Associate Prof., M.D., Serdar Ceylaner, Dept. Genetic, Intergen Genetic Diseases Diagnostic Research and Application Center, Ankara, Turkey
Associate Professor, M.D., Gulali Aktas, Dept. Internal Medicine, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Associate Prof., M.D., Suleyman Ipekci, Dept. Endocrinology, Selcuk University, Faculty of Medicine, Konya, Turkey
Professor, M.D., Amir Hossain, Chattagram International Medical College (CIMC), Chittagong, Bangladesh
Professor, M.D., Kahraman Ozturk, Dept. of Hand Surgery, Health Sciences University, Istanbul, Turkey
Professor, M.D., Ahmet Ural, Department of Otorhinolaryngology, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Associate Prof., M.D., Mükremin Uysal, Dept. Oncology, Afyon Kocatepe University, Medical School, Afyon, Turkey
Associate Prof., M.D., Mehmet Ozen, Dept. Hematology, Ufuk University, Medical School, Ankara, Turkey
Professor, M.D., Yasar Bukte, Dept. Radiology, Health Sciences University, Istanbul, Turkey
Professor, M.D., Nebil Yildiz, Dept. Neurology, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Professor, M.D., Ramazan Topsakal, Dept. Cardiology, Erciyes University, Medical School, Kayseri, Turkey
Associate Prof., M.D., Hikmet Tekce, Dept. Internal Medicine-Nephrology, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Professor, M.D., Hasan Orucoglu, Dept. Endodontics, Faculty of Dentistry, Bolu Abant Izzet Baysal University, Bolu, Turkey
Professor, M.D., Fuat Akpınar, Dept. Orthopedics and Traumatology, Istanbul Medeniyet University, Istanbul, Turkey
Associate Prof., M.D., Furkan Erol Karabekmez, Dept. Plastic and Reconstructive Surgery, Health Sciences University, Ankara, Turkey
Professor, M.D., Muhammed Guzel Kurtoglu, Dept. Microbiology, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Associate Prof., M.D., Memis Hilmi Atay, Dept. Hematology, Ondokuz Mayıs University, Medical School, Samsun, Turkey
Professor, Ph.D., Erol Ayaz, Dept. Parasitology, Bolu Abant Izzet Baysal University, Medical School, Bolu, Turkey
Professor, M.D., Gokhan Kirbas, Dept. Chest Diseases, Dicle University, Medical School, Diyarbakir, Turkey
Associate Prof., M.D., Basri Cakiroglu, Dept. Urology, İstanbul Atlas University, Medical School, İstanbul, Turkey
Professor, M.D., Kemal Nas, Dept. Physical Medicine and Rehabilitation, Sakarya University, Medical School, Sakarya, Turkey
Professor, M.D., Huseyin Buyukbayram, Dept. Chest Diseases, Dicle University, Medical School, Diyarbakir, Turkey

Experimental Biomedical Research is licensed under a Creative Commons Attribution 4.0 International License
AUTHOR GUIDELINES
INSTRUCTIONS FOR AUTHORS

Author Guidelines
Instructions for Authors
Experimental Biomedical Research publishes articles in English. Since the journal does not offer translation services, if the language of the manuscripts is not enough, the editors may refuse the manuscript or ask the author to seek language editorial services to bring the manuscript to minimum standards for the review process. If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

If you would like to submit a Review, please contact Editor-in Chief at info@experimentalbiomedicalresearch.com.

**Online Submission**
The articles must be submitted by the corresponding author via the Online Submissions System. If authors encounter technical problems with online submission, they may contact with support team at info@experimentalbiomedicalresearch.com.

**Corresponding author**
The corresponding author’s must do: complete submission of manuscript files; storage of the article and all related documents and giving original data when necessary; contributions of the authors and explanations of conflict of interest disclosures; approval for submission; and the final proof control.

