcus faecium Isolated from Different Sources in Southwest Iran. Iran J Public Health. 2018;47(2):264–72.

24. Comerlato CB, Resende MC, Caierao J, d'Azevedo PA. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. Mem Inst Oswaldo Cruz 2013; 108(5): 590–95.

25. Worth LJ, Slavin MA, Vankerckhoven V, Goossens H, Grabsch EA, Thursky KA. Virulence determinants in vancomycin-resistant Enterococcus faecium vanB: clonal distribution, prevalence and significance of esp and hyl in Australian patients with haematological disorders. J Hosp Infect 2008; 62(2):137–44.

26. Akpaka P, Kissoon S, Jayaratne P. Molecular Analysis of Vancomycin-Resistant Enterococci Isolated from Regional Hospitals in Trinidad and Tobago. Advances in Medicine. 2016, 1-8. http://dx.doi.org/10.1155/2016/876269.

27. Oancea C, Klare I, Witte W,Werner G. Conjugative transfer of the virulence gene, esp, among isolates of Enterococcus faecium and Enterococcus faecalis. J Antimicrob Chemother. 2004;45(1):232–35.

28. Hasani A, Sharifi Y, Ghotaslou R, Naghili B, Hasani A, Aghazadeh M, et al. Molecular screening of virulence genes in high-level gentamicin-resistant Enterococcus faecalis and Enterococcus faecium isolated from clinical specimens in Northwest Iran. Indian J Med Microbiol. 2012;30:175–81.

29. Hällgren A, Claesson C, Saeedi B, Monstein HJ, Hanberger H, Nilsson LE. Molecular detection of aggregation substance, enterococcal surface protein, and cytolysin genes and in vitro adhesion to urinary catheters of Enterococcus faecalis and Enterococcus faecium of clinical origin. Int J Med Microbiol. 2009;299(5): 323–32.

30. Yang JX, Li T, Ning YZ, Shao DH, Liu J, Wang S, Liang GW. Molecular characterization of resistance, virulence and clonality in vancomycin-resistant Enterococcus faecium and Enterococcus faecalis: A hospital-based study in Beijing, China. Infect Genet Evol. 2015;33:253–60.

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):316-20

Research Article Doi: 10.36472/msd.v6i12.328

The effect of various treatment modalities in idiopathic sudden sensorineural hearing loss: A retrospective evaluation

Şeyda Belli[1]*

Abstract

Objective: In this study, we retrospectively evaluated the effects of treatment modalities on healing in patients diagnosed with idiopathic sudden sensorineural hearing loss.

Material and Method: In this study, we retrospectively reviewed the records of 65 patients diagnosed with idiopathic sudden sensorineural hearing loss and treated as inpatients. The treatment modalities applied to the patients were determined randomly.

Results: The effect of intra-tympanic steroid and / or hyperbaric oxygen therapy in addition to intravenous steroid on 500, 1000, 2000 and 4000 Hz frequencies and improvement in bone conduction was not statistically significant.

Conclusion: Although intra-tympanic steroid or hyperbaric oxygen therapy in addition to intravenous steroid therapy, which is the standard treatment of idiopathic sudden sensorineural hearing loss, does not increase the rate of improvement and speed, it may help to reduce the undesirable effects of the steroid dose.

Key Words: Idiopathic sudden sensorineural hearing loss, intravenous steroid, intra-tympanic steroid, hyperbaric oxygen treatment

Introduction

Idiopathic sudden sensorineural hearing loss (ISSHL) is a sudden decrease in sensorineural hearing sensitivity of unknown etiology (1, 2). For ISSHL, hearing impairment may range from 30 dB at three frequencies to 120 dB losses at higher frequencies (3). ISSHL is diagnosed when an audiometry approves 30 decibel (dB) hearing loss at three consecutive frequencies and no underlying condition has been detected (3). Several theories explaining ISSHL have been recommended, including viral infection (4), vascular occlusion, and breakage of the labyrinth membranes, immune-mediated mechanisms, and abnormal cellular stress responses within the cochlea. However, none of these hypotheses has been convincingly proven (5). For smaller losses, natural route may be favorable due to limited repair capacity of cochleas; however, the chance of full recovery in deep cases is quite low (6).

The treatment of patients with ISSHL continues to vary between autological centers. There is no universally accepted standard protocol. The currently accepted treatment for ISSHL is the use of systemic steroids (7), despite the result of the Cochrane meta-analysis, which claims that the efficacy of steroids remains unproven in the treatment of ISSHL (8).

Intra-tympanic steroid injection and hyperbaric oxygen therapy are also used in combination with oral steroid therapy or alone, in many otology clinics for different treatment methods of ISSHL (9).

Possible side effects of systemic steroids limit its use in treatment in some cases. In 2000, intra-tympanic administration of steroid directly into the middle ear began to be used in the treatment of ISSHL. In this method; since it is delivered directly to the middle ear by trans-tympanic and transmitted to the inner ear through a round window, the steroid concentration in the perilymph can reach higher levels than in systemic use and may be of greater benefit (10,11,12,13,14).

The cochlea is an organ with impressive activity; therefore, it is always dependent on adequate oxygen levels in the blood (15). However, due to the protected position of the cochlea in the temporal bone, blood supply is limited (16). One of the possible mechanisms that play a role in ISSHL is of vascular origin: oxygen deficiency. This vascular etiology was defined by Ruben et al. in ISSHL (17). Some authors have also found a vascular etiology for sudden deafness (18,19,20,21,22). Hyperbaric oxygen treatment (HBOT) can increase the oxygen load to the cochlea by eliminating hypoxia (23).

Received 20-11-2019 **Accepted** 23-12-2019 **Available Online** 25-12-2019 **Published** 30-12-2019
1 University of Health Science, Bagcılar Research and Education Hospital, Dept. of Otorhinolaryngology, Istanbul, TR
*** Corresponding Author:** Şeyda Belli **E-mail:** drseydabelli@gmail.com

In recently, HBOT has been used for ISSHL; however, due to the lack of randomized controlled trials, the role of HBOT in the treatment protocol for acute hearing loss remains unclear (23).

In this study, we aimed to compare the efficacy of intra-tympanic steroid or HBOT in addition to intravenous steroid and intravenous steroid in patients hospitalized in our clinic.

Material and Methods

The study was approved by the local Ethics Committee and carried out in accordance with the ethical principles described by the Helsinki Declaration (2019.10.2.04.077).

This study; the patient presented to our clinic within the first 10 days after the onset of his complaints, between January 2017- December 2019. The diagnosis of ISSHL was made by audiometric examination and hospitalized and treated. The records of 65 patients without tumor diseases were retrospectively reviewed. Diabetic patients, concomitant disease with contraindicated steroid treatment, bilateral sudden hearing loss, patients with hearing loss from the onset of treatment more than 10 days were excluded from the study.

Routine examinations for patients and investigations (blood tests, pure tone audiogram, speech audiometry, acoustic impedance and radiological imaging examinations). Included in the study the patients age, gender, risk factors, hearing loss presence of concomitant tinnitus and / or vertigo; and treatments were evaluated.

The choice of treatment applied to patients; patients which referred to the clinic and the start of treatment randomly determined by day. Patients were divided into three groups: systemic steroid therapy group, steroid therapy and intra-tympanic steroid therapy group and hyperbaric oxygen therapy and systemic steroid treatment group.

All patients received intravenous steroid (1mg/kg metilprenisolone) treatment. In addition to intravenous steroid treatment, 10 patients received 1cc intra-tympanic steroid treatment (1 cc metilprednisolone) once a day for 5 days. In addition to intravenous steroid treatment, the number of patients receiving HBOT in 20 sessions was 14. Hearing tests were performed by the same audiologist. In pure tone audiometry, pure tone thresholds were examined at 500-1000-2000-4000 Hertz (Hz) frequencies.

In audiological examinations before and after treatment, the threshold frequency of 500, 1000, 2000 and 4000 and the resulting SSO changes were found in all three groups. These differences were also analyzed according to factors such as age, sex, vertigo, tinnitus, time to start treatment.

Statistical Analyses: Mean, standart deviation, median, minimum and maximum values were given for the statistical definition of the groups. In comparison of groups, Independent t-test, a parametric test, was used for variables with normal distribution. For the variables that do not show normal distribution, the Mann-Whitney U test, which is an anti-parametric Independent t-test, was used. The significance of the difference between the groups was evaluated at $p < 0.05$.

Results

The mean age of the intravenous steroid group, intravenous steroid+intra-tympanic steroid group and intravenous steroid+hyperbaric oxygen treatment group were 44.07± 12.13, 52.18± 18.01 and 39.5±18.45, respectively. There was no difference between the three groups in terms of age, sex, time to sudden onset of hearing loss (Table 1). When tinnitus and dizziness were questioned, there was no significant difference between the groups (Table 2).

Bone pathway improvement was observed in audiometric tests in all treatment groups compared to pretreatment, and a statistically significant difference was observed (Table 3). 500, 1000, 2000 and 4000 Hz frequencies separately examined bone pathway improvement intravenous steroid and intravenous steroid + hyperbaric O2 treatment were statistically significant compared to pre - treatment. Intravenous steroid + intra-tympanic steroid treatment areas only bone at a frequency of 2000 Hz pathway was not statistically significant, but it was statistically significant at other frequencies (Table 3).

Bone path thresholds; In 41 patients receiving intravenous steroid therapy, a mean improvement of 17.68 dB; intravenous steroid + intra-tympanic steroid treatment in 10 patients, mean 17.63 dB improvement; intravenous steroid + HBOT in 14 patients, mean 16.52 dB improvement was seen. There was no significant difference between the three treatment groups in terms of improvement in bone pathway (Table-4).

Table 1: Evaluation of the groups in terms of age, sex and time to sudden onset of hearing loss

	IV steroid group (n:41)	IVsteroid+Intra-tympanic steroid group (n:10)	IVsteroid+Hyperbaric oxygen group (n:14)	p
Age	44,07± 12,13	52,18± 18,01	39,5±18,45	0,111
Sex Male	19 46,34%	5 50,00%	8 57,14%	0,783
Female	22 53,66%	5 50,00%	6 42,86%	
Time to sudden onset of hearing loss	3,39±2,11	4,2±2,53	5±4,9	0,208

Table 2: Accompanying symptoms and affected ear

		IV steroid group (n:41)		IVsteroid+Intra-tympanic steroid group (n:10)		IVsteroid+Hyperbaric oxygen group (n:14)		p
Tinnitus	No	3	7,32%	1	10,00%	1	7,14%	
	Yes	38	92,68%	9	90,00%	13	92,86%	0,956
Dizziness	No	31	75,61%	8	80,00%	9	64,29%	
	Yes	10	24,39%	2	20,00%	5	35,71%	0,630
Side	Right	23	56,10%	2	20,00%	5	35,71%	
	Left	18	43,90%	8	80,00%	9	64,29%	0,082

Table 3: Bone pathway improvement was observed in audiometric tests in all treatment groups compared to pretreatment

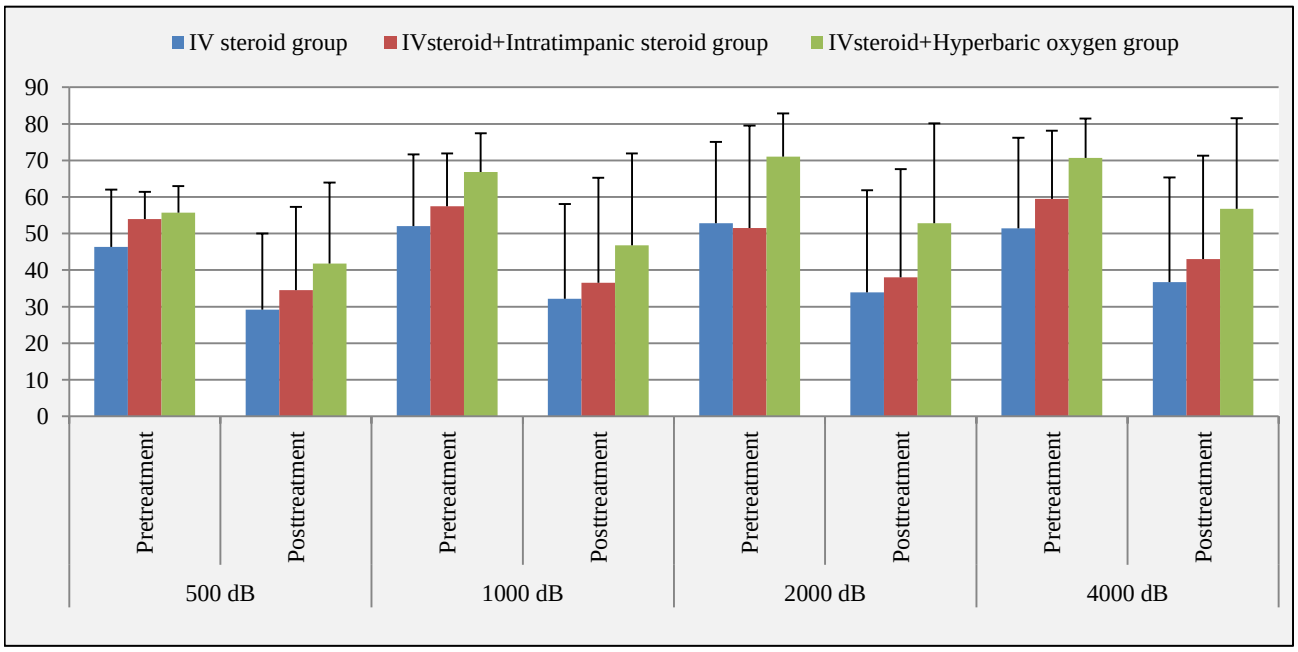


Table 4: Improvement rates according to average and speech frequencies in all groups

	IV steroid group (n:41)	IVsteroid+Intra-tympanic steroid group (n:10)	IVsteroid+Hyperbaric oxygen group (n:14)	
Average improvement (dB)	17,68±16,31	17,63±18,42	16,52±19,82	0,976
Improvement per frequency 500	17,2±16,17	19,5±18,78	13,93±21,68	0,738
Improvement per frequency 1000	19,88±19,28	21±21,32	20±21,39	0,987
Improvement per frequency 2000	18,9±21,05	13,5±21,22	18,21±19,96	0,763
Improvement per frequency 4000	14,76±17,43	16,5±22,37	13,93±21,68	0,948

Discussion

Successful treatment of a disease depends on the underlying cause of etiopathogenesis. Although there are many hypotheses about the causes of ISSHL, it is still unclear at present (24). In the study conducted by Chau et al., 71% idiopathic, 13% infection, 5% primary otologic events, 4% trauma, 3% vascular and hematologic, 2% neoplastic and 2% other causes were detected in the etiology (25).

In the treatment of ISSHL; many different treatment methods are used such as steroids, antivirals, vitamins, antioxidants, vasodilators, heparin, HBOT and intra-tympanic steroids. Systemic steroid therapy is the most frequently used and accepted treatment for sudden hearing loss (25,26).

In sudden hearing loss, the inflammatory response occurs as part of pathophysiology and therefore it is considered necessary to stop the inflammatory response (9). In the treatment of ISSHL, the study on the use of corticosteroids was conducted by Wilson and his colleagues, and according to the group used in the placebo, improvement in the corticosteroid group was statistically significant (27). Moskowitz and Cole have also reported that systemic steroid use is successful in the detection of ISSHL (28,29). In our study, the improvement was statistically significant in the group where we used intravenous steroids.

Inflammatory response plays a role in the pathophysiology of sudden hearing loss (9). Therefore, it is necessary to stop the inflammatory reaction.

Corticosteroids increase oxygen consumption by mobilizing amino acids for gluconeogenesis. This reduces the partial oxygen pressure in the perilymph. This has shed light on the literature in the literature investigating the combined use of HBOT and corticosteroids. In the study of D'Aldin et al., combination therapy was found to be statistically more successful than the control group using only corticosteroids (30). Alimoglu et al. have achieved higher hearing gains in patients treated with combination therapy compared to monotherapies (31). In another study conducted by Capuano et al. reported that combination therapy with HBOT and intravenous corticosteroids had significantly higher mean gains at 0.5, 1, 2, and 4 kHz compared to both HBOT and intravenous corticosteroids as monotherapies in ISSHL patients (32). In our study, in the group where we administered intravenous steroid and HBOT, hearing gains were found to be statistically significant compared to the pre-treatment group.

Intra-tympanic steroid therapy is recommended as a redemptive treatment in cases where it cannot be used in the literature due to side effects of systemic steroid therapy (1,7,14). It is also reported that intra-tympanic steroid administration will help reduce the dose of systemic steroids to be used (10). In the study conducted by Kargin Kaytez et al., the patient who administered intra-tympanic steroid therapy in combination with systemic steroids, only received systemic steroid therapy, and the recovery started earlier, but in the long term hearing there was no statistically significant difference between earnings (10). Bae et al. reported that they could not find a statistically significant difference in recovery between the group given by the intra-tympanic steroid in combination with systemic steroids and the group in which they gave a single systemic steroid (33). In their study, Battaglia et al. found improvement higher in the study where intra-tympanic steroids were combined with systemic steroids, but this difference was not statistically significant (34). In our study, the improvement in 2000 Hz frequencies was not statistically significant in the group where the intra-timpanic steroid was given in combination with systemic steroids, and the improvement in other frequencies was statistically significant. In line with the literature, we could not find a statistically significant difference between the combined treatment group and the combined treatment group compared only to the group given systemic steroids.

Cho et al. compared the group receiving systemic steroids and intra-tympanic steroids and the group receiving additional HBOT for this treatment, and the higher improvement in discrimination scores in the group receiving additional HBOT but did not detect this improvement at a statistically significant level in terms of hearing thresholds (35). In our study, we found no significant difference between the two groups, although the improvement in hearing thresholds was statistically significant in both treatment groups compared to pre-treatment. Overall hearing improvement was higher for patients treated with HBOT and systemic steroids than those treated with only systemic steroids or systemic and intra-tympanic steroids. In our study, the rate of improvement between all three groups was not statistically significant.

Conclusion

In this study, we retrospectively examined the effect of intra-tympanic steroids and HBOT in addition to basic systemic steroid therapy. However, the differences between the numbers of groups led to a limitation in the statistical evaluation of our results. It is more appropriate to refresh this study with larger sample groups.

Consequently, systemic steroid therapy is the main treatment for ISSHL. In addition to systemic steroid therapy, the addition of intra-tympanic steroids and/or HBOT may result in further improvements in hearing thresholds, although not statistically significant. It also reduces the likelihood of side effects by providing lower doses of systemic steroids.

Author Belli S declares that she has no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: SB; Research concept and design, Research the literature, preparation of the article, Revision of the article.

References

1. Levy E, Sela E, Letichevsky V, Ronen O. Nationwide Survey of Intratympanic Steroids for the Management of Sudden Sensorineural Hearing Loss. Isr Med Assoc. 2019 Feb;21(2):105-109. PMID: 30772961

2. Plontke SK, Bauer M, Meisner C. Comparison of pure tone audiometry analysis in sudden hearing loss studies: lack of agreement for different outcome measures. Otol Neurotolog 2007; 28 (6): 753-63. DOI: 10.1097/mao.0b013e31811515ae PMID: 17948355

3. Stachler RJ, Chandrasekar SS, Archer SM, Rosenfeld RM, Schwartz SR, Barrs DM, et al. Clinical practice guideline: sudden hearing loss. Otolaryngol Head Neck Surg 2012; 146: S1-35. DOI: 10.1177/0194599812436449.

4. Blum A, Simsolo C. Acute unilateral sensorineural hearing loss due to H1N1 infection. IMAJ 2010; 12 (7): 450. PMID: 20862833

5. Merchant SN, Adams JC, Nadol JB Jr. Pathology and pathophysiology of idiopathic sudden sensorineural hearing loss. Otol Neurotolog 2005; 26 (2): 151-60. DOI: 10.1097/00129492-200503000-00004 PMID: 15793397

6. Ryan AF, Kujawa SG, Hammill T, Le Prell C, Kil J. Temporary and permanent noise-induced threshold shifts: a review of basic and clinical observations. Otol Neurotol. 2016;37(8):e271–e275. DOI: 10.1097/MAO.0000000000001071.

7. Spear SA, Schwart SR. Intratympanic steroids for sudden sensorineural hearing loss: a systematic review. Otolaryngol Head Neck Surg 2011; 145 (4): 534-43. DOI: 10.1177/0194599811419466.

8. Wei BP, Stathopoulos D, O'Leary S. Steroids for idiopathic sudden sensorineural hearing loss. Cochrane Database Syst Rev 2013 Jul 2;(7). CD003998. DOI:10.1002/ 14651852.CD003998.pub3

9. Bayoumy AB, de Ru JA. The use of hyperbaric oxygen therapy in acute hearing loss: a narrative review. Eur Arch Otorhinolaryngol. 2019 Jul;276(7):1859-1880. DOI: 10.1007/s00405-019-05469-7.