**ORCID ID**
ORCiD IDs of the corresponding author and other authors must be submitted during the registration process. This section is mandatory.

As part of our commitment to ensuring an ethical, transparent and fair peer review process, Experimental Biomedical Research is a publisher who signed ORCID open letter. ORCID provides a unique and persistent digital identifier that distinguishes researchers from every other researcher, even those who share the same name, and, through integration in key research workflows such as manuscript and grant submission, supports automated linkages between researchers and their professional activities, ensuring that their work is recognized.

The collection of ORCID iDs from corresponding authors is now part of the submission process of this journal. If you already have an ORCID iD you will be asked to associate that to your submission during the online submission process. We also strongly encourage all co-authors to link their ORCID ID to their accounts in our online peer review platforms. It takes seconds to do: click the link when prompted, sign into your ORCID account and our systems are automatically updated. Your ORCID iD will become part of your accepted publication’s metadata, making your work attributable to you and only you. Your ORCID iD is published with your article so that fellow researchers reading your work can link to your ORCID profile and from there link to your other publications.

If you do not already have an ORCID iD please follow this [link](#) to create one.

**Author Declaration, Funding and Financial Conflicts of Interest**
Authors should provide a cover letter declares: that the article submitted has not been published elsewhere and is not under review; that the submission has been approved by all co-authors and, if necessary, by the responsible authorities and the institute. The publisher will not be responsible in cases of any claims for compensation.

All authors should disclose commercial ties or consulting, stock or share interests or patent license arrangements that can be viewed as a conflict of interest in relation to the manuscript presented ([Author Declaration Form & Conflict Of Interest Statement](#)).

**Permissions**
Obtaining permission form the copyright owner/owners is obligatory for figures, tables or texts that previously published elsewhere if the authors want to add them to their manuscripts. Without this evidence, any material used in the article will be deemed to be an original product of the authors.

**Units of measurement**
The International System of Units (SI) is the modern form of the metric system, and is the most widely used system of measurement. Therefore, units of measurement should be presented using the International System of Units in Experimental Biomedical Research.

**Abbreviations**
Abbreviations are defined at the first mention and are then used continuously. The authors should always be used standard abbreviations and generic names of the drugs. Additionally, the abbreviations presented in the Tables and Figures must be compatible with SI. If registered trademarks are used, the name and country of the manufacturer must be given in parentheses following the generic name on the first use.

**Preparation of Manuscript**

**Title Page**
The title page should include: manuscript title, the name(s), the affiliation(s) and address(es) of the author(s). The corresponding author information should include the e-mail address, the 16-digit ORCID ID, telephone number(s) and full mailing address.

Disclosure of conflict of interest, funding organizations and acknowledgments of people, grants, funds, etc. should be placed in the last section on the title page.

**Abstract**
Abstracts must not exceed 250 words. The abstract should describe with subheadings; *Aim, Method, Results, and Conclusions*.

Abstracts should not contain any unexplained abbreviations or references. It is crucial that the abstract be an accurate summary of the contents of the paper.

**Keywords**
4 to 6 keywords are sufficient which can be recommended by the "Index Medicus Subject Headings": MeSH (http://www.nlm.nih.gov/mesh/meshhome.html).

**Main Text**
The main text should describe with subheadings; *Introduction, Methods and Materials, Results, Discussion and Conclusions*.

Manuscripts should be submitted in Microsoft Office Word formats and arranged as 12-point Times New Roman for text. References to literature, figures and tables should be placed in the order of their citation in the text. The Author(s) should not use italics, bold or underlined words in the texts. Please use only generic names of drugs.

*Introduction:* Introduction to a research report should provide a context for the study and specify the particular aims of the reported study. In this section, the emphasis should be on brevity, for the introduction is not meant to be a detailed review but merely a capsule summary that provides a rationale for the second and most important part which is a clear statement as to why the study was undertaken.

*Methods and Materials:* In this section, the researcher should clearly write the methods used. The materials section should contain the information requested when the reported results need to be expanded and elaborated. It is also important to carry out appropriate statistical tests and to state the sources of the drugs and chemicals used.

*Results:* In this section, the authors should clearly written information collected using the methods described to achieve the objectives of the study.

*Discussion:* The discussion section is critical, the information collected is evaluated in relation to the objectives of the study and the context in which the study begins, and any inconsistency between the results is explained and elaborated.

*References:* It is important that the authors cite appropriate and up-to-date articles for information and comments in the text.

**Conflicts of Interest**
Authors must declare all relevant interests that could be perceived as conflicting. Authors should explain why each interest may represent a conflict. If no conflicts exist, the authors should state this. Submitting authors are responsible for coauthors declaring their interests.

**References**
Number references in the order they are mentioned in the text; do not alphabetize. Reference citations in the text should be identified by numbers in square brackets. In listing references (Format AMA), follow NLM Style Guide, abbreviating names of journals according to Index Medicus. Indicate each author’s family name followed by a space and initials closed up without periods. Author names should be separated with a comma, never using the conjunction “and” between entries. All authors must be listed for papers with 1 to 3 authors. For papers with more than 3 authors, only the first 3 authors must be listed, followed by et al.

For online journals or articles published online ahead of print, provide the DOI number, if possible, rather than the URL. URLs used in references will not be made hyperlinks.

**Journal article**
List the first three authors;

More than three authors followed by et al.

Chapter in a book

Online document

The authors are responsible for the accurate and in full presentation in accordance with the journal’s style of references.

Preparation of Figures and Tables
The figures and tables should be uploaded electronically by a separate file and should be stated consecutively in the text. Each table should have an explanatory heading, and if numerical measurements are made, the units should be added to the column header. Figures should be presented in vector image formats (Illustrator, EPS, WMF, FreeHand, CorelDraw, PowerPoint, Excel etc.) or in bitmap formats (Photoshop, TIFF, GIF, JPEG, etc.). Bitmap images should be at least 300 dpi resolution.

Supplementary Materials
Authors can submit one file of supplementary material such as audio files, video clips, or datasets. A section titled “Supplementary Material” should be included before the references list with a concise description for each supplementary material file. Authors are responsible for providing the final supplementary materials files that will be published along with the article.

English Language Editing
Editors and reviewers should ensure the clarity of English language of the article in assessment of the manuscript.

If any help needed in writing in English one can consider the following:
- Ask for help from a co-worker who is a native English speaker in sake of clarity of the text.
- Applying to a professional english language editing service to improve the quality of the language and grammar of the text.
Authors should aware that the use of a language editing service does not warrant an article to be accepted for publication in this journal.

ETHICAL STANDARDS
Ethical Responsibilities of Authors
Experimental Biomedical Research journal will follow the Committee on Publication Ethics (COPE) guidelines on how to deal with potential acts of misconduct. For this reason, authors should protected the journal trust, the professionalism of the scientific authorship, and must refrain from misrepresenting the consequences of research that could destroy all scientific effort.

Plagiarism checking
Articles sent to Experimental Biomedical Research journal are checked for possible plagiarism by using an appropriate software (iThenticate). However, corresponding and co-authors are responsible for any fraud, intentional or unintentional malpractice.

Research involving human participants and/or animals
Experimental Biomedical Research adopt ICMJE Recommendations on Protection of Research Participants. For more information, click here!

In addition to ICMJE recommendations, we also support 3Rs principals (Replacement, Reduction and Refinement) for humans and animals usage in research. Briefly 3Rs are mentioned below, and more information can be accessed here!

Replacement: approaches which avoid or replace the use of animals
Reduction: approaches which minimise the number of animals used per experiment
Refinement: approaches which minimise animal suffering and improve welfare
All work should be done with the permission of local human subjects or animal care committees (institutional and national) and clinical trials should be registered to legislation. The official numbers from these committees must be found in the Materials and Methods section (or text describing the experimental procedures).