10. Kargın Kaytez S, Şafak MA, Demirci M. The effect of combined use of ıntratympanic steroid theraphy on sudden hearing loss. KBB ve BBC Dergisi 2019;27(2):51-6. DOI: 10.24179/kbbbbc.2019-65586

11. Kopke RD, Hoffer ME, Wester D, O'Leary MJ, Jackson RL. Targeted topical steroid therapy in sudden sensorineural hearing loss. Otol Neurotol. 2001;22(4):475-9. DOI: 10.1097/00129492-200107000-00011 PMID: 11449103

12. Chandrasekhar SS. Intratympanic dexamethasone for sudden sensorineural hearing loss: clinical and laboratory evaluation. Otol Neurotol. 2001;22(1):18-23. DOI: 10.1097/00129492-200101000-00005 PMID: 11314710

13. Kiliç R, Safak MA, Oğuz H, Kargin S, Demirci M, Samim E, et al. Intratympanic methylprednisolone for sudden sensorineural hearing loss. Otol Neurotol. 2007;28(3):312-6. DOI: 10.1097/MAO.0b013e31802fba7a PMID: 17414035

14. Haynes DS, O'Malley M, Cohen S, Watford K, Labadie RF. Intratympanic dexamethasone for sudden sensorineural hearing loss after failure of systemic therapy. Laryngoscope. 2007;117(1):3-15. DOI: 10.1097/01.mlg.0000245058.11866.15 PMID: 17202923

15. Tabuchi K, Nishimura B, Tanaka S, Hayashi K, Hirose Y, Hara A. Ischemia-reperfusion injury of the cochlea: pharmacological strategies for cochlear protection and implications of glutamate and reactive oxygen species. Curr Neuropharmacol. 2010;8(2):128–134. DOI: 10.2174/157015910791233123.

16. Shi X. Physiopathology of the cochlear microcirculation. Hear Res. 2011;282(1–2):10–24. DOI: 10.1016/j.heares.2011.08.006.

17. Ruben RJ, Distenfeld A, Berg P, Carr R. Sudden sequential deafness as the presenting symptom of macroglobulinemia. JAMA. 1969;209(9):1364–1365. PMID: 4979452

18. Sone M, Mizuno T, Naganawa S, Nakashima T. Imaging analysis in cases with inflammation-induced sensorineural hearing loss. Acta Otolaryngol. 2009;129(3):239–243. DOI: 10.1080/00016480802226163 PMID: 18720058

19. Rudack C, Langer C, Stoll W, Rust S, Walter M. Vascular risk factors in sudden hearing loss. Thromb Haemost. 2006;95(3):454–461. DOI: 10.1160/TH05-08-0554 PMID: 16525573

20. Gussen R. Sudden deafness of vascular origin: a human temporal bone study. Ann Otol Rhinol Laryngol. 1976;85(1 Pt 1):94–100. DOI: 10.1177/000348947608500117 PMID: 1259320

21. Fisch U, Nagahara K, Pollak A. Sudden hearing loss: circulatory. Am J Otol. 1984;5(6):488–491. PMID: 6440439

22. Elwany S, Kamel T. Sensorineural hearing loss in sickle cell crisis. Laryngoscope. 1988;98(4):386–389. DOI:10.1288/00005537-198804000-00005 PMID: 3352436

23. Eryigit B, Ziylan F, Yaz F, Thomeer H. The effectiveness of hyperbaric oxygen in patients with idiopathic sudden sensorineural hearing loss: a systematic review. Eur Arch Oto-Rhino-Laryngol. 2018;275(12):2893–2904. DOI: 10.1007/s00405-018-5162-6.

24. Chen L, Zhang G, Zhang Z, Wang Y, Hu L, Wu J. Neutrophil-to-lymphocyte ratio predicts diagnosis and prognosis of idiopathic sudden sensorineural hearing loss: A systematic review and meta-analysis. Medicine (Baltimore). 2018 Sep;97(38):e12492. DOI: 10.1097/MD.0000000000012492. Review. PMID: 30235752

25. Chau JK, Lin JR, Atashband S, Irvine RA, Westerberg BD. Systematic review of the evidence for the etiology of adult sudden sensorineural hearing loss. Laryngoscope. 2010;120(5):1011-21. DOI: 10.1002/lary.20873.

26. Haberkamp TJ, Tanyeri HM. Management of idiopathic sudden sensorineural hearing loss. Am J Otol. 1999;20(5):587-92. PMID: 10503580

27. Wilson WR, Byl FM, Laird N. The efficacy of steroids in the treatment of idiopathic sudden hearing loss: a double-blind clinical study. Arch Otolaryngol. 1980;106(12):772–776. DOI: 10.1001/archotol.1980.00790360050013 PMID: 7002129

28. Moskowitz D, Lee KJ, Smith HW. Steroid use in idiopathic sudden sensorineural hearing loss. Laryngoscope. 1984;94(5 Pt 1):664- 6. PMID: 6717224

29. Cole RR, Jahrsdoerfer RA. Sudden hearing loss: an update. Am J Otol. 1988;9(3):211-5. PMID: 3140670

30. d'Aldin C, Cherny L, Devriere F, Dancer A. Treatment of acoustic trauma. Ann N Y Acad Sci. 1999;884:328–344. DOI: 10.1111/j.1749-6632.1999.tb08652.x PMID: 10842604

31. Alimoglu Y, Inci E, Edizer DT, Ozdilek A, Aslan M. Efficacy comparison of oral steroid, intratympanic steroid, hyperbaric oxygen and oral steroid + hyperbaric oxygen treatments in idiopathic sudden sensorineural hearing loss cases. Eur Arch Oto-Rhino-Laryngol. 2011;268(12):1735–1741. DOI: 10.1007/s00405-011-1563-5 PMID: 21431435

32. Capuano L, Cavaliere M, Parente G, Damiano A, Pezzuti G, Lopardo D, et al. Hyperbaric oxygen for idiopathic sudden hearing loss: is the routine application helpful? Acta Otolaryngol. 2015;135(7):692–697. DOI:10.3109/00016489.2015.1023355 PMID: 25813083

33. Bae SC, Noh HI, Jun BC, Jeon EJ, Seo JH, Park SY, et al. Efficacy of intratympanic steroid therapy for idiopathic sudden sensorineural hearing loss: comparison with systemic steroid therapy and combined therapy. Acta Otolaryngol. 2013;133(5):428-33. DOI: 10.3109/00016489.2012.749520 PMID: 23356871

34. Battaglia A, Burchette R, Cueva R. Combination therapy (intratympanic dexamethasone+high-dose prednisone taper) for the treatment of idiopathic sudden sensorineural hearing loss. Otol Neurotol. 2008;29;(4):453-60. DOI: 10.1097/MAO.0b013e318168da7a PMID: 18401285

35. Cho I, Lee HM, Choi SW, Kong SK, Lee IW, Goh EK, et al. Comparison of two different treatment protocols using systemic and intratympanic steroids with and without hyperbaric oxygen therapy in patients with severe to profound idiopathic sudden sensorineural hearing loss: a randomized controlled trial. Audiol Neuro-otol. 2018;23(4):199–207. DOI: 10.1159/000493558 PMID: 30380530

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):321-6

Research Article Doi: 10.36472/msd.v6i12.329

The Evaluation of Upper Airway: Point of Care Ultrasound vs. Traditional Tests

Burak Yıldız[1], Banu Çevik[1]*, Yılmaz Karaduman[1], Özlem Sezen[1], Kemal Tolga Saraçoglu[1]

Abstract

Objective: The ultrasound-guided interventions have gained widespread popularity in several aspects of anesthesia practice. In this study, we aimed to compare the preoperative evaluation tests and sonographic measurements of the upper airway for the prediction of a potentially difficult airway.

Material and Methods: In this prospective observational study, we enrolled 136 adult patients undergoing elective surgery under general anesthesia. The Modified Mallampati classification, thyromental distance, sternomental distance, and Cormack-Lehane scores were recorded. Sonographic measurements included pre-epiglottic space (PES), the distance between the midpoints of vocal cords and epiglottis (EVC). The ratio was interpreted. Main outcome is to determine the sensitivity and specificity of the upper airway ultrasound for the prediction of a potentially difficult airway.

Results: There was no statistically significant relationship between body mass index value and thyromental distance, Thyromental/Sternomental Ratio and PES/EVC ratio, Cormack-Lehane, Mallampati classification and thyromental/sternomental distance ratio ($p>0.05$). The sonographic measurements of airway have no significance to predict the difficult intubation and the comparison between PES, EVC and the PES/EVC ratio and assessment tests (Cormack-Lehane, Mallampati classification, thyromental and sternomental distances) was insignificant. The sternomental distance measurement was predictive for the difficult airway only in patients having body mass index more than 31.6 kg m-2.

Conclusion: Ultrasound is a useful tool for identifying the upper airway prior to anesthesia but the validity for the prediction of difficult airway is not clear. By increasing the clinical experiences and further investigations, a greater insight into its use will be gained.

Keywords: airway management; endotracheal intubation; laryngoscopy; ultrasonography

Introduction

The prediction of difficult airway remains the major troublesome of anesthesiology practice. Many bedside airway assessment tests are available in clinical practice but the sensitivity and specificity of these tests are not strong enough to predict the difficult airway (1). The difficult airway was defined as the experience of difficulty with face mask ventilation, difficulty with tracheal intubation, or both by a conventionally trained anesthesiologist (2). However, it's subjective to determine the degree of difficulty. For this purpose, an intubation difficulty scale has been introduced to categorize the difficulty as easy, slightly and very difficult intubation in 1997 (3).

Recently, point of care ultrasound has gained rapid popularity to visualize the airway structures (4-6). Sonographic measurements at the level of hyoid bone and thyrohyoid membrane levels demonstrated as a predictor for the distinction of difficult laryngoscopy (7). Mallampati score is widely used pre-anesthesia evaluation of airway difficulty in daily practice but this screening tool shows considerable inter-observer variation and is influenced by gagging, phonation and posture of the patient (8,9). Thyromental and sternomental distance are the anthropometric measurements to predict the difficult intubation but they were found to be poor single predictors of airway difficulty (10). Cormack-Lehane (grade1-4) classification was based on the direct laryngoscopic view of the anatomic features and strongly associated with the prediction of difficult intubation (grade3-4) (11).

Received 20-11-2019 **Accepted** 23-12-2019 **Available Online** 25-12-2019 **Published** 30-12-2019

1 University of Health Sciences, Kartal Dr.Lutfi Kırdar Training and Research Hospital, Dept. of Anesthesiology and Reanimation, Istanbul, TR

*** Corresponding Author:** Banu Çevik **E-mail:** banueler@yahoo.com

http://dx.doi.org/10.36472/msd.v6i12.329

Rana et al. (12) demonstrated that the assessment of the pre-epiglottic space and the distance from the epiglottis to the midpoint of the distance between the vocal cords is a better predictor of Cormack-Lehane grading as compared to hyomental distance ratio. On the other hand, in another study it was concluded that the measurements do not correlate with Body Mass Index although they correlate with Mallampati score (13) .

This present study was undertaken to compare the utility of sonographic measurements of pre-epiglottic space and the distance from the mid-point of the vocal cords to epiglottis to predict the difficult intubation comparing with Modified Mallampati classification, thyromental distance, sternomental distance, and Cormack-Lehane classification.

Material and Methods

This prospective observational study was conducted after approval of the Institutional Ethics Committee (Decision no: 2017/514/105/3) and written informed consent of all the participants, according to the Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

We enrolled 136 patients over 18 years of age with American Society of Anesthesiology (ASA) physical status I-III who were scheduled to undergo elective surgery under general anesthesia conducted with endotracheal intubation in a tertiary training hospital. The patients were selected randomly from the operation lists of each operating room in a sequential order among the patients requiring the general endotracheal anesthesia. If the ordered patient refused to enroll in the study, following patient in the list was offered to participate.

Exclusion criteria: Emergency surgery, patients with limited mouth, head and neck movement, patients with temporomandibular joint impairment, the history of previous head and neck surgery, fracture or tumors of maxillofacial region, patients requiring awake intubation, uncooperative patients, any cervical spine deformity, patients with severe arthritis, patients with prominent teeth deformities or lost and pregnant patients were excluded from study.

According to institution protocol, an anesthesiologist conducted the preoperative assessment for ASA risk stratification by evaluation of laboratory findings, chest X-ray and electrocardiography. All preoperative assessments have been performed by the anesthesiologists having at least 5 years experienceyears' experience in anesthesia who were participated in this study.

Airway assessment tests: The Modified Mallampati score (MMS) was specified in a sitting position with the patient's head in a neutral position. The patient was asked to open her/his mouth as widely as possible, protrude the tongue out of her/his mouth as much as possible. The observer provided a score of I-IV according to the visibility of the soft palate, uvula and faucial pillars. The thyromental distance (TMD) was measured from the mental prominence to the thyroid cartilage while the patient's neck was fully extended in a supine position. The sternomental distance (SMD) was measured from the suprasternal notch to mentum with the neck fully extended in the supine position.

Ultrasound measurements: Ultrasound measurements were performed by the primary investigator and obtained by using a Sonoline Adara, Siemens ultrasound system. All measurements have been conducted in the supine position with the maximal head and neck extension. The ultrasound probe was placed in the submandibular area and rotated in the transverse plane from cephalad to caudad direction without changing the probe position. Epiglottis and posterior part of the vocal folds with arytenoids was visualized in one 2-dimensional view. Epiglottis visualized as hypoechoic curvilinear structure and its anterior border demonstrated a hyperechoic structure named pre-epiglottic space (PES). Vocal cords appeared as hyperechoic lateral V-shaped structure identified by the movement of two linear structures during breathing or phonation. The distance between the midpoints of vocal cords and epiglottis (E-VC) was measured. The ratio of PES to E-VC was estimated.

After all measurements were completed, patients were premedicated with 0.05 mg kg-1 midazolam intravenously and transferred to the operating room. Standard monitoring recommended in ASA guideline has been employed to the patients and 100% oxygen has been administered before induction for a minimum of 3 minutes. Anesthesia was induced with 0.5 µg kg-1 fentanyl and 2-3 mg kg-1 propofol intravenously. After checking the loss of consciousness and adequate mask ventilation, 0.6 mg kg-1 rocuronium has been administered intravenously to facilitate the endotracheal intubation. For laryngoscopy, a re-usable metal Macintosh blade 3-4 was used depending on the patient's body structure and patients were intubated by another anesthesiologist with more than 5 years of anesthesia experience blinded to the preoperative airway assessments. This anesthesiologist noted the Cormack-Lehane classification according to the position of vocal cords without compressing the larynx. After insertion of the appropriate size of an endotracheal tube, the maintenance of anesthesia was provided depending on the clinical condition of the patient. Guide-wire was not attached to the endotracheal tubes to achieve standardization. If the intubation process was difficult, guide-wire was inserted into the endotracheal tube to facilitate the procedure.

Data collection: Demographic characteristics of the patients including age, gender, height, weight, body mass index (BMI) and ASA physical status were recorded. Thyromental and sternomental distance were recorded for each patient. The ratio of thyromental to sternomental distance was estimated. Modified Mallampati score is graded from I to IV as follows; Grade I: faucial pillars, uvula, soft and hard palate visible; Grade II: uvula, soft and hard palate visible; Grade III: Only base of uvula visible; Grade IV: Only hard palate visible. Grade III and IV are predicted as difficult intubation. The patients with a thyromental distance less than 6-6.5 cm and sternomental distance $\leqslant$12.5 cm are pre-diagnosed as intubation difficulty. Cormack-Lehane classification including four grades (Grade I: full view of the glottis, Grade II: supraglottis not seen, Grade III: visible epiglottis, not the glottis, Grade IV: neither glottis nor epiglottis visible). Grade III and IV imply airway difficulty. Ultrasonographic

measurements of PES, E-VC, the calculation of PES E-VC ratio and the airway assessment test results were recorded in a sheet prepared for each patient. The primary outcome of this study was to determine the sensitivity and specificity of the upper airway ultrasound for the prediction of a potentially difficult airway.

Statistical analysis: For statistical analysis, IBM SPSS Statistics (Version 22.0) was used. Continuous data were expressed as means ± standard deviation (SD); categorical data were expressed as numbers of occurrences (percents). Student-t test was used in the 2-group comparisons of the normally distributed parametric values. Mann-Whitney U test was used to decide the significance between 2-group comparisons not showing normal distribution. Correlation analysis was performed using the Pearson test in normally distributed data. Spearman rank correlation test was used to analyze the correlation between data not showing normal distribution. The level of statistical significance was $p<0.05$.

In the sample size analysis based on 80% power and 95% confidence interval, the minimum sample size to be reached was 78 participants to detect a reasonable change in sensitivity and specificity. The sensitivity and specificity values for calculation were based on a previous study by Reddy et al. (14).

Results

The data of 136 patients were evaluated. None of the patients were excluded. Patient demographics are listed in Table 1. Ninety-two patients (67.6%) had short thyromental distance (⩽ 6 cm). Forty-two patients (30.9%) have a sternomental distance of ⩽12.5 cm. According to Mallampati classification, 107 patients had a score of 1-2 (78.7%) and 29 had a score of 3-4 (21.3%). Cormack-Lehane scores varied between 3-4 for 26 patients (19.1%) and 1-2 for 110 patients (80.9%). All study parameters were shown in Table 2.

Based on the Cormack-Lehane scoring, the sensitivity (D) of detecting difficult intubation of thyromental distance test was 76.9%, specificity (S) was 34.5%, positive predictive value (PPD) was 21.7% with a negative predictive value (NPD) of 86.4%. Sternomental distance test identified the sensitivity (D) of 42.3%, the specificity (S) of 71.8%, the positive predictive value (PPD) of 26.2% and the negative predictive value (NPD) of 84%. There was no statistically significant relationship between Cormack-Lehane and Mallampati classification and TMM / SMM ratio ($p>0.05$). The Mallampati classification was able to detect difficult intubation in 7 of 26 difficult intubation cases, and difficult intubation in 22 of 110 easy intubations comparing to Cormack-Lehane scoring. The statistical sensitivity (D) of the Mallampati classification was 26.9%, the specificity (S) was 80%, the positive predictive value (PPD) was 24.1% and the negative predictive value (NPD) was 82.2% (Table 3).

There was an inverse, moderate (40.4%) and statistically significant relationship between BMI level and sternomental distance ($p<0.0001$). There was a similar, weak (29.2%) statistically significant relationship between BMI and Mallampati classification ($p=0.001$). There was a statistically significant relationship between BMI and Cormack-Lehane (20.7%, $p=0.016$). There was no statistically significant relationship between BMI value and thyromental distance, thyromental/sternomental ratio and PES/E-VC ratio ($p>0.05$) (Table 4). However, the mean BMI of Mallampati class 1-2 patients was 28.42 kg m-2 and 32.42 kg m-2 for subjects with Mallampati class 3-4(p=0,001). The BMI of the patients (31.6 kg m-2) with the sternomental distance of 12.5 cm or less was significantly higher than the BMI (28.02kg m-2) of 12.5 cm ($p = 0.001$).

According to US measurements, there was no statistically significant difference between PES/EVC ratio and Cormack-Lehane, Mallampati classification, thyromental and sternomental distances (Table 5).

Table 1: Patients' characteristics

Variables	Results
[1]**Age (years)**	49.71±13.61
[1]**Height (cm)**	164.78±9.25
[1]**Weight (kg)**	78.89±16.34
[1]**BMI (kg m^{-2})**	29.13±6.04
[2]**Gender Male/Female**	59 (43.4)/77 (56.6)
[2]**Comorbidity Yes/No**	50(36.8)/86 (63.2)

BMI: Body Mass Index . Data were expressed as [1] Mean±SD or [2] numbers (percentage)

Table 2. The study parameters

	Minimum	Maximum	Mean±SD
Thyromental Distance (cm)	4	12	6.19±1.55
SternomentalDistance (cm)	9	18	13.54±1.81
Thyromental/Sternomental ratio	0.31	0.74	0.45±0.1
MallampatiScore	1	4	1.94±0.72
Cormack-Lehane	1	4	1.78±0.82
Perepiglottic Space (PES) (mm)	4	20	10.19±3.53
Epiglottis-Vocal Cord Distance (E-VC) (mm)	3	19	9.17±2.55
PES/E-VC	0.33	28	1.39±2.35

http://dx.doi.org/10.36472/msd.v6i12.329

Table 3. The correlations between Cormack-Lehane score and preoperative airway assessement tests

Thyromental Distance (cm)	r	-0.252
	p	0.003*
Sternomental Distance (cm)	r	-0.245
	p	0.004*
Mallampati Classification	r	0.157
	p	0.068
Thyromental/Sternomental Ratio	r	-0.109
	p	0.205

Spearman's rho correlation test, *p<0.05; statistically significant

Table 4. The correlation between BMI and study parameters

ThyromentalDistance	r	-0.063
	p	0.467
SternomentalDistance	r	-0.404
	p	0.000*
Thyromental/Sternomental Ratio	r	0.161
	p	0.061
MallampatiClassification	r	0.292
	p	0.001*
PES/E-VC	r	-0.024
	p	0.779
Cormack-Lehane	r	0.207
	p	0.016*

BMI: Body Mass Index, PES/E-VC: Perepiglottic Space/ Epiglottis-Vocal Cord Distance *Pearson correlation coefficient *p<0.05; statistically significant*

Table 5. The correlations between PES/E-VC and airway assessment tests

		PES/E-VC
Cormack-Lehane	r	-0.006
	p	0.948
Mallampati Classification	r	-0.012
	p	0.891
Thyromental Distance	r	0.034
	p	0.697
Sternomental Distance	r	0.035
	p	0.690
Thyromental/Sternomental ratio	r	0.003
	p	0.971

PES/E-VC: Perepiglottic Space/ Epiglottis-Vocal Cord Distance, *Spearman's rho correlation test*

Discussion

In this study, we compared the role of ultrasonographic assessment of upper airway with the traditional three preoperative airway assessment tests including Modified Mallampati classification, thyromental distance, and sternomental distance. Our results showed that the sonographic evaluation of airway for the prediction of difficult intubation was not supportive of traditional assessment tests. Moreover, the Cormack-Lehane classification indicated no correlation with the sonographic measurements. The comparison between preoperative bed-side assessment tests revealed that the sternomental distance predicted the difficult airway significantly in patients having body mass index over 31.6 kg m-2.

The prediction of difficult airway is a challenging issue and the expectations from the assessment tests are to be highly sensitive and specific with minimal false positive and negative results (15). The reliability of these tests depends on the correct measurements and the optimization of inter-observer variability. Seo at al. (16) studied 7 airway assessment test and estimated a total airway score (TAS) with the sum of all scores. They suggested that the TAS>6 was a better method than using only one score for the prediction of difficult intubation.