1) Statement of human rights
The studies involving human participants should state that the research has been endorsed by the institutional and / or national research ethics committee and that it is conducted in accordance with the ethical standards set out in the Helsinki Declaration of 1964, and that subsequent changes are also met (1).
2) Statement on the welfare of animals
If you have done experimental research on animals, authors should indicate whether the international, national and / or institutional guidelines for the care and use of the animals are followed, and whether the work has been approved by an institutional research ethics committee.

Informed consent
If manuscripts report the results of an experimental research of human subjects, all authors must fulfill the International Committee of Medical Journal Editors (ICMJE) requirements on confidentiality and informed consent from patients and study participants. Therefore;
1- Informed consent is obtained from all participants before they are included in the work.
2- Distinguishing details of the participants examined (name, date of birth, identification numbers and other information) should not be published in print, photographs and genetic profiles.
3- Where someone is deceased, please make sure that you have written permission from the family or estate.
4- If the identification features are changed to protect anonymity as in genetic profiling, the authors should assure that the changes do not distort scientific meaning.
Authors may use this Patient Consent Form, which sent to the journal if requested.
The journal reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines.

Publication charges
There are no submission fees or page charges for Experimental Biomedical Research journal.

Copyright Policy
Articles published in Experimental Biomedical Research are open-access, distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Upon acceptance of an article, authors will be asked to transfer copyright. This transfer will ensure the widest possible dissemination of information. A letter will be sent to the corresponding author confirming receipt of the manuscript. A form facilitating transfer of copyright will be provided. (Copyright Transfer Agreement Form).
If the article contains a figure or table produced from a book or other journal article, the authors must obtain permission from the copyright owner before submitting the manuscript and they will be entirely liable for legal and / or financial consequences if such authorization documents are not obtained.
If you wish to use PDF, HTML, XML files or any artwork published in this journal for any commercial purpose, please contact the publisher at info@experimentalbiomedicalresearch.com.

Proofs
Accepted articles are sent as portable document format (PDF) files, along with proof by e-mail to the relevant author for approval. Corrections to PDF evidence should be limited to posting errors only, and no significant additions / deletions should be made. Authors are responsible for all statements made in their work, including changes made by the copy editor and authorized by the author concerned. Authors are strongly advised to thoroughly examine the PDF evidence and return the proofs within 3 days.

Experimental Biomedical Research
E-mail: info@experimentalbiomedicalresearch.com
Completed authorship forms may be mailed to this address.

Reference

Editorial Assessment and Peer Review Policy-Process
Experimental Biomedical Research is an online-only, international, peer-reviewed, open access journal and is committed to maintaining the high quality of the peer-review process. Additionally, the peer review process ensures that the articles published, meet the accepted standards of the discipline. Experimental Biomedical Research (Editor) reviews new submissions according to its guidelines. When they meet all criteria, they are sent to two referees (double blind) and all manuscripts are read by reviewers, and revisions to the manuscript may be required. If the decision conflicts between two reviewers, it will be send to third peer reviewer. The typical review will take in 2-4 weeks. When the manuscript is received from peer reviewer there will be one of the following outcome: 1) accepted manuscript without revisions, 2) invite authors to resubmit the manuscript after minor or major
changes while the final decision is kept pending, 3) or reject the manuscript. When the manuscript is returned for revision prior to acceptance, the revised manuscript must be submitted within 30 days after the author's receipt of the referee's reports. Editorial review again (re-peer review/accepted/rejected). The final decision is sent to the authors.

**Double blinded peer review process**

**Manuscript Submission**
- New submission via online system or e-mail
- Cover letter, author and co-author details, manuscript and separate files

**Pre-Quality Associate Editorial Assessment**
- Plagiarism check
- Qualification in the English language editing
- Ensuring that the manuscript adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines (Experimental Biomedical Research- Submission and Publication Checklist)
- Sent back to author for approval of edits

**Peer Review**
- Double-blind peer review undertaken by experts in the field
- Revision made by authors on the basis of reviewer recommendations (revisions must be highlighted and accompanied by a letter in response to each comment by the reviewers)
- Revised article: Accept/Reject/Re-revise

**Editor-in-Chief Decision**
- Re-checks the revised manuscript to ensure that it meets the journal requirements
- Final decision: Accept/Reject/Re-write and Re-submit

**Copy Editing**
- Professional checking for the composition and organization (formatting) of the paper against the journal guidelines
- Reference styling and proof corrections
- Author’s confirmation of the final edited manuscript before publication
- In this version, corrections to PDF evidence should be limited to posting errors only, and no significant additions / deletions should be made

**Publishing**
- Accepted article is sent for generating the galley proof
- Online publication of the manuscript

**Copyright Notice**
Experimental Biomedical Research journal is licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](http://creativecommons.org/licenses/by-nc/4.0/).

**Privacy Statement**
The names and email addresses entered in this journal site will be used exclusively for the stated purposes of this journal and will not be made available for any other purpose or to any other party.
The efficacy of active warming in preventing unplanned hypothermia during perioperative period in pediatric surgery patients in a tertiary care center

Ganime Esra Soysal1 · Arzu Ilce1 · Ummuhan Yigit1 · Hulya Ozturk2 · Murat Bilgi3
1 Surgical Nursing Department, Faculty of Health Sciences, Bolu Abant Izzet Baysal University, Bolu, Turkey
2 Department of Pediatric Surgery, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey
3 Department of Anesthesia and Reanimation, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey

ABSTRACT

Aim: To investigate the effects of using the active warming method on children with hypothermia in the perioperative period and examining the effects of hypothermia on awakening time, pain, shivering and hospital stay.

Methods: This study included patients 18 years of age and younger who underwent surgery in the pediatric surgery department of a tertiary hospital. The active warming group consisted of 28 patients and the control group consisted of 29 patients. The first group of patients was actively warmed during the surgery and the second group was treated as the control group and did not actively receive any warming therapy. Preoperative body temperatures of the cases were measured. In addition, body temperatures were recorded every 15 minutes in the intraoperative and postoperative periods.

Results: There was no statistically significant difference in preoperative body temperature between the control group and the active warming group. When body temperature were examined in the intraoperative period, there was no significant difference between the groups at the first 15 minutes of operation; however, the mean of the body temperature in active warming group was significantly higher than the control group at the 30th, 45th, and 60th minutes of operation. It was determined that patients in the control group had a longer stay in the hospital and the amount of time for waking at the end of the anesthesia was shorter in the active heating group than in the control group.

Conclusions: The results of our study suggest that active heating with a carbon fiber resistant system is an effective method to prevent unplanned hypothermia in operated children.

Keywords: Body temperature, surgical procedures, hypothermia, active warming, child.

Introduction
General anesthesia affects the body’s internal temperature in all children undergoing surgery. There is an increased risk of hypothermia, especially in prolonged and open body cavity operations [1]. Heat loss occurs more easily in children [2]. Hypothermia occurs when body temperature drops below 36°C in adults and below 36.5 °C in newborns. Cold stress in newborns is between 36-36.5°C, moderate hypothermia is considered 32-36°C and severe hypothermia is below 32°C [3,4]. During surgery, heat loss from the body occurs by radiation, convection, conduction and
evaporation, and radiation and convection make up 85% of this heat loss [5]. In addition, preterm, low birth weight and sick infants are much more susceptible to hypothermia due to the absence or scarcity of brown adipose tissue, and newborns also have a limited amount of movement, shaking and stretching [6,7]. Therefore, infants and newborns have a higher risk of developing intraoperative hypothermia during surgery. Since thermoregulation centers of babies are not developed, they should be supported in the perioperative period to maintain the hemodynamic state and maintain normal body temperature [8]. In addition, it has been reported that perioperative hypothermia causes wound infections, increases surgical site infections, increases oxygen demand, changes the pharmacokinetics of drugs, impairs coagulation, and causes cardiac arrhythmias and ventilation problems, especially in children [9–12]. Therefore, the quality of care and safety of children who have undergone surgery is very important in the perioperative period.