Thyromental distance (TMD) is a frequently used preoperative assessment test for the prediction of the difficult airway. However, the discussion about its sensitivity and specificity has been going on.

The accepted cut-off value is 6.5 cm but there are many contradictions about this value. Baker et al. (17) suggested that the cut off value of TMD ranged between 6 and 8 cm. In a similar study new score estimated by the ratio of the height of the patient to TMD showed better accuracy for the prediction of airway difficulty (18). Selvi et al (19) indicated that TMD measurement was not being the alone tool to predict the difficulty in intubation with the cut off value of 6.5 cm. The sensitivity of TMD has been found %10.5 in a large case study and the value of the positive and negative predictive values were 20% and 92% respectively (20).

The sensitivity of TMD was 76.9%, specificity was 34.5%, the positive predicting value was 21.7% and the negative predicting value was 86.4% in our study. This result indicated that the TMD alone is not sensitive and specific for the prediction of the difficult airway with the cut of the value of 6.5 cm.

Since it's introduced to clinical practice, the Mallampati score has been widely used preoperative assessment test. However, its accuracy has been discussed in a previous meta-analysis due to large variation among studies (21). Controversially, a large meta-analysis had been indicated that bedside airway examination tests should be used with caution due to challenges in the literature but the Mallampati test had the highest sensitivity among all the screening tests (22).

Acer et al. (23) suggested that the Mallampati itself was not sufficient to predict the difficult intubation so; conjunction with measurement of neck circumference should be used. Sternomental and thyromental distances together with neck length found more useful in preoperative assessment tests. In our study, the sensitivity of the Mallampati test was 26.9%. The specificity was 80%, the positive predicting value was 24.1% and the negative predicting value was 82.2% in our study. This indicated that the sensitivity and specificity of the Mallampati test were not strong enough to predict the airway difficulty.

Sternomental distance (SMD) provides a rapid, simple and objective test to identify the difficult airway. The validity of this test increases when combined with the other bed-side assessment tests (24). Our results indicated that the sensitivity of 42.3%, the specificity of 71.8%, the positive predicting value of 26.2% and negative predicting value of 84% in SMD assessments in our patient population for the prediction of the difficult intubation.

Cormack-Lehane (C-L) classification is an invasive assessment test so, it's not be used as a prediction method. The accuracy of this test is still a challenging issue. Selvi et al. (19) reported that 28 of 37 patients who were accepted as difficult intubation had been graded C-L classification 3 and 4. The sensitivity and the specificity of C-L classification have been reported as 96.43 and 97.64% respectively. In our study, the number of the patient having C-L grade III and IV was 23 and 3 respectively. The correlation between preoperative screening tests and C-L classification revealed no significance.

Due to non-invasive characteristics, the use of ultrasound (US) in operating rooms has been increasing. The usage of US for the assessment of airway structures gains popularity during the last years. The measurement of anterior neck soft tissue (ANS) thickness at the hyoid bone, thyrohyoid membrane, and anterior commissure levels were found as independent predictors of difficult laryngoscopy (6). Ultrasonographic measurement of ANS- vocal cords was found a potential predictor of difficult intubation. A value of 0.23 mm was shown to be more sensitive than the preoperative screening tests (MMS, TMD, SMD) (14).

Gupta et al. (25) measured the distance from the epiglottis to the midpoint of the distance between vocal cords (E-VC), the depth of the pre-epiglottic space (PES) and compared with C-L grade of the patient. They found a weak correlation with 87% sensitivity and 30% specificity to predict the airway difficulty. In a similar study, the ratio of PES to E-VC showed a weak correlation with the C-L grade (26). We measured the PES, E-VC distance and estimated the PES to E-VC ratio by ultrasound. The results showed a weak correlation with the preoperative assessment tests and C-L classification. Our results indicated that ultrasonographic measurements of airway were not an accurate tool for the prediction of difficult intubation before anesthesia.

Limitations: The study group is not homogenous so the further comprehensive studies based on gender, age, body mass index, ASA physical status may give more revealing results. The evaluation of the presence of co-existing disease, the anatomical differences between patients in respect of head, neck and dental status may change the measurements. The number of patients C-L grade III and IV was limited in our study. The more patients having high graded C-L classification may change the statistical results.

Conclusion

The prediction of difficult airway is still one of the main topics of anesthesia practice and the researches to find the most accurate assessment tool has been going on. The evaluation of upper airway with US is a promising issue and the further investigations on this area will encourage the clinicians to use US in daily anesthesia practice for the prediction of difficult airway.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: BY, BÇ, YK, OS, KTS; Research concept and design, Research the literature, preparation of the article, Revision of the article.

References

1. Roth D, Pace NL, Lee A, Hovhannisyan K, Warenits AM, Arrich J, et al. Airway physical examination tests for detection of difficult airway management in apparently normal adult patients. Cochrane Database Syst Rev 2018; 5(5): CD008874. Published 2018 May 15. doi:10.1002/14651858.CD008874.pub2

2. Apfelbaum JL, Hagberg CA, Caplan RA, Blitt CD, Connis RT, Nickinovich DG, et al; American Society of Anesthesiologists Task Force on Management of the Difficult Airway. Practice guidelines for management of the difficult airway: an updated report by the American Society of Anesthesiologists Task Force on Management of the Difficult Airway. Anesthesiology 2013; Feb; 118(2): 251-70.

3. Adnet F, Borron SW, Racine SX, Clemessy JL, Fournier JL, Plaisance P, et al .The intubation difficulty scale (IDS): proposal and evaluation of a new score characterizing the complexity of endotracheal intubation. Anesthesiology 1997; Dec; 87(6): 1290-7.

4. Prasad A, Yu E, Wong DT, Karkhanis R, Gullane P, Chan VW. Comparison of sonography and computed tomography as imaging tools for assessment of airway structures. J Ultrasound Med 2011; Jul; 30(7): 965-72.

5. Kristensen MS. Ultrasonography in the management of airway. Acta Anaesthesiol Scand 2011; Nov; 55(10): 1155-73.

6. Wu J, Dong J, Ding Y, Zheng J. Role of anterior neck soft tissue quantifications by ultrasound in predicting difficult laryngoscopy. Med Sci Monit 2014; Nov 18; 20: 2343-50.

7. Adhikari S, Zeger W, Schmier C, Crum T, Craven A, Frrokaj I, et al. Pilot study to determine the utility of point-of-care ultrasound in the assessment of difficult laryngoscopy. Acad Emerg Med 2011; Jul; 18(7):754-8.

8. Sankal A, Halemani KR, Bhadrinath N. Assessment of the effect of supine posture and phonation on modified mallampati grading and its applicability in prediction of difficult airway. J Mahatma Gandhi Inst Med Sci 2018; 23: 19-24.

9. Khan ZH, Eskandari S, Yekaninejad MS. A comparison of the Mallampati test in supine and upright positions with and without phonation in predicting difficult laryngoscopy and intubation: A prospective study. J Anaesthesiol Clin Pharmacol 2015; Apr-Jun; 31(2):207-11.

10. Liaskou C, Vouzounerakis E, Moirasgenti M, Trikoupi A, Staikou C. Anatomic features of the neck as predictive markers of difficult direct laryngoscopy in men and women: A prospective study. Indian J Anaesth 2014 Mar; 58(2): 176-82.

11. Cormack RS, Lehane J. Difficult tracheal intubation in obstetrics. Anaesthesia 1984; 9(11): 1105-11.

12. Rana S, Verma V, Bhandari S, Sharma S, Koundal V, Chaudhary SK. Point-of-care ultrasound in the airway assessment: A correlation of ultrasonography-guided parameters to the Cormack-Lehane Classification. Saudi J Anaesth 2018; 12: 292-296.

13. Hall EA, Showaihi I, Shofer FS, Panebianco NL, Dean AJ. Ultrasound evaluation of the airway in the ED: a feasibility study. Crit Ultrasound J 2018; Jan 18; 10(1):3. doi: 10.1186/s13089-018-0083-6.

14. Reddy PB, Punetha P, Chalam KS. Ultrasonography- A viable tool for airway assessment. Ind J Anaesth 2016; 60:807-13.

15. Aktas S, Atalay YO, Tugrul M. Predictive value of bedside tests for difficult intubation. Eur Rev Med Pharmacol Sci 2015; 19 (9): 1595-9.

16. Seo SH, Lee JG, Yu SB, Kim DS, Ryu SJ, Kim KH. Predictors of difficult intubation defined by the intubation difficulty scale (IDS): predictive value of 7 airway assessment factors. Korean J Anesthesiol 2012; Dec; 63(6):491-7.

17. Baker PA, Depuydt A, Thompson JM. Thyromental distance measurement-fingers don't rule. Anaesthesia 2009 Aug; 64(8):878-82.

18. Baker P. Assessment before airway management. Anesthesiol Clin 2015; Jun;33(2):257-78.

19. Selvi O, Kahraman T, Senturk O, Tulgar S, Serifsoy E, Ozer Z. Evaluation of the reliability of preoperative descriptive airway assessment tests in prediction of the Cormack-Lehane score: A prospective randomized clinical study. J Clin Anesth 2017; Feb; 36:21-26.

20. Javaher Foroosh Zadeh F, Safaii S. Comparison of four methods for predicting difficult intubation. Int J Bioassays 5.1 2016; 4720-22.

21. Lundstrøm LH, Vester-Andersen M, Møller AM, Charuluxananan S, L'hermite J, Wetterslev J; Danish Anaesthesia Database. Poor prognostic value of the modified Mallampati score: a meta-analysis involving 177 088 patients. Br J Anaesth. 2011 Nov; 107(5):659-67.

22. Roth D, Pace NL, Lee A, Hovhannisyan K, Warenits AM, Arrich J et al. Bedside tests for predicting difficult airways: an abridged Cochrane diagnostic test accuracy systematic review. Anaesthesia 2019; Jul; 74(7):915-928.

23. Acer M, Akkaya A, Tuğay BU, Öztürk A. A Comparison of Cormeck-Lehane and Mallampati tests with mandibular and neck measurements for predicting difficult intubation. Balkan Med J 2011; 28: 157-163.

24. Prakash S, Mullick P, Bhandari S, Kumar A, Gogia AR, Singh R. Sternomental distance and sternomental displacement as predictors of difficult laryngoscopy and intubation in adult patients. Saudi J Anaesth 2017; Jul-Sep;11(3):273-278.

25. Gupta D, Stirajakalidindi A, Ittiara B, Apple L, Toshniwal G, Haber H. Ultrasonographic modification of Cormack-Lehane classification for pre-anesthetic assessment. Middle East J Anaesthesiol 2012; Oct; 21(6):835-42.

26. Mohammadi SS, Saliminia A, Nejatifard N, Azma R. Usefulness of ultrasound view of larynx in pre-anesthetic airway assessment: A comparison with Cormack-Lehane classification during direct laryngoscopy. Anesth Pain Med 2016; 6(6): e39566.

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):327-32

Research Article Doi: 10.36472/msd.v6i12.330

Clinical results and importance of next-generation sequencing (NGS) in detecting targeted mutations in the treatment of metastatic Lung Cancer: Single center initial results

Cem Mirili[1]*, Çiğdem Kahraman[2], Ali Yılmaz[1], Mehmet Bilici[1], Salim Başol Tekin[1], Abdulgani Tatar[2], Ömer Yakar[2], Pelin Ercoşkun[2]

Abstract

Objective: In Lung cancer (LC), which is one of the most deadly cancers, longer survival has been achieved with targeted agents. For this reason, it is important to find the patients who are suitable for targeted therapies. Next-generation sequencing (NGS) is a method that allows multiple genetic variants to be detected simultaneously by performing massive parallel DNA sequencing at the same time. We wanted to reveal the clinical effects and benefits of genetic variant analysis with NGS for our patients.

Material and Methods: Patients with stage IV non-squamous and not otherwise specified (NOS) non-small cell LC who underwent genetic variant analysis with NGS were included in the study, retrospectively.

Results: Total of the 51 patients, 41 (80.4%) were male and the median age was 64 (35-85) years. According to TNM, 21 (41.2%) patients were stage 4A, 30 (58.8%) patients were stage 4B and 39 (76.5%) patients had adenocarcinoma and 12 (23.5%) had NOS histology. NGS analyzes were performed in median 14 days (8-43) and determined 24 pathogenic variants in 17 (%25) patients: 9EGFR (%17,6), 6PIKC3A (%11,7), 5KRAS (%9,8), 2PTEN (%3,9), 1BRAF (%1,9), 1MET (%1,6) (7 of them concomitantly). Cytotoxic chemotherapy was recommended in 41, anti-EGFR agents in 8 (afatinib in 4, erlotinib in 4 patients) patients and anti-BRAF+MEK inhibitor agent (dabrafenib+trametinib) in 1 patient.

Conclusion: With the NGS, in just two weeks, both target and resistance genetic variants of our patients were detected at the same time and individualized treatments were applied. In this way, both time and cost were saved.

Key words: Lung neoplasm, DNA Mutational Analysis, DNA Sequence Analysis, ErbB Receptors.

Introduction

Lung cancer (LC) is the leading cause of cancer-related deaths worldwide (1). Histologically LC is classified primarily as small cell (15%) and non-small cell carcinomas (85%), whereas non-small cell lung cancers (NSCLC) are divided into two main groups as adenocarcinoma and squamous cell carcinoma (2). NSCLC, which accounts for the majority of LC, generally diagnosed in the advanced stage, while the 5-year survival rate is still low, around 16% (3). Unfortunately, the 5-year survival rate of stage IV patients treated with classical cytotoxic chemotherapy is <5% (3). However, it has been determined that this rate has increased to 15-25% by using targeted and immunotherapy agents (4-5).

Targeted therapies in LC have initiated with the detection of driver mutations such as *EGFR*, *ALK*, *ROS1* over a decade ago.

EGFR mutation is detected by 15-25% (more than 50% in Asian race), *ALK* mutation is around 2-6% and *ROS1* mutation is around 1% by conventional methods such as Real time Polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), or Sanger sequencing (SS). Anti-*EGFR*, such as gefitinib, afatinib, erlotinib and osimertinib, anti-*ALK* therapies such as crizotinib, alectinib, brigatinib, lorlatinib provided both the advantage of progression-free survival (PFS) and overall survival (OS) compared to cytotoxic chemotherapy in advanced LC (6). In addition due to oral administration, more manageable side effect profile, and rapid response, targeted agents have been superior to chemotherapeutics (7). Therefore, all updated guidelines such as the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) require mutation analyses of

Received 23-11-2019 **Accepted** 15-12-2019 **Available Online** 26-12-2019 **Published** 30-12-2019
1 Ataturk University School of Medicine, Dept of Medical Oncology, Erzurum, TR
2 Ataturk University School of Medicine, Dept of Medical Genetics, Erzurum, TR
*** Corresponding Author:** Cem Mirili **E-mail:** cemirili@gmail.com

http://dx.doi.org/10.36472/msd.v6i12.330

EGFR, ALK and ROS1 to all advanced non-squamous (adenocarcinoma, large cell carcinoma) and not otherwise specified (NOS) NSCLC (category 1). And they also recommended to detect mutations such as *MET, RET, HER2, BRAF* in a low level of evidence and to use targeted agents in appropriate patients (8). In spite of this information, it is now a known fact that detection of a mutation in step by step procedure by conventional methods such as FISH or SS causes both time and material wasting (9). Recently, with the completion of the cancer genome atlas project (TCGA), next generation sequencing method (NGS) has discovered. The most important advantage of this method over conventional methods is that it can perform multiple sequencing at the same time (massive parallel sequencing). Thus, more genetic variants can be detected at the same time, cheaper and faster. In addition, it enables the detection of variants using both tissue and liquid biopsies, thus reducing invasive interventions and also allowing the detection of genetic variants that are resistant to targeted agents (10).

In this study, we aimed to present the NGS results of our patients with advanced lung cancer and to show the effect of these results on our patient management.

Material and Methods

A total of fifty-one patients who were diagnosed as stage 4 non-squamous and NOS NSCLC between November 2018 and November 2019 at Atatürk University Medical Oncology and who underwent genetic variant analysis using NGS method were included in our study retrospectively.

Ethics committee approval was obtained from the ethics committee of Erzurum Ataturk University. All the procedures were performed according to the 1964 Helsinki declaration.

Sample and DNA Isolation

FFPE tumor specimens and liquid biopsy materials were collected from lung cancer patients referred to the medical genetics clinic. All patients had a clinical indication for molecular testing and were informed about the purpose of the molecular analysis by the treating physician.

DNA was isolated using the GeneRead™ DNA FFPE Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. The nucleic acid concentration was measured with the Qubit dsDNA HS Assay kits on the Qubit 2.0 Fluorometer (Thermo Fisher Scientific).

GeneReader Assay and Sequencing

In total, 40 ng of each DNA sample was used as a template for the QIAGEN GeneRead QIAact Lung DNA Panel UMI Kit according to the manufacturer's instructions. The GeneRead QIAact Lung DNA UMI Panel is designed to enrich specific target regions in select genes (*AKT1, ALK, BRAF, DDR2, EGFR, ERBB2/HER2, ESR1, FGFR1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, RICTOR, ROS1*). The amplicons in the panel cover multiple exons in 19 genes. -*Libraries were prepared using the QIAGEN GeneRead DNA Library Kit and an automated protocol on a QIAcube. PCR-enriched DNA and GeneRead libraries were qualified and quantified using a QIAGEN QIAxcel Advanced System. Emulsion PCR and bead enrichment steps were carried out using the GeneRead Clonal Amp Q Kit on a GeneRead QIAcube. Following clonal amplification, amplicon libraries were sequenced using the QIAGEN GeneRead Sequencing Q Kit and after an upgrade during the testing period, the GeneRead Advanced Sequencing Q Add-On on a GeneReader instrument (all protocols available on http://www.qiagen.com). QIAGEN Clinical Insight Analyze (QCI-A) software performed the secondary analysis of FASTQ reads generated by the GeneReader. Variants were imported into the QCI-interpret (QCI-I) web interface for data interpretation and report generation.

Results

Total of the 51 patients included in the study, 41 (80.4%) were male and 10 (19.6%) were female and the median age was 64 (35-85) years. 39 (76.5%) patients had adenocarcinoma and 12 (23.5%) had NOS histology. According to TNM, 21 (41.2%) patients were stage 4A, 30 (58.8%) patients were stage 4B. Metastasis of the patients were detected in 16 (31.4%) contralateral lobe, 13 (25.5%) bone, 6 (11.8%) cranial, 6 (11.8%) liver, 6 (11%, 8) adrenal, 4 (7.8%) pleura.

Mutation analysis with NGS was performed from tissue biopsy in 27 (52.9%) patients and liquid biopsy in 24 (47.1%) patients. As a result of these analyzes, no genetic variant was detected in 29 patients but 7 variants of uncertain clinical significance in 5 patients and 24 pathogenic variants in 17 patients were detected. Detailed results of uncertain clinical significance and pathogenic genetic variants are shown in Tables 1 and 2. Totally, 9*EGFR*, 6*PIKC3A*, 5 *KRAS*, 1*BRAF*, 2*PTEN*, 1*MET* pathogenic variants were determined. Seven of 24 pathogenic variants had concomitant mutations: *EGFR* + *PIK3CA* in 2 patients, *EGFR* + *KRAS* in 1 patient, *EGFR* + *PTEN* in 1 patient, *EGFR* + *MET* in 1 patient and *KRAS* + *PIK3CA* in 2 patients.

NGS analyses were performed in median 14 days (8-43), and according to the data, standard cytotoxic chemotherapy was recommended in 41 patients, anti-*EGFR* agents in 8 patients (afatinib in 4 patients, erlotinib in 4 patients) and anti-*BRAF* + MEK inhibitor agent (dabrafenib + trametinib) in 1 patient. Table 2 shows the treatment agents applied to patients with pathogenic mutations in detail. In one patient, no variant could be detected by liquid biopsy, *ALK* mutation was detected by FISH and alectinib, which is an anti-*ALK* agent, was used. In order to detect resistance mutations, variant analysis with NGS was performed in 2 patients due to showing progression while using anti-*EGFR* agents and *EGFR* T790M mutations were detected in both of them.