Passive and active warming methods are used to prevent unplanned hypothermia in the perioperative period. Passive warming methods consist of cotton or wool blankets, stockings, hats, and surgical drapes [13,14]. Active warming techniques include compressed air systems, resistive systems (electric, carbon fiber, jelly coats, etc.), radiant heaters, intravenous fluid, blood and blood products heaters, and heat and moisture exchanger filters [1-14]. There are few studies in the literature on active warming of children in the operating room. In addition, it is not known exactly whether an active warming method will have any effect on preventing unplanned hypothermia in the perioperative period. Therefore, the aim of this study is to investigate the effects of hypothermia on recovery time, pain, shivering, and duration of hospital stay in operated children, and the effects of perioperative active warming on preventing unplanned hypothermia in children.

Materials and Methods
The study was conducted on 57 cases, who underwent surgery in the pediatric clinic at the University Hospital between October 1, 2016, and December 30, 2016. Ethical approval was obtained from Clinical Trials Ethical Board at Bolu Abant Baysal University (Date and decision number: 2016/17-182). Necessary permissions were obtained from the hospital and families. 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.

We included all children under the age of 18 with normal weight and ranked ASA I-II-III and were operated upon. The exclusion criteria were those who needed intensive care, had infectious diseases, scored ASA IV and above, and had congenital anomalies.

The accidental sampling method was used by the investigator to divide the patients into groups and to prevent bias. The sampling size was determined by power analysis in a computer program. Two groups of 60 patients were placed randomly but patients who did not want to participate were excluded from the groups. For this reason, the control group consisted of 29 patients and the experimental group consisted of 28 patients. The operations included in the study were hernia, hernia + circumcision, neck mass excision, undescended testis + circumcision, cholecystectomy and pyeloplasty. The first group of patients were actively warmed during the perioperative period (Istanbul Medical- Medwarm resistive
system-W-500D + 80*50 cm or 120*50). The system was set to 38°C from 40°C and disposable mattress covers were used for each patient in order to prevent surgical area infections. The second group was assigned as a control group. As a result of hospital procedures, all children were dressed in surgical gowns until they were taken to surgery. They were then wrapped in green covers. Apart from this, no application was made to the control group.

Body temperatures of all patients were measured with non-contact thermometer from temporal artery at 15 minute intervals during the preoperative, intraoperative and postoperative period. Body temperature measurement of children continued for 3 hours in the recovery room and pediatric clinic after surgery. The hospital staff measuring the patient's body temperature did not know to which group the patients were assigned. Blood pressure, pulse, respiration, O₂ saturation and pain scores were evaluated synchronously. Additionally, surgery room ambient temperature (°C), surgery time, IV (intravenous) solution quantity (ml), type of anesthesia and ASA score were recorded. Visual pain scale (VAS) scale was used for pain measurement. Those whose body temperature was measured below 36 degrees were considered hypothermic.

**Results**

If we look at the identifying characteristics of patients, 57 patients, that consisted of 28 patients in active warming group and 29 patients in control group, were included in the study. The anesthesia technique used was similar in all children. General anesthesia was applied to all patients.

All patients were normothermic before induction. It was determined that age average of all patients was approximately 3.5 years old, the operating room temperature mean was 25.9±1.3 °C, awakening time from anesthesia was 8.4±3.0 minute, and the length of stay in hospital mean was 1.5±2.5 day in active warming group. Shivering was seen less frequently in patients who were warmed (Table 1).