Table 1. Uncertain significance genetic variants detected by Next-Generation Sequencing

Mutation	DNA Code	Protein	Allelic Fraction (%)	Community frequency (%)	Concomitant Mutation	DNA Code/Protein
PIK3CA	c.1173A>G	P1391M	21	0	-	-
ERBB2	c.1963A>G	P1655V	35	0	-	-
ERBB2	c.1963A>G	P1655V	33	0	-	-
BRAF	c.2128-5dupT		1,96	0	*PIK3CA*	c.1173A>G/ 1391M
BRAF	c.1860+66A>C		8,74	0	*EGFR*	c.1006+15G>A

Table 2. Pathogenic genetic variants detected by Next-Generation Sequencing

Mutation	DNA Code	Protein	Allelic Fraction (%)	Community Frequency (%) (gnomAD)	Concomitant Mutation	DNA Code/Protein	Treatment
EGFR	c.2235_2249del	p.E.746_A750del	4,22	0	-	-	erlotinib
EGFR	c.1787C>G	P596R	1,14	0	-	-	erlotinib
EGFR	c.2235_2249del	E.746_A750del	3,2	0	-	-	erlotinib
EGFR	c.2235_2249del	E.746_A750del	62	0	-	-	afatinib
EGFR	c.2235_2249del	E.746_A750del	0,8	0	*PIK3CA*	c.3140A>G/ H1047R	erlotinib
EGFR	c.2127_2129del	E.709_T710del	42	0	*PIK3CA*	c.1633G>A/ E545K	afatinib
EGFR	c.2237A>G	E.746G	1,06	0	*KRAS*	c.20T>G/ V7G	chemoterapy
EGFR	c.2573T>G	L858R	31	0	*PTEN*	c.697C>T/ R233	afatinib
EGFR	c.323G>A	R108K	0,45	0	*MET*	Amplifikasyon	afatinib
PIK3CA	c.1633G>A	E545K	8,58	0	-	-	chemoterapy
PIK3CA	c.1624G>A	E542K	17	0	-	-	chemoterapy
KRAS	c.34G>T	G12C	12	0	-	-	chemoterapy
KRAS	c.34G>T	G12C	54	0	-	-	chemoterapy
KRAS	c.35G>T	G12V	14	0	*PIK3CA*	c.1634A>C/ E545A	chemoterapy
KRAS	c.34G>T	p.G12C	17	0	*PIK3CA*	c.1633G>A/p.E545K	chemoterapy
PTEN	c.697C>T	R233	20	0	-	-	chemoterapy
BRAF	c.1799T>A	V600E		0	-	-	Dabrafenib+ trametinib

Discussion

The use of targeted agents in LC yields satisfactory results for both the patient and the clinician. These agents provide faster response and longer survival. In our study, a total of 10 druggable genetic variants were detected, 6 of which had concomitant mutations, and 8 of them were initiated with anti-*EGFR* and 1 with anti-*BRAF* agents. Cytotoxic chemotherapy was planned for 1 patient because of concomitant *EGFR* resistance mutation. And these target mutations were detected in median 14 (8-43) days, allowing first line using targeted therapies without the need for cytotoxic chemotherapy. Since some lung cancer patients do not have a single day to wait for treatment, it is seen that the rapid and simultaneous detection of target mutations by NGS is very beneficial for our patients.

Cancer is a genetic disease characterized by the uncontrolled growth of cells. Chromosome dissociation problems, replication errors or DNA damage, which cannot be corrected by repair mechanisms over the years, cause somatic mutations. These mutations are called driver mutations and give growth and survival advantages to certain cell groups by inhibiting apoptosis, accelerating cell proliferation (11). According to current data, the most important driver mutations in LC are *EGFR, ALK, ROS1, BRAF, KRAS, HER2, MET, RET, NTRK* (12). Detection of these mutations at the time of diagnosis and the use of targeted agents improve the quality of life and survival. Prior to NGS, target mutations allowed detection of a mutation in step by step procedure by conventional methods (13).

Although the recommended time is 2 weeks, in our country, the general approach is to detect *EGFR* mutation first by Real time PCR (mean 10 working days) and if negative results, *ALK* (10 working day) and *ROS* (10 working day) mutations are detected by FISH method, respectively. On average, treatment decisions for these three mutations were made within 3-4 weeks. However, there are situations where patients need urgent treatment, so our patients may have to take chemotherapy even if their mutations are detected in the first place. In addition to these 3 important mutations, many different mutations are detected by NGS method and results are obtained within 2 weeks on average (14). In our study, mutation analyzes were performed in median 14 days in accordance with the literature and treatment opportunities were provided to the patients. It is one of the most important advantages of NGS to introduce so many different driver and resistance mutations in such a short time.

The epidermal growth factor (EGF) family includes 4 receptor kinases: EGFR, EGFR2 (HER2 or ERBB2), HER3 (ERBB3), HER4 (ERBB4). When the activating ligand binds to these receptors, EGFR is active and sends intracellular growth, invasion, angiogenesis and anti-apoptotic signals (15). The most common *EGFR* mutations are detected in exon 19 and 21. In the Asian race, the mutation rate increases to 50% and in western it is around 15-20% (16-17). In our patient group, this rate is similar to western societies with 17.6%. Gefitinib, one of the first anti-*EGFR* agents, was first applied in the group of patients

who progressed after chemotherapy regardless of mutation status and the expected results could not be obtained (18). Thereafter, gefitinib and erlotinib were compared with chemotherapy in the *EGFR* mutant group and PFS prolongation was detected (11 months vs 6 months). According to these results, anti-*EGFR* agents has been approved in first-line treatment with *EGFR* mutant patients (19). In the following years, second-generation anti-*EGFR* agents, afatinib and dacomitinib, were introduced and showed superiority of PFS compared with gefitinib. The difference of these agents from the first generation is that they inhibit irreversible EGFR with HER2 and HER4. In addition, afatinib provided longer median OS versus chemotherapy in exon 19 mutant patients (20). Although targeted agents show very promising results at the beginning, they lose their effectiveness due to resistance mechanisms developed within the median 10-16 months. The most important resistance mutation is *EGFR* T790M, which is detected at around 50% and is much less at the time of diagnosis (21). In order to overcome this resistance mechanism, the third generation of anti-*EGFR* agent called osimertinib is used. The FLAURA study has shown that osimertinib provides significant PFS contribution to gefitinib and erlotinib in the first-line treatment in the *EGFR* mutant group (18.9 months vs. 10.2 months; 0.46; 95% confidence interval [CI], 0.37 to 95). 0.57; P <0.001) (22). In addition, ESMO 2019 published OS data for this study and showed that osimertinib provides longer OS with 38.8 months versus 31.8 months (p: 0.0462) (23). With this result, osimertinib can be considered to be rapidly displaced from second-line treatment to first line. In our study, 9 activating *EGFR* pathogenic variants were detected and afatinib was started in 4 and erlotinib was started in 4 of these patients. *KRAS* mutation is another common mutation in NSCLC and it is known to be around 25% on average. Although the survival of the KRAS mutant group was found to be shorter, targeted agents did not benefit. However, the *KRAS* mutation is thought to be a cause of intrinsic resistance to anti-*EGFR* agents (24). Despite the negative effects against anti-*EGFR* agents, recent studies have shown that *KRAS* mutation is associated with increased tumor-infiltrating lymphocyte, PD-L1 and tumor mutation burden and is thought to be a biomarker for immunotherapy agents (25). In our study, although 5 patients (9.8%) were *KRAS* mutated, 1 was found to be concomitant with activating *EGFR* mutation. In this patient, chemotherapy was given because of anti-*EGFR* resistance. If *EGFR* mutation was examined by conventional method and positivity was detected, anti-*EGFR* agents would be prescribed. As a result, treatment would be unresponsive and would cause unnecessary costs. However, better and more personalized treatments can be planned for patients because NGS can be detected at the same time in both targeted mutations and other mutations that are resistant to them.

Phosphatidylinositol 3-kinases (PIK3) play a major role in cell metabolism, migration, growth, and proliferation. PIK3 and AKT are important components of the EGFR pathway and induce oncogenesis and progression in LC. *PIK3CA* mutation is detected in 27% of glioblastoma, 25% of gastric cancer, 32% of colon cancer, and 1-4% of lung adenocarcinoma and the most common type of *PIK3CA* mutation is E545K (57.1%) (26-27). It is more common concomitantly with other mutations in LC cancer and the role of resistance to anti-*EGFR* agents is still controversial. However, in several studies, it was found that the survival of patients with concomitant *EGFR* and *PIK3CA* mutation with anti-*EGFR* agents was not different (28), *PIK3CA* c.1633G> A (p.E545K) mutation was found to be resistant to gefitinib in one trial (29). In our study, *PIK3CA* was found to be singular in 2 patients (3.9%) and concomitantly in 4 patients (7.8%). Two of the *PIK3CA* mutations were concomitantly with *EGFR* and c.1633G> A (p.E545K) was detected in one of them. Afatinib was applied instead of gefitinib in that patient due to possible resistance. It is thought that with the increasing use of NGS, the term known as class effect of drugs in oncology will lose its place to personalized medicine. The class effect is when an *EGFR* mutation detected any anti-*EGFR* agents can be given, but the concomitant mutations show us there is resistance to some of these drugs but not some the others. In this way, the clinician can determine which drug is given to the patient and get better results by NGS method.

Phosphatase and tensin homologously deleted in chromosome 10 (*PTEN*) acts as a very strong tumor suppressor, in case of mutation the tumor passes through the PI3K / mTOR / Akt pathway to an uncontrolled growth phase (30). According to TCGA, it is 15% positive in lung squamous carcinoma and 3% in adenocarcinoma and is generally accepted as a poor prognostic factor (31). On the other hand, in a study with 162 Korean NSCLC patients were included, *PTEN* mutation was detected in 4 (2.5%) cases by NGS method (32). In addition, in two separate studies, it was found that decreased *PTEN* expression caused resistance to gefitinib and erlotinib (33-34). In our study, *PTEN* mutation (R233) was present in 2 patients (3.9%), one of which was associated with *EGFR* mutation. In order to prevent possible resistance to erlotinib and gefitinib, second-generation anti-*EGFR* afatinib was given to the patient with this concomitant mutation.

B-Raf (*BRAF*) is one of the most important protooncogenes and is detected between 2-4% in NSCLC, while it is around 3-7% mutant according to TCGA. The most common type of mutation is the *BRAF* V600E mutation, which is mostly detected in women and non-smokers (35). After the success of anti-*BRAF* treatments in malignant melanoma, and detection of *BRAF* mutant patients in LC have been found to have a shorter survival, it has been decided to use *BRAF* targeted therapy in lung cancer (36). *BRAF* and *MEK* inhibitor combination therapies have been tried since single-agent *BRAF* inhibitors did not provide superiority to chemotherapy in the first studies. In the Phase 2 study, dabrafenib was administered to previously treated patients in cohort A (78 patients), dabrafenib + trametinib to previously treated patients in cohort B (57 patients), and dabrafenib + trametinib to previously untreated patients in cohort C (36 patients). In the Cohort C, complete response was obtained in 2 patients and the overall response rate was 64%. In addition, median PFS was found to be 10.9 months and OS 24.6 in this cohort (37). FDA approval was obtained after these results and the guidelines were

included *BRAF+MEK* inhibitor therapy in the first-line treatment of NSCLC with *BRAF* V600E mutated. In our study, *BRAF* V600E mutation was detected in a non-smoking female patient and dabrafenib + trametinib treatment was planned.

The use of NGS, which was gradually increasing after finishing TCGA, was at a high cost in the early days, but over the years have been reduced to affordable prices (38). Detecting individual mutations by conventional methods seems to be more cost-effective at first, but the clinical results suggest the opposite. Pennel et al. found that the use of NGS in a health plan involving 1000,000 people was associated with both shorter time and significant cost savings than conventional methods (39). In our country, in fact, one-to-one comparative evaluation of *EGFR, ALK, ROS1* should be spent on average 100-150 USD (approximately 600 Turkish Liras) for conventional methods, while the cost of our NGS panel used in our hospital is 300-350 USD (approximately 1850 Turkish Liras) (according to the data obtained from the purchasing unit of our hospital). However, as the number of patients increases, it is expected that NGS costs will decrease. In addition, for example, if the *EGFR* mutation and concomitant resistance mutation cannot be detected by conventional methods at the diagnosis, the targeted agent to be administered may be ineffective. There are two similar examples in our patient group: gefitinib resistance EGFR mutant patients were detected with NGS and afatinib was applied. As a result, more money is given to the test, but no more money is unnecessarily spent on the whole treatment of the patient. In our country, the health insurance of individuals are covered by the state and have an important place in the budget of the state. Therefore, it is thought that the use of NGS method will be more beneficial for both the patients and the health service provider.

In addition to the positive features of the NGS method, there are also disadvantages (40): The first of these is the ability to detect error in continuously repeating sequence regions. However, when this error persists, it can be understood by the geneticist that it is a mistake. Another is that sometimes certain decisions about the effect of genes cannot be made due to the finalization of the resulting raw data through many software programs. For example, in our study, 2 *BRAF*, 2 *HER2*, 2 *PIK3CA*, and 1 *EGFR* mutations were identified as clinical uncertain significance variants. If these mutations were considered pathogenic, targeted agents could be added to the treatment of 4 of these patients. The last disadvantage is that, although it is gradually decreasing due to the increase in the use of NGS, false-negative results are seen in the liquid biopsy around 20-30%. In our study, we did not detect any *ALK* mutations with NGS, but we detected that one of our patients had *ALK* mutation by FISH method and we achieved a complete metabolic response at 3 months after alectinib use. Therefore, it is the role of the clinician to evaluate the results carefully and confirm them with another method if there is discordance.

The limiting points of our study were retrospective nature, small number of patients, and lack of response to treatment. However, the results of our article are important as there are very few studies showing the effect of NGS in clinical use.

Conclusion

LC is one of the deadly cancers. With the help of targeted agents, patients' survival is extended in a more comfortable way than chemotherapy. Therefore, it is the first duty of the clinician to determine the appropriate patients for the targeted therapies and to initiate the treatment. NGS, which have increased using in recent years, is a method that quickly and accurately identifies all targeted genetic variants and resistance variants at the same time. According to the results, we have shown, NGS has helped us to apply more personalized and more effective treatments to patients for achieving longer survival. In addition, the use of NGS method instead of conventional methods has saved time and cost for both patients and health service providers. For these reasons, it would be more beneficial to use NGS method wherever appropriate.

Acknowledgments: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Author's Contributions: CM, ÇK: field surveys, collection and analysis of data, preparation of the manuscript, ÇK, ÖY, AT, PE: NGS analysis. AT, MB, SBT: reading and revision of the manuscript. CM, AY, ÇK: collection data and preparation of the manuscript. CM, ÇK: development of the manuscript. CM, AY, ÖY, PE: reading, manuscript correction and literature search

References

1. Barta JA, Powell CA, and Wisnivesky JP. Global Epidemiology of Lung Cancer. Ann Glob Health. 2019; 85(1): 8.

2. Oser MG, Niederst MJ, Sequist LV, Engelman JA. Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. Lancet Oncol. 2015; 16(4): 165–172.

3. Siegel RL, Miller KD, Jemal A. Cancer Statics, 2015. CA Cancer J Clin. 2015; 65: 5–29

4. Lu M, Su Y. Immunotherapy in non-small cell lung cancer: The past, the present, and the future. Thorac Cancer. 2019; 10(4): 585–586.

5. Rivero JD, Enewold L, Thomas A. Metastatic lung cancer in the age of targeted therapy: improving long-term survival. Transl Lung Cancer Res. 2016; 5(6): 727–730.

6. Chan BA, Hughes BGM. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res. 2015; 4(1): 36–54.

7. Mayekar MK, Bivona TG. Current Landscape of Targeted Therapy in Lung Cancer. Clin Pharmacol Ther. 2017; 102(5):757-764

8. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med. 2018; 142(3):321-346.

9. Shim HS, Choi YL, Kim L, Chang S, Kim WS, Roh MS, et al. The Korean Cardiopulmonary Pathology Study Group, and The Korean Molecular Pathology Study Group. Molecular Testing of Lung Cancers. J Pathol Transl Med. 2017; 51(3): 242–254.

10. Garinet S, Laurent-Puig P, Blons H, Oudart JP. Current and Future Molecular Testing in NSCLC, What Can We Expect from New Sequencing Technologies? J Clin Med. 2018; 7(6): 144.

11. Kruglyak KM, Lin E, Ong FS. Next-Generation Sequencing and Applications to the Diagnosis and Treatment of Lung Cancer. Adv Exp Med Biol. 2016; 890:123-36.

12. Bernicker EH, Allen TC, Cagle PT. Update on emerging biomarkers in lung cancer. J Thorac Dis. 2019; 11(Suppl 1): 81–88.

13. Mehrad M, Roy S, Bittar HT, Dacic S. Next-Generation Sequencing Approach to Non-Small Cell Lung Carcinoma Yields More Actionable Alterations. Arch Pathol Lab Med. 2018; 142(3):353-357

14. Friedlaender A, Banna G, Malapelle U, Pisapia P, Addeo A. Next Generation Sequencing and Genetic Alterations in Squamous Cell Lung Carcinoma: Where Are We Today? Front Oncol. 2019; 9: 166.

15. Wapiszewski R, Pawlak SD, Adamkiewicz K. Anti-EGFR Agents: Current Status, Forecasts and Future Directions. Targ Oncol. 2016; 11: 739–752

16. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018; 553(7689):446-454

17. Dearden S, Stevens J, Wu YL, Blowers D. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). Ann Oncol. 2013; 24(9): 2371–2376.

18. Nurwidya F,Takahashi F, Takahashi K. Gefitinib in the treatment of nonsmall cell lung cancer with activating epidermal growth factor receptor mutation. J Nat Sci Biol Med. 2016; 7(2): 119–123.

19. Kanthala S, Pallerla S, Jois S. Current and future targeted therapies for non-small-cell lung cancers with aberrant EGF receptors. Future Oncol. 2015; 11(5): 865–878.

20. Takeda M, Nakagawa K. First- and Second-Generation EGFR-TKIs Are All Replaced to Osimertinib in Chemo-Naive EGFR Mutation-Positive Non-Small Cell Lung Cancer? Int J Mol Sci. 2019; 20(1): 146.

21. Wang J, Wang B, Chu H, Yao Y. Intrinsic resistance to EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer with activating EGFR mutations. Onco Targets Ther. 2016; 9: 3711-26

22. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B1, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med. 2018; 378(2):113-125.

23. Ramalingam SS, Gray JE, Ohe Y, Cho BC, Vansteenkiste J, Zhou C, et al. Osimertinib vs comparator EGFR-TKI as first-line treatment for EGFRm advanced NSCLC (FLAURA): Final overall survival analysis. Annals of Oncology (2019) 30 (suppl_5): 851-934.

24. Román M, Baraibar I, López I, Nadal E, Rolfo C, Vicent S, et al. KRAS oncogene in non-small cell lung cancer: clinical perspectives on the treatment of an old target. Mol Cancer. 2018; 19;17(1):33

25. Adderley H, Blackhall FH, Lindsay CR. KRAS-mutant non-small cell lung cancer: Converging small molecules and immune checkpoint inhibition. EBioMedicine. 2019; 41: 711–716.

26. Cantley CL. The phosphoinositide 3-kinase pathway. Science. 2002; 296, 1655–1657

27. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004; 304(5670):554.

28. Wu SG, Chang YL, Yu CJ, Yang PC, Shih JY. The Role of PIK3CA Mutations among Lung Adenocarcinoma Patients with Primary and Acquired Resistance to EGFR Tyrosine Kinase Inhibition. Sci Rep. 2016; 6: 35249.

29. Scheffler M, Bos M, Gardizi M, König K, Michels S, Fassunke J, et al. PIK3CA mutations in non-small cell lung cancer (NSCLC): genetic heterogeneity, prognostic impact and incidence of prior malignancies. Oncotarget. 2015; 6(2):1315-26.

30. Papa A, Pandolfi PP. The PTEN–PI3K Axis in Cancer. Biomolecules. 2019; 9(4): 153

31. Gkountakos A, Sartori G, Falcone I, Piro G, Ciuffreda L, Carbone C, PTEN in Lung Cancer: Dealing with the Problem, Building on New Knowledge and Turning the Game Around. Cancers (Basel). 2019; 11(8): 1141.

32. Ku BM, Heo MH, Kim JH, Cho BC, Cho EK, Min YJ, et al. Molecular Screening of Small Biopsy Samples Using Next-Generation Sequencing in Korean Patients with Advanced Non-small Cell Lung Cancer: Korean Lung Cancer Consortium (KLCC-13-01). J. Pathol. Transl. Med. 2018; 52: 148–156

33. Zhu Z, Yu T, Chai Y. Multiple primary lung cancer displaying different EGFR and PTEN molecular profiles. Oncotarget 2016; 7: 81969–81971

34. Sun H, Ma H, Wang J, Xia L, Zhu G, Wang Z , et al. Phosphatase and tensin homolog deleted on chromosome 10 degradation induced by NEDD4 promotes acquired erlotinib resistance in non-small-cell lung cancer. Tumour Biol. 2017; 39(7): 1010428317709639. doi: 10.1177/1010428317709639.

35. Lin Q, Zhang H, Ding H, Qian J, Lizaso A, Lin J, et al. The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients J Transl Med. 2019; 17: 298.

36. Alvarez JGB, Otterson GA. Agents to treat BRAF-mutant lung cancer. Drugs Context. 2019; 8: 212566.

37. Planchard D, Smit EF, Groen HJM, Mazieres J, Besse B, Helland Å, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. Lancet Oncol. 2017; 18(10): 1307-1316

38. Gong J, Pan K, Fakih M, Pal S, Salgia R. Value-based genomics. Oncotarget. 2018; 20; 9(21): 15792–15815

39. Pennell NA, Mutebi A, Zhou ZY, Ricculli ML, Tang W, Wang H, et al. Economic impact of next generation sequencing vs sequential single-gene testing modalities to detect genomic alterations in metastatic non-small cell lung cancer using a decision analytic model. Journal of Clinical Oncology 2018; 36(15): 9031-9031.

40. Besser J, Carleton HA, Gerner-Smidt P, Lindsey RB, Trees E. Next-Generation Sequencing Technologies and their Application to the Study and Control of Bacterial Infections. Clin Microbiol Infect. 2018; 24(4): 335–341.

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):333-9

Research Article Doi: 10.36472/msd.v6i12.331

A comparison of two supraglottic airway devices in general anaesthesia: baska mask® vs. I-gel®

Ozlem Sezen[1]*

Abstract

Objective: The aim of this was to compare the Baska® mask and the I-gel® airway in paralyzed patients during general anaesthesia in terms of clinical performance, the risk of aspiration, and intraoperative and postoperative characteristics.

Material and Methods: The two devices were compared in 100 paralyzed anesthetized adult patients. Primary outcomes of the study were to evaluate the characteristics of the airway devices within respect to the success of first insertion attempt, the insertion time, the ease of insertion, leak volume, and peak airway pressure. The blood staining on the mask, and the presence of gastric reflux or sore throat two hours postoperatively were evaluated. Hemodynamics, end-tidal CO2 and the peripheral oxygen saturation measurements were secondary outcomes.

Results: No statistically significant difference was observed between the groups in the criteria of first attempt success rate, ease of insertion, blood staining upon removal of the masks, gastric regurgitation, or sore throat two hours after the procedure. The insertion time was significantly longer for the Baska® mask compared with the I-gel® airway. The leak volume was significantly higher in the Baska® mask patients throughout the surgical procedure. The heart rate and mean arterial pressure measurements were significantly higher in the Baska® mask patients.

Conclusions: Both the Baska® mask and the I-gel ® device can be used effectively for selected paralyzed patients under general anaesthesia. The insertion time was significantly longer for the Baska® mask compared with the I-gel® device.

Keywords: Airway management, Baska, I-gel, Laryngeal mask, gastric regurgitation

Introduction

Since Archie Brain introduced the LMA-Classic® (Teleflex Medical Europe Ltd., Westmeath, Ireland) to anaesthesia practice, many supraglottic airway devices (SADs) have been produced. Following the first-generation devices with only a breathing lumen, second- generation SADs with an additional lumen for gastric drainage became available in the market (1). The I-gel® airway (Intersurgical Ltd., Wokingham, Berkshire, UK) is a second- generation SAD with a medical-grade, thermoplastic, elastomeric, soft gel-like structure. It has a non- inflatable cuff designed to fit over the pharynx, larynx and perilaryngeal structures (2). More recently, Australian anesthetists Kanag and Meena Baska designed a new third generation device called the Baska® mask (Logical Health Products PTY Ltd., Morisset, NSW, Australia). This mask brings together clinical characteristics of the LMA-ProSeal®, the LMA-Supreme® (Teleflex Medical Europe Ltd., Westmeath, Ireland), the I-gel® and the SLIPA® device (SLIPA Medical Ltd., London, UK). It has a cuffless membranous bowl, which inflates and deflates with positive pressure ventilation improving the seal, an inbuilt “tab” permitting ease of placement and allowing control of the degree of flexion, a dual drainage system for the prevention of the aspiration of gastric contents, and a bite block to reduce the risk of patients’ biting down on and blocking the airway(3). The structures of two devices are shown in Figure 1.

Since the introduction of Baska® mask many studies have been published that compare the Baska® mask with other airway devices in different patient populations (4-9). The efficacy of this mask has been demonstrated in both spontaneously breathing and paralyzed patients (10-12).

In this study, the aim was to compare the performance of the Baska® mask and the I-gel® airway in paralyzed patients during general anaesthesia in terms of clinical performance, the risk of aspiration, and intraoperative and postoperative characteristics. The secondary objectives were to assess hemodynamic parameters, peripheral oxygen saturation, and end-tidal CO2 variability induced by intraoperative mask placement.

Received 24-11-2019 **Accepted** 22-12-2019 **Available Online** 26-12-2019 **Published** 30-12-2019
1 University of Health Sciences, Kartal Dr. Lutfi Kırdar Training and Research Hospital, Istanbul, TR
*** Corresponding Author:** Özlem Sezen **E-mail:** drozlemsezen@hotmail.com

http://dx.doi.org/10.36472/msd.v6i12.331

Material and Methods

The study was conducted according to the ethical principles outlined in the Helsinki Declaration and the Guideline of Good Clinical Practice. After obtaining approval from the Ethics Committee (decision no: 2018/514/124/9) and written informed consent, the study was conducted with 100 patients of American Society of Anesthesiologists (ASA) physical status I-II and aged 18 years or older of either genders. (Figure 2) The patients were not assessed to be at risk for a difficult airway in the preanaesthetic evaluation and were scheduled for an elective, flexible ureterorenoscopy in the supine position for which a no 4 size Baska® mask (Group B) or I-gel® (Group I) was suitable for airway management. Patients were randomly assigned to either Baska® mask (Group B; n=50) or I-gel® (Group I; n=50) by a computer-generated randomization method (13).

Exclusion criteria included obese patients (Body mass index ⩾30 kg/m2), patients with known gastrointestinal reflux or, upper respiratory tract infections, planned surgical duration of ⩾2 hours, patients with neck pathology and patients with oral or dental deformities. The patients fasted for at least eight hours prior to the surgical procedure, including both solids and clear liquids.

All of the patients were premedicated intravenously with 0.03 mg/kg midazolam about 30 minutes before the induction of anaesthesia. Prior to the operation, standard monitoring included a 3-lead electrocardiogram (ECG) with continuous ST-segment analysis, and evaluation of peripheral oxygen saturation (SpO2) and intermittent non-invasive blood pressure. Following preoxygenation with 100% oxygen for three minutes, general anaesthesia was induced with propofol (3mg/kg) a few minutes after a fentanyl (2µg/kg) injection. Neuromuscular paralysis was achieved with rocuronium (0.5 mg/kg) in all patients. In the event of coughing, gagging, or body movement, an additional dose of propofol was administered. Mask ventilation was continued with 100% oxygen until the adequate jaw relaxation was confirmed. The patient's head was placed on a silicone pillow in the sniffing position. All device insertions were performed by personnel with significant experience in laryngeal mask insertion.

The standard pre-use test was performed to check structural integrity. A well-lubricated size- 4 Baska® mask or I-gel® airway device was chosen according to the prior randomization protocol. According to the manufacturer-recommended approach, the mask size was based on the patient's weight. When the adequate depth of anaesthesia and jaw relaxation were achieved, the mask was held away from the airway tube with the dominant hand facing the cuff outlet anteriorly and pushed against the hard palate until encountering resistance. The non-dominant hand was used to extend the inter-incisor distance and compress the tongue while the device was advanced into the mouth. A maximum of three attempts per patient was permitted to determine successful placement. In the event of a failed insertion, another SAD or endotracheal intubation was used and the patient was excluded from the study. Successful insertion was confirmed with the inspection of bilateral chest movements, auscultation of both lungs and a capnography interpretation. After successful ventilation was ascertained, the mask was connected to a breathing circuit and fixed by taping the device in place. Anaesthesia was maintained with sevoflurane of 1 to 2% volume with a mixture of 50% oxygen-air in a fresh gas flow of 2 L/minute.

All patients received 1 gr paracetamol and 1 mg/kg tramadol for postoperative analgesia. At the end of the surgery, the anesthetic gas mixture was replaced with 100% oxygen and the neuromuscular block was reversed using a neostigmine (0.05 mg/kg)-atropine (1mg) combination. After adequate ventilation, protective airway reflexes and the patients' response to verbal commands were established, the mask was removed. Immediately after removal, the pH of the posterior surface of the mask was measured with a pH indicator strip (DIRUI H11, DIRUI Industrial Co., Ltd. Changchun, China) and the pH⩽6 (the normal pH of saliva is 6.2-7.6) was accepted as evidence of gastric regurgitation. Any blood staining on the mask was recorded.

Data collection: The patients' characteristics including age, gender, body weight, Mallampati score, American Society of Anesthesiologists (ASA) classification of physical status and the duration of anaesthesia were recorded. The insertion time (the time between picking up the mask by the anesthesiologist and successful placement), number of attempts needed for correct placement of the mask, the ease of insertion (very easy, easy, difficult), the leak volume (calculated by the difference between inspiratory and expiratory volume), peak airway pressure, the presence of blood staining on the mask, whether or not there was gastric reflux in the oral cavity, and a sore throat were the primary outcome measurements of this study. The presence of a sore throat was determined by another blinded observer 2 hours after the operation.

Secondary outcomes included the end-tidal CO2 (EtCO2), peripheral oxygen saturation (SpO2) and the hemodynamic variability throughout the surgical procedure.

Statistical analysis: The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± SD while frequency and percentage was computed for gender, ASA status, Mallampati score, insertion attempts, ease of insertion and postoperative outcomes. The Kolmogorov-Smirnov test was used to test for a normal distribution of data. Student's t-test was used to compare the differences in quantitative measurements between groups. A chi-square and Fisher's exact test were used to evaluate the between-group differences in categorical variables. The Mann-Whitney U-test was applied to unpaired and independent observations. A value of $p<0.05$ was considered significant in this study.

For two independent and continuous group comparisons with 0.05 confidence level (Type I error) and 0.80 power (Type II error), the sample size was calculated to be at least 17 for each group. However, in order to make the study more reliable, 50 patients for each group were included in the study.

http://dx.doi.org/10.36472/msd.v6i12.331

Results

The analysis of patients' characteristic indicated that there was a statistical significance in gender representation due to randomization (p=0.016).

However, the mean body length and weight of the patients in the groups were similar. The majority of patients in both groups had an ASA II score; the figure was significant in Group I (p=0.002).

There was no between-group difference in the Mallampati scores (Table 1). The duration of surgery was 55.80±21.10 minutes and 47.20±15.65 minutes respectively in Group B and Group I (p=0.023).

The number of mask insertion attempts was similar in both groups. The insertion time was significantly longer in Group B (p <0.001). One patient in each group was defined as a case of difficult insertion. No blood staining, gastric reflux, or sore throat was recorded two hours after the procedure (Table-2).

Analysis of intraoperative variables revealed that the heart rate and the mean arterial pressure measurements were significantly high until the 60th minutes of surgery in Group B.

The EtCO2 variables were similar in the groups. There was small but significant decline in the SpO2 value after the insertion of the mask in Group I patients, but there was no significant difference between groups during the remainder of the procedure (Figure 3).

The peak airway pressure difference was comparable in both groups but the leak volume was significantly greater in Group B patients (p <0.001) (Figure 4).

No untoward effect was recorded throughout the study period.

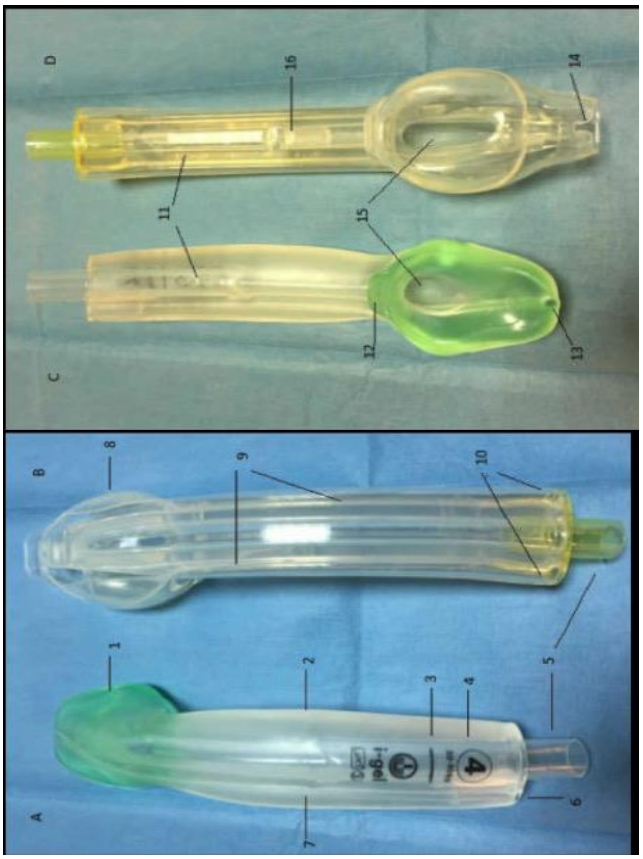


Figure 1. The structural characteristics of the I-gel® airway (A,C) and Baska® mask (B, D). Posterior aspect of the I-gel® device demonstrating the non-inflatable cuff made from a soft gel-like material (1), buccal cavity stabilizer eliminating the potential for rotation (2), position guide for confirmation of the depth of insertion (3), the size and weight guide for the mask (4), proximal end of the gastric channel (6) and the gastric channel (7). The standard connector of both masks is suitable for circle-system connection and catheter mount (5). The cuffless, membraneous bowl of the Baska® mask (8) with 2 gastric channels (9) and 2 openings to the atmosphere (10). An anterior aspect view, illustrating the integral bite block to reduce airway occlusion (11), the epiglottic rest of the I-gel® device to prevent the epiglottic "down fold" and airway obstruction (12), the distal ends of the gastric channels (13,14), the airway orifice of both masks (15) and the insertion "tab" of the Baska®mask to ease the manual curve during insertion (16).

Table 1. The demographics of the study groups

Variable		Group B	Group I	Test Statistics	P value
Gender [1]	Female Male	32 (64) 18 (36)	20 (60) 30 (40)	Pearson Chi-square:5,769 df:1	**0.016***
Age (years) [2]		47.32±13.82	51.16±13.77	Independent sample t test:0.167 df:98	0.167
Body length (cm)[2]		166.36±7.76	164.76±7.22	Independent sample t test:-0,167 df:98	0.288
Body weight (kg)[2]		76.16±15.15	72.34±13.31	Independent sample t test:-1,339 df:98	0.184
ASA class[1]	I II	14 (28) 36 (72)	2 (4) 48 (96)	Fisher's exact test	**0.002***
Mallampati score[1]	I II	22 (44) 28 (56)	30 (60) 20 (40)	Pearson Chi-square:2,564 df:1	0.109

Table 2. Device characteristics

Variables		Group B	Group I	P Value
Number of attempts[1]	**1**	47(94)	49(98)	0.617
	2	3(6)	1(2)	
Insertion time		27.97± 12.97	12.73 ±2.01	<0.001*
				95% Confidence Interval of the Difference
(sec)[2]				Lower Upper
				-18,68170 -10,92710
Blood staining[1]	+/-	0/50 (100)	0/50 (100)	---
Sore throat after 2 hrs[1]	+/-	0/50 (100)	0/50 (100)	---
Gastric reflux [1]		0/50 (100)	0/50 (100)	---

Data are expressed as 1 the number of the patients (n) and the percentage (%) or 2 (mean± SD). * p<0.001, statistically highly significant

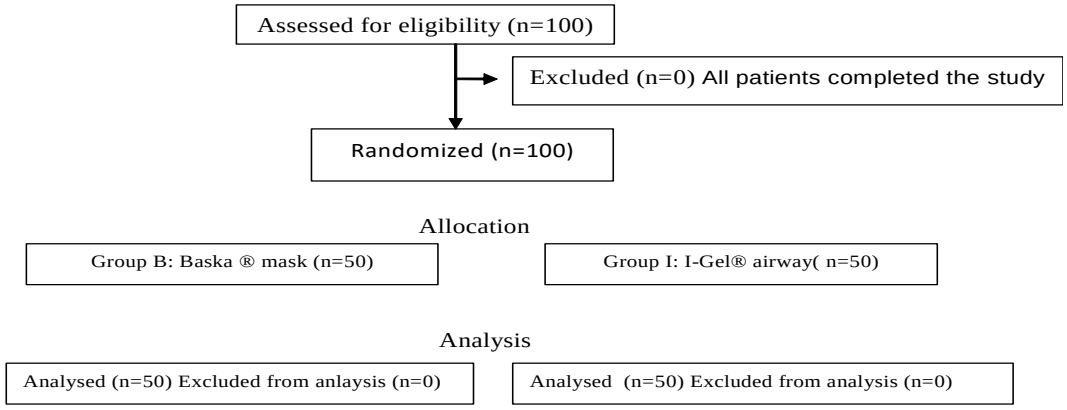


Figure 3. Intraoperative variables of the study groups. A. Heart rate (HR), B. Mean arterial pressure (MAP), C. Peripheral oxygen saturation (SpO2), D. End-tidal carbondioxide (EtCO2) Group B: Baska® mask; Group I: I-gel® airway

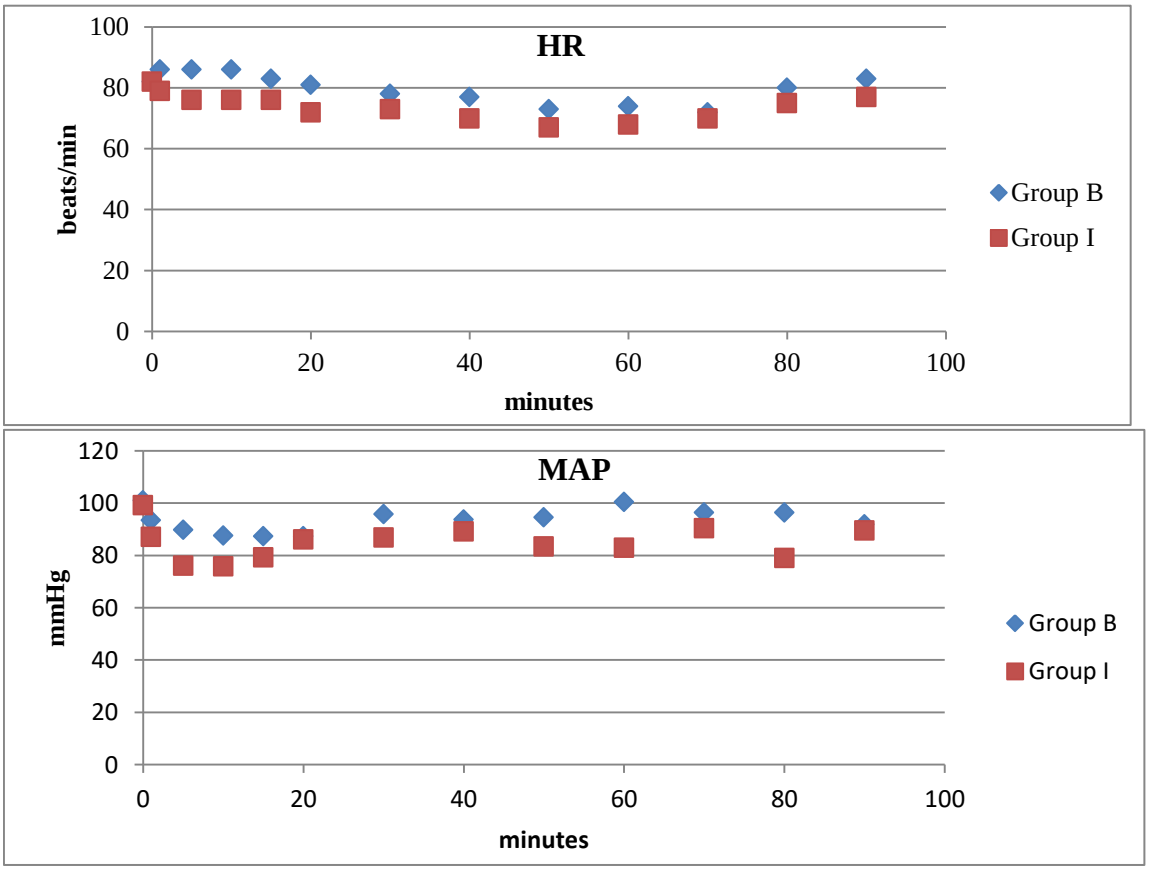


Figure 3. Intraoperative variables of the study groups. **A.** Heart rate (HR), **B.** Mean arterial pressure (MAP), C. Peripheral oxygen saturation (SpO2), D. End-tidal carbondioxide (EtCO2) Group B: Baska® mask; Group I: I-gel® airway

http://dx.doi.org/10.36472/msd.v6i12.331

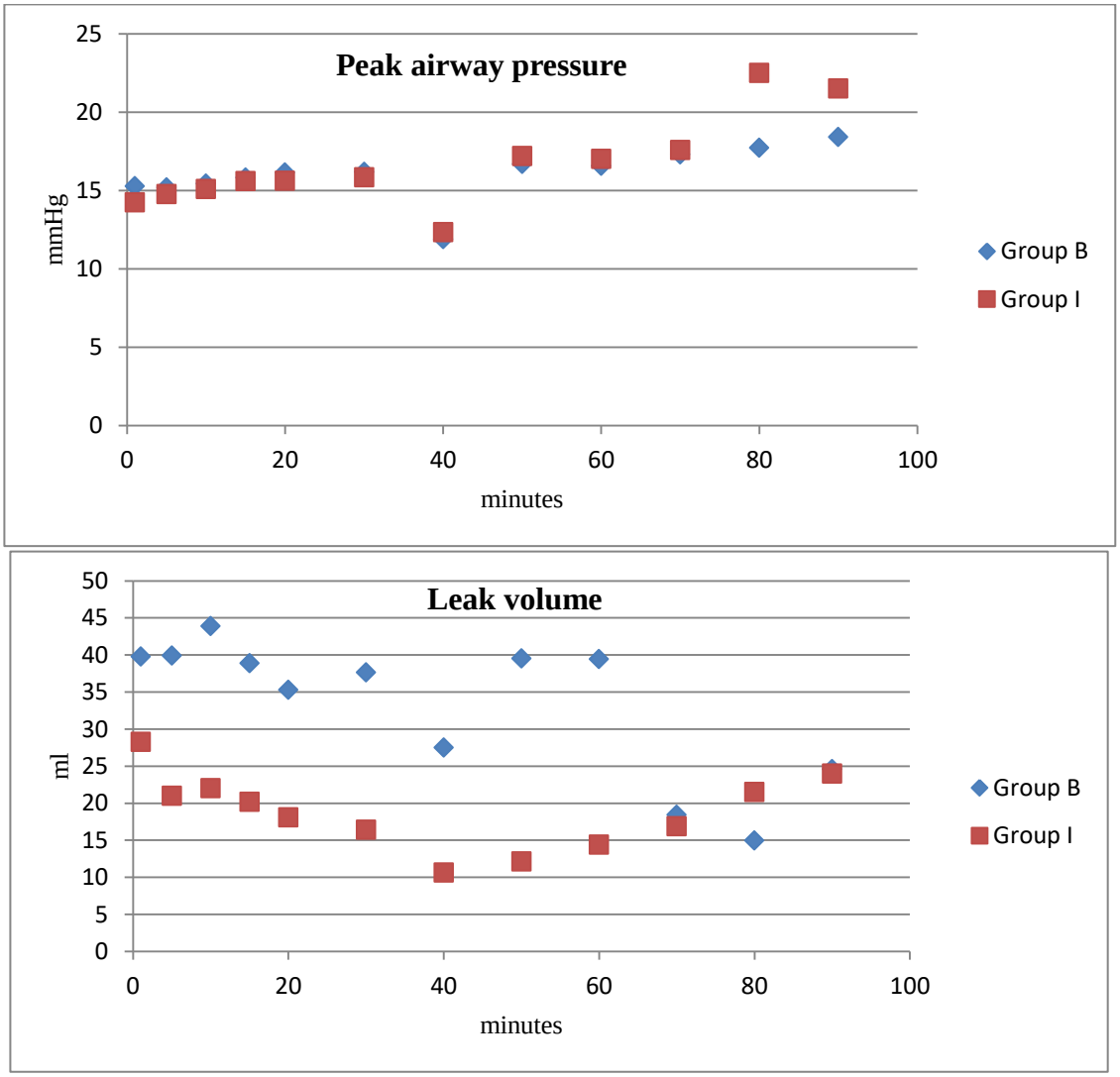


Figure 4. The measurements of peak airway pressure **(A)** and leak volume **(B).** Group B: Baska® mask; Group I: I-gel® airway

Discussion

This study indicated that results obtained using the I-gel® mask were superior to those using the Baska® mask in paralyzed patients during general anaesthesia in terms of a lower leak volume and more stable intraoperative hemodynamic characteristics. The I-gel® device provided easier and faster airway management than the Baska® mask. There was no significant difference regarding blood staining, regurgitation, or a sore throat after the operation. Both devices provided a safe airway management under positive pressure ventilation.

According to the earliest data published related to the Baska® mask, the first-time insertion success rate was reported to be 73%, 76.7% and 88% (6,11,12). In a recent study, the percentage was reported as 92.5%10. This may be explained by increasing experience with the device over time. Studies comparing the Baska ® mask to other SADs have yielded varied results.

Aziz et al. (5) reported that first-attempt insertion of the Baska® was better than that of I-gel® (90% vs 83.3%). Shanmugavelu et al. (4) demonstrated in an observational study that the first attempt success rate was comparable between the Baska® mask and the I-gel® airway. The results were similar to those seen with the ProSeal® laryngeal mask (7). In our study, the difference in the success rate for insertion on the first attempt was insignificant, with 94% for the Baska® mask and 98% for the I-gel® airway (p=0.617).

The insertion time has been a controversial issue. Some earlier studies found the Baska® mask to be difficult to insert and suggested that it was time-consuming (4,6,9,10,12). Yet a comparative analysis of the Baska® mask with the I-gel® and the ProSeal® devices indicated superiority of the Baska® mask (5,7). Our results revealed that the insertion time was significantly shorter in the I-gel® group (p=0.000); however the Baska® mask device is a new device for us so the insertion time may improve over time with clinical practice.

The one of the primary outcomes of this study was to compare the leak volume and peak airway pressure created by both devices. Aziz et al. (14) suggested that the head and neck position reflected no significant changes in respect of oropharyngeal leak and peak airway pressure with the Baska® mask in a comparative clinical trial. The Baska® device was also demonstrated to be a suitable airway device for positive pressure ventilation with a minimum leak even in the event of increased intra-abdominal pressure (4,5,15). Alexiev et al. (6,12) emphasized the importance of anaesthesia depth during mask insertion and suggested that the leak amount showed parallelism to the depth of anaesthesia. They reported that positive pressure ventilation had a positive effect on the leak around the mask secondary to cuff-seal improvement with each inflation. Our results demonstrated indicated a significantly higher leak volume in patients enrolled in the Baska® mask group.

The analysis of intraoperative hemodynamics revealed a significant difference in favor of the I-gel® group in heart rate and mean arterial pressure. This result contradicted some previous reports. Fotedar et al. (9) demonstrated that the I-gel® and the Baska® devices provided similar intraoperative hemodynamics. In a comparative study with a single-use laryngeal mask airway (LMA), the Baska® mask demonstrated no significant hemodynamic differences (6). Nonetheless, in the literature, many authors have concentrated the characteristics of the Baska® mask and the data concerning the effects on the perioperative hemodynamic parameters are limited. Our results may be re-evaluated in subsequent clinical trial with a larger sample size. This may be more beneficial to understanding whether or not the Baska® mask has a negative effect on intraoperative hemodynamics. As in some previous reports, the SpO2 and EtCO2 results were comparable in our study (5,9).

Pharyngolaryngeal morbidity is a frequent postoperative adverse outcome associated with patients' satisfaction and delayed post-operative discharge. The larynx has a mucosal structure covered by a cartilaginous framework and can be easily damaged during endotracheal intubation or placement of a SAD. Fotedar et al. (9) reported a 5% occurrence of a sore throat and cough that resolved within 6 hours of postoperatively in spontaneously breathing patients after Baska® mask insertion during anaesthesia. In order to decrease the incidence of sore throat after SADs use, lubrication of the posterior aspects of the mask with a water-soluble jelly is the preferred method. Alternative techniques, including a cuff wash, lidocaine gel and washing the mouth with saline before the mask removal have showed no benefit (16). It has been reported that the use of lidocaine may result in a delay in recovering the protective reflexes or may trigger the allergic reactions (17). In our clinical protocol, we use a water-soluble lubricant to ease the insertion of SADs. No blood staining or a sore throat was recorded two hours after the operation in either study groups in this trial.

Despites its many advantages, positive pressure ventilation with an LMA is considered by many authors as a risk factor for pulmonary aspiration, as well as gastric insufflations (18-22). Second generation SADs with a gastric lumen were introduced to decrease this risk. The suction port of the device can be helpful throughout the procedure or during the removal of the mask in the risk of gastric regurgitation. This port may also be used for the placement of a gastric tube to empty the stomach (11). In a comparative study with an LMA, the incidence of gastric insufflation was significantly lower in I-gel® patients (23). In a recent study of a geriatric population, the I-gel® had superior results in terms of gastric insufflation when compared with the LMA-Supreme™ (24). A cadaver study demonstrated that an inspiratory pressure of 20 mbar is a safe airway pressure to prevent gastric insufflation during SADs insertions (25). Saracoglu et al. (26) reported that the I-gel device can be used safely in both the supine and lateral positions. In our study, the mean peak airway pressures throughout the procedure was 16.04±1.63 mmHg in Group B and 16.60±2.89 mmHg in Group I ($p<0.05$). There was no instance of gastric reflux with either device at these pressure levels.

The sample size may be a limitation of this study. As previously mentioned, the I-gel airway has been used in our clinical practice for a long time but the Baska® mask was a new device for us. Those participating in this research had no prior training with the Baska® mask before the trial. This was a randomized clinical study designed to provide information about the Baska® mask and its' clinical characteristics. Both airway devices have been introduced by the investigators. So, the study was not blinded for researchers; there is a risk of bias in subjective measures. All of the researchers had similar experience with other SADs.

Another limitation is that we enrolled only Mallampati I and II patients in this study. The effectiveness of both masks in an airway predicted to be difficult was not observed. Furthermore, neither mask was used in high-risk patients. These points may be the subjects of new clinical trials in the future.

Conclusion

In our opinion, the Baska® mask demonstrated clinical utility as a useful supraglottic airway device. Both the Baska ® and the I-gel® device provided a safe airway management in paralyzed patients under positive pressure ventilation. The observed difference in insertion time may reflect a required-learning period with the Baska® mask. Although the leak volume in the Baska® mask was significantly greater than that of the I-gel®, this did not create an untoward clinical effect in our study. This subject requires further evaluation, but these results may re-enforce new clinical studies.

Acknowledgments, Funding: None

Conflict of interest and financial disclosure: The authors declare that there is no conflict of interest and financial relationships.

Author's contiributions: OS; Research concept and design; patient examination, data collecting, analysis and interpretationof data, preparation article and revision.

Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Michalek P, Donaldson W, Vobrubova E, Hakl M. Complications associated with the use of supraglottic airway devices in perioperative medicine. Biomed Res Intern. 2015; 2015:746560. doi: 10.1155/2015/746560. Epub 2015 Dec 13.
2. https://www.intersurgical.com/info/igel (access date 29/10/2018).
3. http://www.baskamask.com.au (access date 29/10/2018).
4. Shanmugavelu G, Kanagarajan T. Comparing the functional analysis of I-gel with Baska mask in laparoscopic surgeries: an observational study. Int J Res Med Sci. 2018; 6(4): 1440-43.
5. Rehab Abdel Raof Abdel Aziz, Yasser Mohamed Osman. Comparison of I-gel with Baska mask airway for controlled ventilation in obese patients undergoing ambulatory surgery: A prospective randomized trial. Journal of Anesthesiology. 2017; 5(4): 29-35.
6. Alexiev V, Ochana A, Abdelrahman D, et al. Comparison of the Baska® mask with the single-use laryngeal mask airway in low-risk female patients undergoing ambulatory surgery. Anaesthesia. 2013; 68 (10): 1026-32.
7. Al-Rawahi SAS, Aziz H, Malik AM, Khan RM, Kaul N. A comparative analysis of the Baska® mask vs. Proseal® laryngeal mask for general anaesthesia with IPPV. Anaesth Pain &Intensive Care. 2013; 17(3): 233-236.
8. Durrani HD, Butt KJ, Sadaf S, Rehan A, Khan AM, Umar A. Comparison of LMA Classic and I-gel in anesthetized spontaneously breathing patients during elective surgical procedures. Anaesth Pain& Intenive Care. 2013; 17(3): 274-78.
9. Fotedar S. A comparison between the i-gel and the Baska mask in spontaneously ventilating anaesthetised patients. Int J Sci Res. 2018; 7(4): 23-6.
10. Shivani Fotedar, Lt (Gen), Sujan Singh, Col RK Tripathi. The Baska Mask trial in patients undergoing laparoscopic cholecystectomies. Intenational Journal of Scientific Research Publications (IJSRP). 2018; 8(3) (ISSN: 2250-3153) DOI: http://dx.doi.org/10.29322/IJSRP.8.3.2018.p7515.
11. Zundert Tv, Gatt S. The Baska Mask®- A new concept in self-sealing membrane cuff extraglottic airway devices, using a sump and two gastric drains: A critical evaluation. J Obstet Anaesth Crit Care. 2012; 2(1): 23-30.
12. Alexiev V, Salim A, Kevin LG, Laffey JG. An observational study of the Baska® mask: a novel supraglottic airway. Anaesthesia. 2012; 67 (6): 640-45.
13. https://www.random.org/(Access date: 12/11/ 2018)
14. Rehab Abdel-Raof Abdel-Aziz, Yasser Mohamed Osman. Evaluation of Head and Neck Position on Oropharyngeal Leak Pressure with Baska Mask and Streamlined Liner of Pharyngeal Airway (SLIPA™): A Randomized Clinical Trial. Journal of Anesthesiology. 2017; 5(5): 36-41.
15. Mahajan SR, Mahajan M, Chaudhary U, Kumar S. Evaluation of Baska mask performance in laparoscopic cholecystectomies. Journal of Dental and Medical Sciences. 2018 17(4): 74-78.
16. Taghavi Gilani M, Miri Soleimani I, Razavi M, Salehi M. Reducing sore throat following laryngeal mask airway insertion: comparing lidocaine gel, saline, and washing mouth with the control group. Rev Bras Anestesiol. 2015; 65(6):450-454.
17. Sengupta S, Roy MB. Use of lubricating jelly for laryngeal mask airways. Indian J Anaesth. 2011; 55(5):545.
18. Sidaras G, Hunter JM. Is it safe to artificially ventilate a paralysed patient through the laryngeal mask? The jury is still out. Br J Anaesth. 2001; 86 (6): 749–53.
19. Devitt JH, Wentsone R, Noel AG, O'Donnell MP. The laryngeal mask airway and positive-pressure ventilation. Anesthesiology. 1994; 80 (3): 550–5.
20. Weiler N, Latorre F, Eberle B, Goedecke R, Heinrichs W. Respiratory mechanics, gastric insufflation pressure, and air leakage of the laryngeal mask airway. Anesth Analg. 1997; 84 (5): 1025–8.
21. Asai T, Kawashima A, Hidaka I, Kawashi S. The laryngeal tube in comparison with the laryngeal mask: insertion, gas leak and gastric insufflation. Br J Anaesth. 2002; 89 (5): 729–32.
22. Miller DM, Light D. Storage capacities of the laryngeal mask and laryngeal tube compared and their relevance to aspiration risk during positive pressure ventilation. Anesth Analg. 2003; 96 (6): 1821–2.
23. Helmy AM, Atef HM, El-Taher EM, Henidak AM. Comparative study between I-gel, a new supraglottic airway device, and classical laryngeal mask airway in anesthetized spontaneously ventilated patients. Saudi J Anaesth. 2010; 4 (3): 131-6.
24. In CB, Cho SA, Lee SJ, Sung TY, Cho CK. Clinical performance of airway management with the i-gel and laryngeal mask airway supreme in geriatric patients: a prospective and randomized study Korean J Anesthesiol. 2019;72(1):39-46. doi: 10.4097/kja.d.18.00121. Epub 2018 Oct 22.
25. Schmidbauer W, Genzwürker H, Ahlers O, Proquitte H, Kerner T. Cadaver study of oesophageal insufflation with supraglottic airway devices during positive pressure ventilation in an obstructed airway. Br J Anaesth. 2012; 109 (3): 454-58.
26. Saracoglu KT, Demir A, Pehlivan G, Saracoglu A, Eti Z. Analysing the efficacy of the I-gel supraglottic airway devices in the supine and lateral decubitus positions. Anaesthesiol Intensive Ther. 2018;50(4):259-262

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):340-6

Research Article Doi: 10.36472/msd.v6i12.333

An investigation of the protective effects of Dehydroepiandrosterone (DHEA) in chemotherapatic Cyclophosphamide (CP) induced ovarian damage on rats

Önder Sakin[1]*, Yasemin Alan[2], Ali Doğukan Anğın[1], Kahyan Başak[3], Murat Alan[4]

Abstract

Objective: In this study, we aimed to investigate the preventive effect of Dehydroepiandrosterone (DHEA) on Cyclophosphamide (CP) induced damage on rat ovarium.

Material and Method: Wistar Albino Rats have been used for the study and three groups have been created. Group 1 (the control Group): no treatment was administered. Intact ovarian tissue was removed and blood samples were taken for anti-mullerian hormone (AMH) test. Group 2 (the CP Group): Rats received CP intraperitoneally at a single dose of 150 mg / kg. Group 3 (the CP + DHEA Group): Rats received CP intraperitoneally at a single dose of 150 mg / kg at baseline and DHEA has been administrated subcutaneously for 10 days at a dose of 60 mg / kg daily. Rats in groups 2 and 3 were sacrificed at the end of 10 days, ovarian tissues were removed and blood samples have been collected for AMH test.

Results: While normal ovarian tissue damage scores were zero, CP showed significant damage and histopathological changes on ovarian tissue in all CP administrated rats. CP group had higher vascular congestion ($p=0.004$) and total damage scores ($p=0.010$) than normal ovarian group. CP + DHEA group had higher edema ($p<0.001$), vascular congestion ($p<0.001$) and total damage scores ($p<0.001$). CP group had a decrease in primordial ($p = 0.001$), primary ($p = 0.043$) and preantral follicles ($p = 0.006$). CP + DHEA group showed a decrease in primordial ($p = 0.001$) and antral follicles ($p = 0.018$). AMH levels did not decrease in both groups.

Conclusions: It was found that the use of DHEA to prevent CP-induced ovarian damage in rats did not produce significant changes in antral follicle counts, ovarian volume, and AMH levels, which were important for clinical practice.

Keywords: CP; dehydroepiandrosterone; anti-mullerian hormone; ovary; rat

Introduction

It is well known that chemotherapy, which is used to treat cancers commonly observed in girls and women, can deplete ovarian reserve and cause infertility or early menopause (1). Cancer treatments increase survival in both pediatric and young patients. However, the fertility problems associated with the anti-tumor treatments administered to this patient group have not yet been resolved. New treatments and new strategies are needed to prevent ovarian damage and to restore ovarian functions (1,2).

The most ovotoxic chemotherapy agents are the nitrogen mustard-derived alkylators (e.g. CP, cisplatin, etc.) (1). Today, approximately 5% of malignant neoplasms occur in women younger than 35 years of age (3). Women who have survived severe treatment protocols are likely to suffer from ovarian infertility problems (4).

CP is widely used in the treatment of many diseases, since it is a low-cost and effective therapeutic agent (5).

Received 28-11-2019 **Accepted** 26-12-2019 **Available Online** 28-12-2019 **Published** 30-12-2019
1 University of Health Sciences, Kartal Training and Research Hospital, Obstetrics and Gynecology Clinic, Istanbul, TR
2 İzmir Metropolitan Municipality Eşrefpaşa Hospital, Obstetrics and Gynecology Clinic, İzmir, TR
3 University of Health Sciences, Kartal Training and Research Hospital, Pathology Clinic, Istanbul, TR
4 University of Health Sciences, Tepecik Education and Research Hospital, Obstetrics and Gynecology Clinic, İzmir, TR
*** Corresponding Author:** Önder Sakin **E-mail:** sakin-onder@hotmail.com

http://dx.doi.org/10.36472/msd.v6i12.333

These include many diseases such as cancers (breast cancer, ovarian cancer, hematological cancers, etc.), hematological diseases, nervous system diseases, immune diseases, rheumatoid arthritis and nephrotic syndrome (6,7). CP belongs to the oxazaphosphorine family of mustard-alkylating agents (8) and has been used for more than 50 years in the clinical practice (9).

It is highly ovotoxic, and its ovarian toxicity and relationship with infertility have been proven (9). It has been reported that it is the agent with the highest impact on women's fertility(10, 11) and is associated with a high risk of ovarian insufficiency (4). Early ovarian insufficiency, premature menopause and impaired reproductive potential after chemotherapy can significantly affect quality of life in this young age group (12,13). Despite efforts to understand the details of ovarian damage and insufficiency caused by CP, the mechanisms remain unclear (14).

Dehydroepiandrosterone (DHEA) is an endogenous steroid produced by the zona reticularis of the adrenal cortex and ovarian theca cells (15). It promotes follicular development and granulosa cell proliferation by increasing intraovarian androgen concentrations in the ovaries (16). The responses given in a worldwide survey conducted in 196 in vitro fertilization (IVF) units in 45 countries, representing a total of 124,700 IVF cycles, revealed that DHEA was included in more than a quarter of treatment protocols concerning patients with reduced ovarian reserve (17). Several reports have shown that DHEA supplementation in patients with low ovarian reserve helps to improve ovarian reserve parameters (18,19), increase ovarian response (20), increase pregnancy rates and reduce age-related aneuploidy (21).

Our aim in this experimental study was to investigate whether DHEA has protective effects on ovarian damage caused by CP.

Materials and Methods

This study was conducted at the Animal Testing Laboratory of the University of Üsküdar in July 2019, after the approval of the Ethics Committee.

Laboratory animals and the care of animals in research: Ten-twelve weeks old, female Wistar Albino (Rattus Norvegicus species) rats weighing 175 to 210 grams were used in this study. Rats were exposed to light for 12 hours a day (from 08:00 to 20:00) and had access to food (standard rodent pellet) and drinking water (tap water) without restriction and were kept at room temperature of 21 to 23ºC and a humidity of 40 to 50%. 4 or 5 rats were placed in every cage. The number of rats was determined in line with the previous studies. Rats were randomly assigned to four groups of 8. Considering bowel transit time, rats were not fed within 6 hours before laparotomy to empty the gut and allow surgery but they had access to drinking water.

Study groups: Group 1 (the control group): These rats underwent a laparotomy procedure at baseline and their ovaries were removed. Blood was drawn from the inferior vena cava for anti-mullerian hormone (AMH) testing.

Group 2 (the CP group): These rats received CP intraperitoneally at a dose of 150 mg/kg at baseline (22) and underwent an oophorectomy procedure at the end of the 10th day of the study. After the rats were sacrificed, at least 2-3 ml of blood was collected for AMH testing. Then laparotomy was performed and both ovaries were excised for histopathological examination.

Group 3 (the CP + DHEA Group): Rats received CP intraperitoneally at a dose of 150 mg/kg at baseline. In addition they received DHEA (Cayman Chemical, Michigan, USA, CAS registry no: 53-43-0, item no:15728) subcutaneously for 10 days at a dose of 60 mg/kg daily as dissolved in 0.1 ml of sesame oil. (23, 24)After the rats were sacrificed, at least 2-3 ml of blood was collected for AMH testing. Then laparotomy was performed and both ovaries were excised for histopathological examination.

CP dose and preparation: CP was administered intraperitoneally only at baseline at a dose of 150 mg/kg. While preparing the drug,we used the central drug preparation unit of our hospital (with Robotic Chemotherapy Drug Preparation System) in a closed environment where microbiological contamination and employee exposure risks were eliminated under conditions that comply with national and international standards.

This Drug Preparation System uses a negative pressure indoor air environment complying with ISO 5, Class 100 and GMP Class A, double HEPA filter air cleaning system, safe waste management system, high capacity laminator current and dose sensitivity information (gravimetric and volumetric) measurement and uses the barcode system during drug preparation process.

Surgical procedures: Laparotomy was performed after decapitation of the animals. Sterile, powder-free, latex gloves were used during all surgical procedures. The procedure was performed while rats were lying in a supine position. Abdominal area was shaved before the procedure and the surgical site was prepped using 10% Povidone-iodine solution (Batticon; Adeka Laboratories, Istanbul, Turkey).

A 5 cm median (on the line between the xiphoid process and pubis) incision was made to enter into the abdominal cavity and each surgical procedure lasted 5 to 10 minutes to protect the drying effect of the room air (Figure 1). After the removal of ovaries for histological examination, animals were disposed of in red waste containers.

Histopathological examinations: Surgically excised ovaries were fixed in 10% formalin. Paraffin blocks were prepared 24 hours after the oophorectomy procedure. Tissue sections of 5 micrometers were taken and follicular activity was assessed in 5 randomly selected samples from each ovary. Slides were stained with hematoxylin eosin and examined under the light microscope. The paraffin blocks were sectioned using a microtome blade (Leica, Nussloch, Germany). Every slide was blindly assessed by the same pathologist. A light microscope (Olympus Clinical Microscope, Tokyo, Japan) was used to analyze the sections. Edema, vascular congestion, inflammation, cellular degeneration and hemorrhage were examined as

http://dx.doi.org/10.36472/msd.v6i12.333

histopathological injury scores. The scores were evaluated as described by Celik et al. Pathological findings were rated. Grade 0 indicated normal alterations, no abnormal findings; Grade 1 indicated mild edema, mild vascular congestion, absence of hemorrhage or leukocyte infiltration; Grade 2 indicated moderate edema, moderate vascular congestion, absence of hemorrhage or leukocyte infiltration; Grade 3 indicated severe edema, severe vascular occlusion, minimal hemorrhage and minimal leukocyte infiltration, Grade 4 indicated severe edema, severe vascular occlusion, hemorrhage and leukocyte infiltration. (Figure 2)

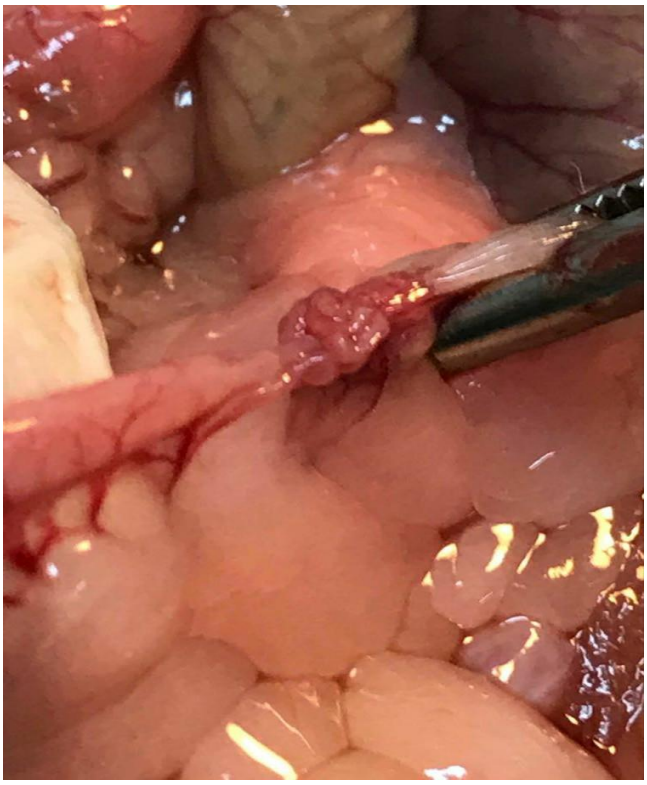


Figure 1. Excision of the ovary

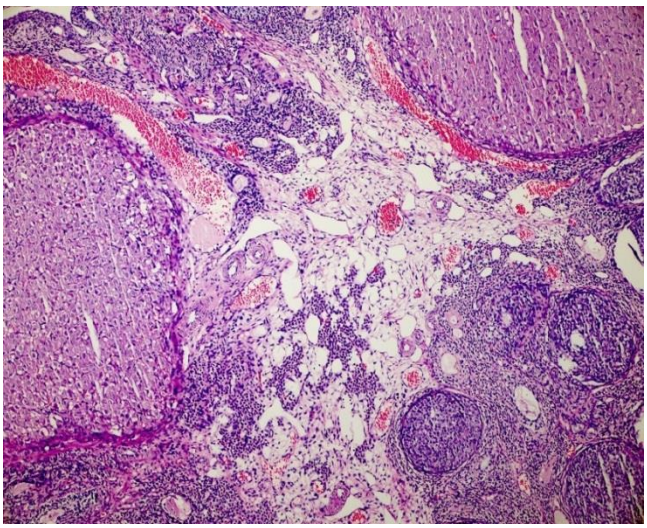


Figure 2. Edema in the medullar region - dilated vessels x200 hematoxylin eosin

All follicles were counted to assess ovarian reserve. Primordial, primary, secondary (pre-antral), tertiary (antral) and atretic follicles were counted (figures 3). Follicles were evaluated as described by Parlakgumus et al. (25). Primordial, primary, secondary (pre-antral) and tertiary (antral) follicles were counted. Primordial follicle is described as an oosit, surrounded with only one layer of epithelial cell layer, primer follicle is surrounded with one or more layers of cuboidal granulosa cells. Secondary/ pre-antral follicle is surrounded with more than two cell layers and consists of antrum folliculi and zona pellucida. The follicle which has an antrum and stratum granulosum and is surrounded with cumulus oophorus is defined as a tertiary follicle. Atretic follicle, the basement that separated the oocyte from granulosa cells often thickens to become the glassy membrane. Fibrous material replaces the granulosa cells and loss of cohesion may also occur in granulosa cells.

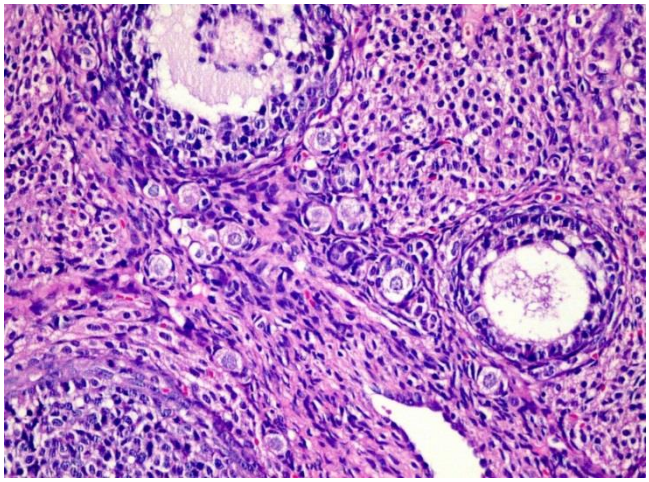


Figure 3. Multiple primary and primordial follicles x400 hematoxylin eosin

AMH assays: Blood samples were collected into tubes containing lithium heparin (BD Vacutainer Plasma tubes, Manchester, England). The concentration of the Lithium Heparin additive in these tubes was 17 international units of heparin/ml of blood. Blood samples were centrifuged within 30 minutes of sampling. After 15 minutes of centrifugation at 1000xg, serum was removed and the remaining plasma was transferred into an Eppendorf tube and stored frozen at -20ºC until the time of analysis. AMH concentrations were measured in “ng/ml” plasma using ELISA method. The rat AMH kit used in study had a sensitivity of 0.10 g/mL, a detection range of 0.16 to 10 ng/mL and a coefficient variation of less than 10% (Elabscience, Rat AMH kit; Houston, Texas, ABD). The laboratory technician of the laboratory of the university hospital was blinded to the study groups and unaware of which samples belonged to which rat. All samples were analyzed in the same assay.

Statistical analysis: Statistical analysis was performed with SPSS version 17.0 program. The suitability of the variables to normal distribution was examined by histogram graphs and Kolmogorov-Smirnov test. Mean, standard deviation, median and IQR values were used to present descriptive analyzes.

http://dx.doi.org/10.36472/msd.v6i12.333

Non-parametric variables were evaluated between the two groups and Mann Whitney U Test was used. Spearman Correlation Test was used to analyze the measured data of each group in relation to each other. The cases where the P-value was less than 0.05 were evaluated as statistically significant results.

Results

Histopathological damage scores were compared between the groups. Vascular congestion and total damage scores were higher in the CP group than in the normal group. CP + DHEA group had higher edema, vascular congestion and total score values than the normal group (Table 1).

Follicle numbers and AMH values were compared according to the groups. In the CP group, the number of Primordial, Primary, Secondary follicles and ovarian volume were lower than the normal group. In the CP + DHEA group, the number of primordial and tertiary follicles and ovarian volume were lower than in the normal group. AMH values do not differ between the groups (Table 2).

The correlation between AMH and rat weight, ovarian volume, total damage score, number of atretic follicles, secondary and tertiary follicles were evaluated between the groups. Accordingly, there was a strong positive correlation between AMH and ovarian volume in the normal group (Table 3).

Table 1. Comparison of histopathological damage scores of normal ovary vs CP, CP + DHEA

	Normal ovary	CP	P*	CP +DHEA	P**
Edema					
Mean SD Median- IQR	0,00±0,00 0,00(0,00-0,00)	0,25±0,46 0,00(0,00-0,50)	0,143	2,50±0,53 2,50(2,00-3,00)	**<0,001**
Vascular congestion					
Mean SD Median- IQR	0,00±0,00 0,00(0,00-0,00)	1,13±0,83 1,00(0,50-2,00)	**0,004**	1,50±0,53 1,50(1,00-2,00)	**<0,001**
Inflammation					
Mean SD Median- IQR	0,00±0,00 0,00(0,00-0,00)	0,00±0,00 0,00(0,00-0,00)	1,000	0,00±0,00 0,00(0,00-0,00)	1,000
Cellular degeneration					
Mean SD Median- IQR	0,00±0,00 0,00(0,00-0,00)	0,25±0,71 0,00(0,00-0,00)	0,317	0,00±0,00 0,00(0,00-0,00)	1,000
Hemorrhage					
Mean SD Median- IQR	0,13±0,35 0,00(0,00-0,00)	0,00±0,00 0,00(0,00-0,00)	0,317	0,00±0,00 0,00(0,00-0,00)	0,317
Total score					
Mean SD Median- IQR	0,13±0,35 0,00(0,00-0,00)	1,62±1,30 1,50(0,50-3,00)	**0,010**	4,00±0,93 4,00(3,00-5,00)	**<0,001**

Mann Whitney U Test

Table 2. Comparison of normal ovary vs CP, CP + DHEA groups in terms of follicle count and AMH values

	Normal ovary	CP	P*	CP +DHEA	P**
Primordialfollicle					
Mean SD Median- IQR	12,75±1,91 12,50(11,50-14,00)	2,88±1,36 2,50(2,00-4,00)	**0,001**	4,63±1,77 4,50(3,00-5,50)	**0,001**
Primerfollicle					
Mean SD Median- IQR	10,50±2,33 11,00(8,50-12,00)	6,13±4,45 3,50(3,00-9,00)	**0,043**	9,50±4,57 10,50(7,50-11,50)	0,671
Secondary (pre-antral) follicle					
Mean SD Median- IQR	12,25±1,83 12,50(10,50-13,50)	8,63±2,39 9,50(6,50-10,00)	**0,006**	12,13±4,05 11,50(8,50-15,50)	0,833
Tertiary (antral) follicle					
Mean SD Median- IQR	21,50±3,21 22,00(19,50-23,50)	17,75±5,97 17,00(12,50-22,00)	0,140	17,00±2,83 16,50(14,50-19,00)	**0,018**
Atreticfollicle					
Mean SD Median- IQR	0,25±0,46 0,00(0,00-0,50)	0,25±0,71 0,00(0,00-0,00)	0,643	0,00±0,00 0,00(0,00-0,00)	0,143
AMH (ng/mL)					
Mean SD Median- IQR	3,42±0,79 3,37(2,64-4,06)	2,96±0,57 3,03(2,57-3,29)	0,189	3,71±0,75 3,64(3,18-4,05)	0,528
Ovaryvolume (mm3)					
Mean SD Median- IQR	55,49±9,14 54,12(50,19-55,53)	23,27±5,04 24,07(19,15-27,68)	**0,001**	38,70±12,16 36,22(27,80-50,19)	**0,017**

Mann Whitney U Test

http://dx.doi.org/10.36472/msd.v6i12.333

Table 3. Correlations between rat weights, ovary volume, total damage score, number of atretic follicles and AMH levels

	Normal rat AMH	CP AMH	CP+DHEA AMH
Rat weight (grams)	0,443	-0,160	-0,562
Ovary volume (mm3)	**0,778***	0,476	-0,168
Total damage score	0,082	0,222	0,095
Atretic follicle count	0,000	-0,412	0,000
Pre-antral+antral follicle count	-0,072	-0,325	0,611

Spearman Correlation Test *p<0,050

Discussion

Many previous studies have investigated the effects of CP on reproductive function in women and have found that it is associated with the highest risk of iatrogenic infertility following the treatment of common cancers frequently observed in women of reproductive age and children (26). CP induces its effects by mitotically diminishing the stock of active cells (4). It was found that ovarian tissues had prominent atrophy, fibrosis, prominent follicular atresia and did not have normal follicular stages (27-29). In the pathological examinations of the ovary, it was generally seen that there was decreased primordial follicles, ovarian blood vessel damage, and ovarian atrophy (30,31). To investigate these effects associated with CP, we conducted histopathological examinations on ovarian tissue and performed volume evaluations. We examined the correlation of AMH with rat weight, ovarian volume, total damage score, atrial follicle count, and secondary and tertiary follicle counts. As such, we determined that there was a strong positive correlation between AMH and ovarian volume in the normal group. However, ovarian volume decreased significantly compared with normal rats in all rats administered with CP. Although the DHEA supplementation reduced this decrease numerically, it could not prevent a statistical decrease. In addition, although ovarian volume decreased in both groups, AMH levels were not affected.

CP causes progressive and irreversible damage due to the destruction of oocytes, follicular depletion and severe vascular damage in the ovary (32,33). Severe ovarian damage develops as a result of ovarian atrophy, destruction of growing follicles, and hence a decrease in follicle counts (34). We evaluated the histopathological damage scores in order to examine ovarian damage. We found that there was a significant increase in vascular congestion and total damage score compared with normal ovarian tissue. We determined that the addition of DHEA to CP application did not lead to a difference in any of these damage scores.

Alkylating drugs such as CP are the most effective agents in inducing ovarian failure(35). In studies, it is usually used as a single dose of 200 mg/kg(36,37).It has been reported that in the treatment of mice with CP, a process related to the p53-upregulated modulator of apoptosis (PUMA), a pro-apoptotic protein, results in a massive net follicle loss within five days(38).

Primordial follicles can also become the target of direct chemotherapy, since cytotoxic agents with a non-specific cell cycle, such as CP, can also damage resting cells(39).

It has been shown that the alkylating agent CP induces primordial follicle loss in mice by resting follicle activation and burn-out (40).

In our study, we examined the effects of CP on ovarian follicles. We determined that compared with normal ovarian tissue, there was a significant decrease in primordial, primary and preantral follicles in the group treated with CP alone. We also found that the addition of DHEA to chemotherapy prevented a decrease in primordial and primary follicles, although preantral follicles still remained at a low level. There was no significant change in terms of atretic follicles in both groups treated with CP.

The anti-mullerian hormone levels are associated with follicular reserves, in particular with preantral follicle reserves (4). The effects of anti-tumoral therapies on the ovaries can be evaluated with many different markers. Serum early phase FSH, estradiol, AMH levels and antral follicle count can be used for these evaluations (41).Although there are many ovarian reserve tests with varying predictive capabilities, antral follicle count (AFC) and AMH have been found to have the best diagnostic accuracy for consistently predicting poor ovarian reserve (42,43).

In rats treated with CP, it was observed that ovarian damage increased and that total vascular congestion and total damage scores increased significantly compared with normal rats. There was a decrease in ovarian volume, primordial, primary and preantral follicle numbers. It was determined that in the group that received DHEA in addition to CP, there was no decrease in primary and preantral follicles. It was also foundthat there were changes in all other parameters, and that there were no significant differences in terms of tissue damage, follicle counts and AMH values, which are important for clinical practice.

Conclusion

It was determined that the use of DHEA to prevent CP-related ovarian damage in rats did not produce significant changes in antral follicle counts, ovarian volume and AMH levels, which are important in clinical practice.

Acknowledgments, Funding: None

Conflict of interest and financial disclosure: The authors declare that there is no conflict of interest and financial relationships.

Author's contiributions: ÖS, YA, ADA, KB, MA; Research concept and design; Animal studies, data collecting, analysis and interpretationof data, **KB;** Pathological examinations, **ÖS;** Preparation of the article and revisions.

Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Nguyen QN, Zerafa N, Liew SH, Findlay JK, Hickey M, Hutt KJ. Cisplatin- and cyclophosphamide-induced primordial follicle depletion is caused by direct damage to oocytes. Molecular human reproduction. 2019.
2. Han M, Cheng H, Wang J, Yu Y, Wang F, Zhu R, et al. Abnormal aggregation of myeloid-derived suppressor cells in a mouse model of cyclophosphamide-induced premature ovarian failure. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2019:1-6.
3. D'Avila AM, Biolchi V, Capp E, Corleta H. Age, anti-mullerian hormone, antral follicles count to predict amenorrhea or oligomenorrhea after chemotherapy with cyclophosphamide. Journal of ovarian research. 2015;8:82.
4. Vural B, Duruksu G, Vural F, Gorguc M, Karaoz E. Effects of VEGF (+) Mesenchymal Stem Cells and Platelet-Rich Plasma on Inbred Rat Ovarian Functions in Cyclophosphamide-Induced Premature Ovarian Insufficiency Model. Stem cell reviews and reports. 2019;15(4):558-73.
5. Zhang BF, Hu Y, Liu X, Cheng Z, Lei Y, Liu Y, et al. The role of AKT and FOXO3 in preventing ovarian toxicity induced by cyclophosphamide. 2018;13(8):e0201136.
6. Zhang CW, Wang CZ, Tao R, Ye JZ. Separation of polyprenols from Ginkgo biloba leaves by a nano silica-based adsorbent containing silver ions. Journal of chromatography A. 2019;1590:58-64.
7. Helsby NA, Yong M, van Kan M, de Zoysa JR, Burns KE. The importance of both CYP2C19 and CYP2B6 germline variations in cyclophosphamide pharmacokinetics and clinical outcomes. 2019;85(9):1925-34.
8. Meng Y, Wang J, Wang Z, Zhang G, Liu L, Huo G, et al. Lactobacillus plantarum KLDS1.0318 Ameliorates Impaired Intestinal Immunity and Metabolic Disorders in Cyclophosphamide-Treated Mice. Frontiers in microbiology. 2019;10:731.
9. Liu X, Zhang Z, Liu J, Wang Y, Zhou Q, Wang S, et al. Ginsenoside Rg3 improves cyclophosphamide-induced immunocompetence in Balb/c mice. International immunopharmacology. 2019;72:98-111.
10. Meirow D, Epstein M, Lewis H, Nugent D, Gosden RG. Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. Human reproduction. 2001;16(4):632-7.
11. Mark-Kappeler CJ, Hoyer PB, Devine PJ. Xenobiotic effects on ovarian preantral follicles. Biology of reproduction. 2011;85(5):871-83.
12. Gui T, Yuan G, Shen K, Cao D, Yang J, Wu M, et al. Protective effect of gonadotropin-releasing hormone analog on the ovarian reserve in rats receiving cyclophosphamide treatment. OncoTargets and therapy. 2015;8:661-7.
13. Dynes J, Osz K, Hooper A, Petrik J. Low-dose metronomic delivery of cyclophosphamide is less detrimental to granulosa cell viability, ovarian function, and fertility than maximum tolerated dose delivery in the mouse. Biology of reproduction. 2017;97(3):449-65.
14. Chen XY, Xia HX, Guan HY, Li B, Zhang W. Follicle Loss and Apoptosis in Cyclophosphamide-Treated Mice: What's the Matter? International journal of molecular sciences. 2016;17(6).
15. Healy MW, Patounakis G, Connell MT, Devine K, DeCherney AH, Levy MJ, et al. Does a frozen embryo transfer ameliorate the effect of elevated progesterone seen in fresh transfer cycles? Fertility and sterility. 2016;105(1):93-9.e1.
16. Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, Van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reproductive biomedicine online. 2012;24(4):381-8.
17. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. Journal of assisted reproduction and genetics. 2007;24(12):629-34.
18. Yilmaz N, Uygur D, Inal H, Gorkem U, Cicek N, Mollamahmutoglu L. Dehydroepiandrosterone supplementation improves predictive markers for diminished ovarian reserve: serum AMH, inhibin B and antral follicle count. European journal of obstetrics, gynecology, and reproductive biology. 2013;169(2):257-60.
19. Zhang HH, Xu PY, Wu J, Zou WW, Xu XM, Cao XY, et al. Dehydroepiandrosterone improves follicular fluid bone morphogenetic protein-15 and accumulated embryo score of infertility patients with diminished ovarian reserve undergoing in vitro fertilization: a randomized controlled trial. Journal of ovarian research. 2014;7:93.
20. Casson PR, Lindsay MS, Pisarska MD, Carson SA, Buster JE. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. Human reproduction. 2000;15(10):2129-32.
21. Gleicher N, Weghofer A, Barad DH. Dehydroepiandrosterone (DHEA) reduces embryo aneuploidy: direct evidence from preimplantation genetic screening (PGS). Reproductive biology and endocrinology : RB&E. 2010;8:140.
22. Khedr NF. Protective effect of mirtazapine and hesperidin on cyclophosphamide-induced oxidative damage and infertility in rat ovaries. Experimental biology and medicine. 2015;240(12):1682-9.
23. Hassa H, Aydin Y, Ozatik O, Erol K, Ozatik Y. Effects of dehydroepiandrosterone (DHEA) on follicular dynamics in a diminished ovarian reserve in vivo model. Systems biology in reproductive medicine. 2015;61(3):117-21.
24. Wang YX, Zhu WJ, Xie BG. Expression of PPAR-gamma in adipose tissue of rats with polycystic ovary syndrome induced by DHEA. Molecular medicine reports. 2014;9(3):889-93.
25. Parlakgumus HA, Aka Bolat F, Bulgan Kilicdag E, Simsek E, Parlakgumus A. Atorvastatin for ovarian torsion: effects on follicle counts, AMH, and VEGF expression. European journal of obstetrics, gynecology, and reproductive biology. 2014;175:186-90.
26. Alarfaj AS, Khalil N. Fertility, ovarian failure, and pregnancy outcome in SLE patients treated with intravenous cyclophosphamide in Saudi Arabia. Clinical rheumatology. 2014;33(12):1731-6.

27. Liu T, Huang Y, Guo L, Cheng W, Zou G. CD44+/CD105+ human amniotic fluid mesenchymal stem cells survive and proliferate in the ovary long-term in a mouse model of chemotherapy-induced premature ovarian failure. International journal of medical sciences. 2012;9(7):592-602.

28. Liu T, Qin W, Huang Y, Zhao Y, Wang J. Induction of estrogen-sensitive epithelial cells derived from human-induced pluripotent stem cells to repair ovarian function in a chemotherapy-induced mouse model of premature ovarian failure. DNA and cell biology. 2013;32(12):685-98.

29. Liu T, Huang Y, Zhang J, Qin W, Chi H, Chen J, et al. Transplantation of human menstrual blood stem cells to treat premature ovarian failure in mouse model. Stem cells and development. 2014;23(13):1548-57.

30. Zhou L, Xie Y, Li S, Liang Y, Qiu Q, Lin H, et al. Rapamycin Prevents cyclophosphamide-induced Over-activation of Primordial Follicle pool through PI3K/Akt/mTOR Signaling Pathway in vivo. 2017;10(1):56.

31. Yuksel A, Bildik G, Senbabaoglu F, Akin N, Arvas M, Unal F, et al. The magnitude of gonadotoxicity of chemotherapy drugs on ovarian follicles and granulosa cells varies depending upon the category of the drugs and the type of granulosa cells. Human reproduction. 2015;30(12):2926-35.

32. Ataya KM, Valeriote FA, Ramahi-Ataya AJ. Effect of cyclophosphamide on the immature rat ovary. Cancer research. 1989;49(7):1660-4.

33. Pydyn EF, Ataya KM. Effect of cyclophosphamide on mouse oocyte in vitro fertilization and cleavage: recovery. Reproductive toxicology. 1991;5(1):73-8.

34. Stefansdottir A, Fowler PA, Powles-Glover N, Anderson RA, Spears N. Use of ovary culture techniques in reproductive toxicology. Reproductive toxicology. 2014;49:117-35.

35. Epstein RJ. Drug-induced DNA damage and tumor chemosensitivity. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1990;8(12):2062-84.

36. Kilic S, Pinarli F, Ozogul C, Tasdemir N, Naz Sarac G, Delibasi T. Protection from cyclophosphamide-induced ovarian damage with bone marrow-derived mesenchymal stem cells during puberty. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2014;30(2):135-40.

37. Song D, Zhong Y, Qian C, Zou Q, Ou J, Shi Y, et al. Human Umbilical Cord Mesenchymal Stem Cells Therapy in Cyclophosphamide-Induced Premature Ovarian Failure Rat Model. 2016;2016:2517514.

38. Nguyen QN, Zerafa N, Liew SH, Morgan FH, Strasser A, Scott CL. Loss of PUMA protects the ovarian reserve during DNA-damaging chemotherapy and preserves fertility. 2018;9(6):618.

39. Lande Y, Fisch B, Tsur A, Farhi J, Prag-Rosenberg R, Ben-Haroush A, et al. Short-term exposure of human ovarian follicles to cyclophosphamide metabolites seems to promote follicular activation in vitro. Reproductive biomedicine online. 2017;34(1):104-14.

40. Jayasinghe YL, Wallace WHB, Anderson RA. Ovarian function, fertility and reproductive lifespan in cancer patients. Expert review of endocrinology & metabolism. 2018;13(3):125-36.

41. Pascuali N, Scotti L, Di Pietro M, Oubina G, Bas D, May M, et al. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Human reproduction. 2018;33(5):844-59.

42. Jayaprakasan K, Campbell B, Hopkisson J, Johnson I, Raine-Fenning N. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertility and sterility. 2010;93(3):855-64.

43. Jayaprakasan K, Narkwichean A, Maalouf WE, Campbell BK. Efficacy of dehydroepiandrosterone to overcome the effect of ovarian ageing (DITTO): a proof of principle randomised controlled trial protocol. BMJ open. 2014;4(10):e005767.

http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):347-50

Case Report Article Doi: 10.36472/msd.v6i12.335

A cervical paravertebral schwannoma: A case report

Atakan Emengen*[1], Anıl Ergen[1], Muhammed Hamza Genç[1], Umay Kiraz[2], Mahmut Konuralp İlbay[1]

Abstract

Objective: Paraspinal schwannomas arise from the dorsal nerve root. Symptoms of schwannomas may depend on their locations and sizes. This case was presented by a female patient with a dorsalgia for 10 years. She had not any specific symptoms but pain and a thick spot on the neck (cervical area). Paraspinal schwannomas involve the dorsal nerve roots, affecting people in the fourth and fifth decades of life. Paraspinal schwannomas are frequently asymptomatic and diagnosed incidentally on imaging of the spine. Total excision is mostly possible and the recurrence rate is low.

Keywords: Paraspinal schwannomas, Extradural schwannomas, Nerve sheath tumors, Periferic nerve tumors

Introduction

Schwannomas are the type of tumors originating from the nerve sheath. Although most of these tumors are benign, they can rarely be malignant. Most of them are extramedullary and are often seen in intradural areas.(1) Neurogenic tumors of the paraspinal region are common in the adult population. Paraspinal schwannomas that develop from the dorsal nerve of the spinal root can cause sensorial symptomatology. Schwannomas may also derivate from last four cranial nerves or autonomic nerves in parapharyngeal space, vagus nerve being the most common(2). Symptoms of schwannomas may depend on its location and size. While Schwannomas randomly cause symptoms, patients may suffer from pain, muscle weakness, tingling sensation, numbness, auditory problems or facial paralysis. In some cases, they may occur in syndromes with multiple manifestations. Treatments of schwannomas are surgical and several techniques are depending on its location. With the correct surgical approach, total excision is possible for these tumors.

Case

A 47-year old female presented with back pain for almost 10 years. She had not any chronic diseases and had not any surgical operations. Apart from the pain and a thickened spot on the neck for a year, she did not have any specific symptoms. In the first couple of years, her VAS score for the pain was 3, but she describes an increase to 7 in the last 2 years. She described her pain as a burning sensation and it did not get affected by movement. Blood tests including acute phase reactants were normal. Physical examination revealed no motor and sensory loss, both in her previous consults to other physicians and in ours. She was diagnosed with chronic muscle stiffness due to excessive use and underwent medical and conservative treatment for a few years. She had continuous NSAI usage for the last 2 years. For the first years, symptomatic treatment was episodic. Before coming to our clinic, she had undergone physiotherapy for 3 weeks and she described no decrease in her symptoms after these treatments. On the light of these facts, we thought of the possibility of a deeply located regional legion like a mass. These kinds of lesions are usually asymptomatic unless they grow and compress adjacent tissues and cause pain. Cervical MRI was planned to search for possible soft tissue problems. The tumor was found in the paravertebral area with intensive contrast enhancement (Fig 1). We thought it to be the cause of the pain that the patient has been suffering, so we planned a surgical approach with the patient's approval.

Under general anesthesia and in the prone position, the skin and dermis were cut on the C6 to the T1 level. After paravertebral fascia was opened and muscles were dissected, a 3-centimeter long soft mass was seen near the spinous processes of C7 and T1. We separated the mass from paravertebral muscles and spinous processes and burned the tail, so we could remove it from the C7 nerve sheath. (Fig2)

The pathology results showed that the tumor was a schwannoma, with residual peripheral tissue on it. The patient had a good recovery. After 8 months, symptoms - especially back pain- were gone and there were no sensorial and motor deficits. A follow-up MRI was planned to check the area (Fig 3).

Received 30-11-2019 **Accepted** 26-12-2019 **Available Online** 28-12-2019 **Published** 30-12-2019
1 Kocaeli University Medicine Faculty, Dept of Neurosurgery, Kocaeli, TR
2 Kocaeli University Medicine Faculty, Dept of Pathology, Kocaeli, TR
*** Corresponding Author:** Atakan Emengen **E-mail:** dratakanemengen@gmail.com

 http://dx.doi.org/10.36472/msd.v6i12.335

Table1: McCormick's Classification- Classification for para-spinal region tumors.

McCormick's Classification	
Type 1	• Dumbell tumours with significant intraspinal and anterior paraspinal components.
Type 2	• Confined to anterior spinal region and near spinal canal without foraminal or intraspinal extension.
Type 3	• Anterior paraspinal tumors with minor foraminal or intraspinal extension.
Type 4	• Involve vertebral, epidural and paraspinal regions.
Type 5	• Involve paraspinal soft tissue only (our case).

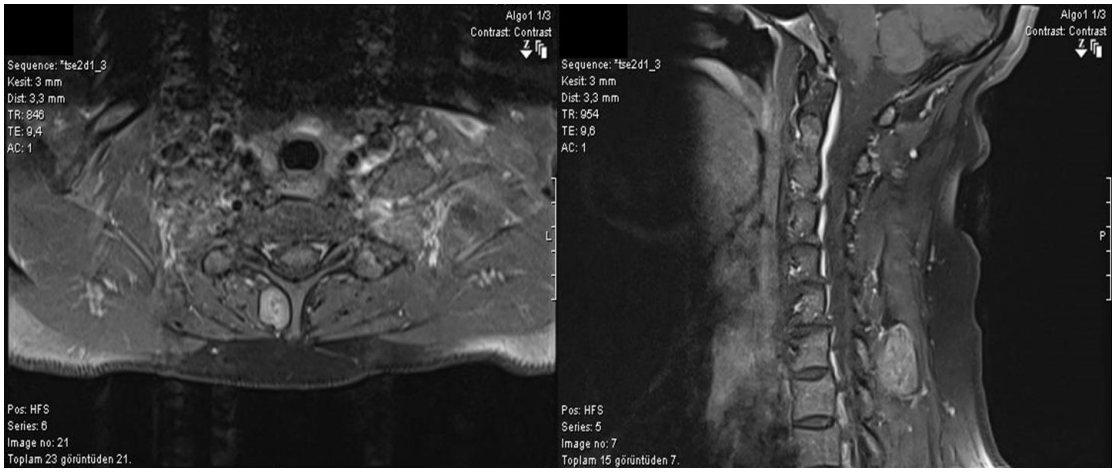


Figure 1: Patient's pre-operative cervical MRI, showing a smooth-contoured mass lesion at C7-T1, near the spinous processes on the para-vertebral area. The lesion is T2-hyper-intense, T1-hypo-intense and has post-contrast series intensive contrast enhancement with a necrotic component at the center. The maximum tumor diameter of the lesion was 18 mm.

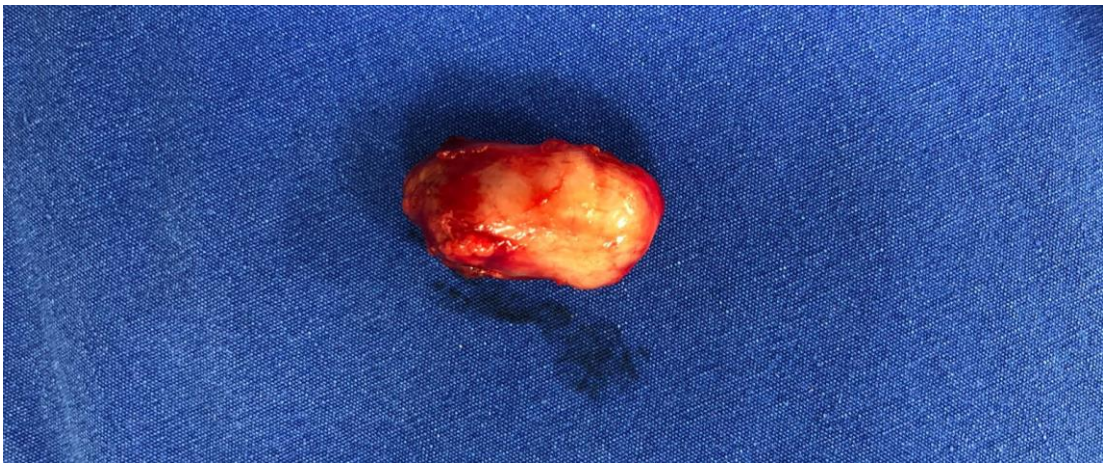


Figure 2: The tumor showed macroscopically. All the contours of the tumor were found to be smooth. The pathology results showed that the mass is a schwannoma with residual peripheral nerve tissue on it.

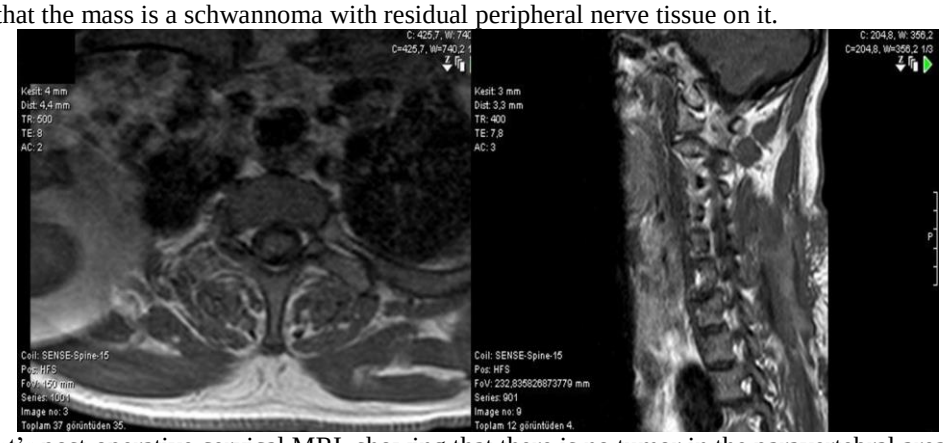


Figure 3: Patient's post-operative cervical MRI, showing that there is no tumor in the paravertebral area and that all tissues were looking normal.

http://dx.doi.org/10.36472/msd.v6i12.335

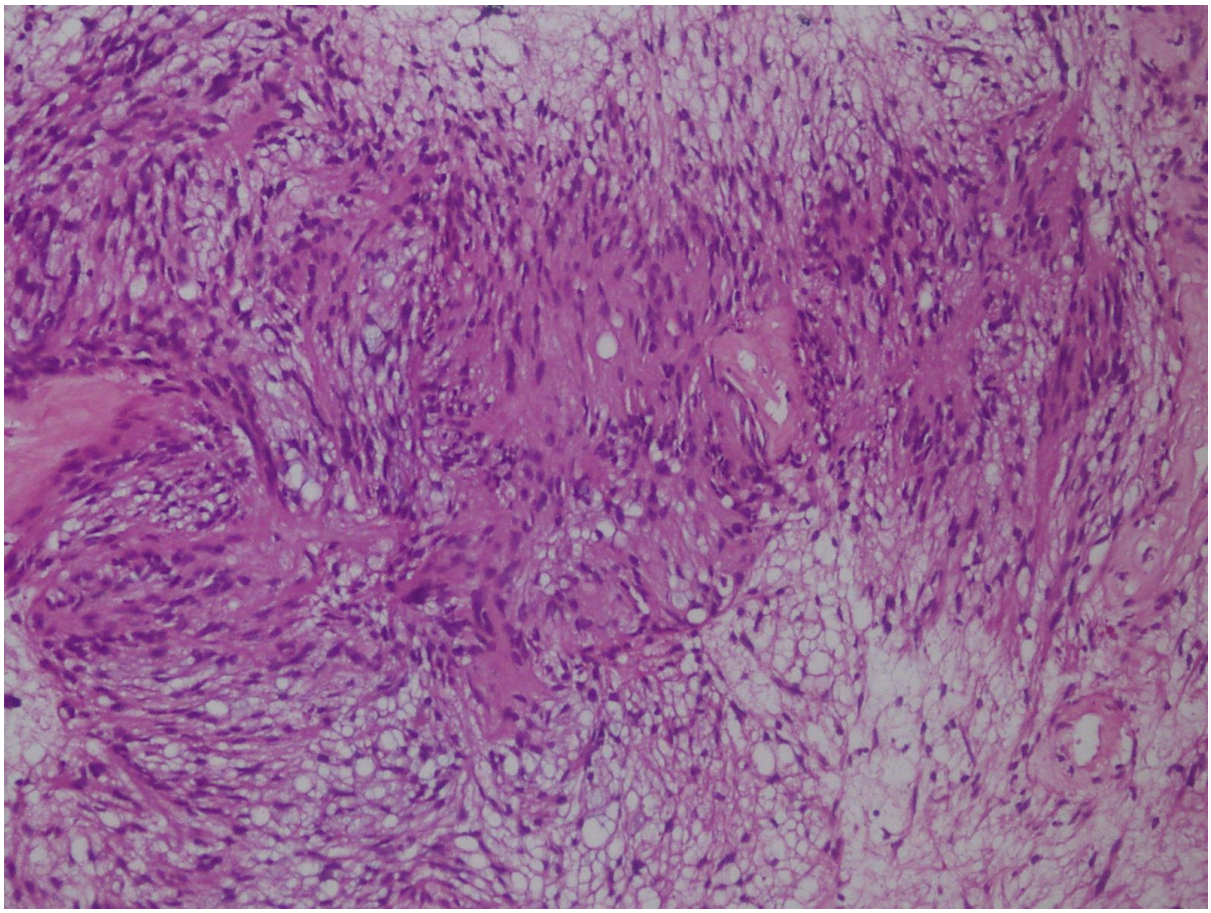


Figure 4: A common finding in schwannoma is the formation of Verocay bodies within the cellular Antoni A zones, defined as palisading rows of nuclei around a pink fibrillary and hypocellular Antoni B zones, contain degenerative changes (H&E, x200).

Discussion

Schwannomas are benign rare tumors that originate the nerve sheath of peripheral nerves. It is the most common cause of intradural extramedullary tumors in the paraspinal area, but it can also be seen extradural. Schwannomas of the cervical sympathetic chain are extremely rare compared to lumbar or thoracic types, and they originate from the superior part of the cervical chain.(3) However, schwannomas can originate from any peripheral nerve in the body, nerves of the head and neck being the most involved ones. Paraspinal schwannomas develop around dorsal nerve roots in the 4t-5th decades of life. This tumor mostly presents as asymptomatic and is found incidentally in spinal imaging. The most common symptoms are lancinating pain and/or paresthesia. They may also compress the nerve roots and cause non-specific abdominal or back pain. Schwannomas may be sporadic and solitary or multiple as in neurofibromatosis type 1.(5) They may extend along the spinal nerve and impose as a dumbbell mass, consisting of both intradural and extradural components. Intramuscular schwannomas are highly uncommon, with palpable masses that present with either no symptoms or lower extremity numbness, but with no characteristic features. Intramuscular schwannomas originate from motor branch nerves. Neurological symptoms, including pain, motor weakness or paresthesia are rarely seen. In our patient who had no radicular pain, motor weakness or tenderness, the tumor was located in the erector spinal muscles.

The histological hallmark of a schwannoma is the alternating pattern of two distinct tissue types: fascicular type (Antoni-A) and reticular type (Antoni-B) (Fig 4). Antoni A regions are composed of more densely arranged cells with specific areas of palisading nuclei arranged in rows, whereas Antoni B regions tend to be more hypocellular, with a loose and disorderly arrangement. In the present case, as the MRI findings were indicative of a benign nerve sheath tumor, we cautiously removed the encapsulated mass from the origin but could not identify the dorsal ramus nerve. Our tissue biopsy confirmed the presence of a very rare intramuscular benign schwannoma, with typical features. Schwannomas usually demonstrated by hypointensity on T1-weighted images and hyperintensity on T2-weighted images.(4) McCormick's Classification is used in the classify paraspinal region tumors.(6) (Table 1).

http://dx.doi.org/10.36472/msd.v6i12.335

Conclusion

The diagnosis of paraspinal tumors is delayed by non-specific symptoms such as pain. In some cases, the tumor may expand and cause motor weakness or sensory deficit. If it is feasible to remove, the main treatment choice is surgery, radiotherapy is in the alternative. Total excision is mostly possible and the recurrence rate sare extremely low after a successful surgery

Acknowledgments, Funding: None

Conflict of interest and financial disclosure: The authors declare that there is no conflict of interest and financial relationships.

Author's contiributions: AE, AE, MHG, UK, MKİ; Research concept and design; Animal studies, data collecting, analysis and interpretationof data, **UK;** Pathological examinations, **AE;** Preparation of the article and revisions.

Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Shah, Kunal Ajay, and Ajay Chimanlal Shah. "Paraspinal Schwannoma of dorsal ramus nerve: A case report." Journal of clinical orthopaedics and trauma 9 (2018): S1-S4.

2. Bocciolini, C., et al. "Schwannoma of cervical sympathetic chain: assessment and management." Acta Otorhinolaryngologica Italica 25.3 (2005): 191.

3. Iacconi, P., et al. "Cervical sympathetic chain schwannoma: a case report." Acta Otorhinolaryngologica Italica 32.2 (2012): 133.

4. Adouly, Taoufik, et al. "An unusual giant schwannoma of cervical sympathetic chain: a case report." Journal of medical case reports 10.1 (2016): 26.

5. Ukaigwe, Anene, Akintomi Olugbodi, and Richard L. Alweis. "Taking it to the next level: lumbar radiculopathy from thoracic nerve schwannoma." Journal of community hospital internal medicine perspectives 5.1 (2015): 25744.

6. Dhar, Arjun, et al. "Approaches to paraspinal tumours–a technical note." Neurological research 39.4 (2017): 315-322.

MSD

Medical Science & Discovery

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

lycians

www.lycians.com