Hypothermia was observed in 27.6% (8:29) of children in the control group during the intraoperative period. In the postoperative period, 13.8% (4:29) of the children were hypothermic in the control group. Hypothermia was not observed in children in the active heating group who were given intraoperative heating.

When body temperature was examined in the preoperative period, there was no statistically significant difference between the body temperatures in the control group (X: 36, 88±0.43) and the active warming group (X: 36, 80±0.35), indicating that the groups were homogeneous (t: .817; p≥.05). The primary outcomes when body temperature were examined in the intraoperative period was that there was no significant difference between the groups at the first 15 minutes of operation (p ≥ 0.05), but the active warming group was significantly higher than the control group in 30th, 45th, 60th minutes in intraoperative period (p ≤ 0.05), (Figure 1).
Table 1. Identifying characteristics of patients (n: 57).

<table>
<thead>
<tr>
<th>Groups*</th>
<th>A (n:28)</th>
<th>C (n:29)</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>12.3</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>36.8</td>
<td>19</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>9</td>
<td>15.8</td>
<td>13</td>
</tr>
<tr>
<td>2-18</td>
<td>19</td>
<td>33.3</td>
<td>16</td>
</tr>
<tr>
<td>ASA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>24</td>
<td>42.1</td>
<td>25</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>5.3</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>Operation time (minute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-60</td>
<td>21</td>
<td>36.8</td>
<td>25</td>
</tr>
<tr>
<td>Over 60</td>
<td>7</td>
<td>12.3</td>
<td>4</td>
</tr>
<tr>
<td>Duration of fasting (hour)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>1</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>5-8</td>
<td>19</td>
<td>33.9</td>
<td>23</td>
</tr>
<tr>
<td>Over 8</td>
<td>8</td>
<td>14.3</td>
<td>3</td>
</tr>
<tr>
<td>Shivering</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1.8</td>
<td>13</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>47.4</td>
<td>16</td>
</tr>
<tr>
<td>Sum</td>
<td>28</td>
<td>49.1</td>
<td>29</td>
</tr>
</tbody>
</table>

* A: Active Warming Group; C: Control Group **p≤0.05

<table>
<thead>
<tr>
<th></th>
<th>Mean± SD</th>
<th>Mean± SD</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating room temperature</td>
<td>25.9±2.0</td>
<td>26.7±1.3</td>
<td>t: 1.853 p: 0.06**</td>
</tr>
<tr>
<td>Awakening time from anesthesia (minute)</td>
<td>8.4±3.0</td>
<td>10.7±3.0</td>
<td>t: 2.832 p: 0.006**</td>
</tr>
<tr>
<td>Length of stay in hospital (day)</td>
<td>1.5±2.5</td>
<td>1.8±1.5</td>
<td>U: 281.0 p: 0.01**</td>
</tr>
</tbody>
</table>

Figure 1. Change of body temperature during preoperative and intraoperative period. *Preop: Body temperature at the time of admission in operating room.
In the postoperative period, there was no statistically significant difference in the body temperatures between the control group and the active warming group \((p\geq .05)\), (Figure 2).

When pain was examined in the postoperative period, it was observed that the pain mean score of control group was significantly higher than the pain mean score of the active warming group only in the 15th minute \((p \leq 0.05)\) (Table 2).

**Table 2. Pain in the postoperative period.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>C</th>
<th>(p^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mn</td>
<td>6.0±3.4</td>
<td>3.5±3.2</td>
<td>.006</td>
</tr>
<tr>
<td>30 mn</td>
<td>3.2±3.1</td>
<td>2.5±3.2</td>
<td>.20</td>
</tr>
<tr>
<td>45 mn</td>
<td>1.5±1.9</td>
<td>0.8±1.8</td>
<td>.11</td>
</tr>
<tr>
<td>1 hour</td>
<td>0.7±1.3</td>
<td>0.2±0.8</td>
<td>.10</td>
</tr>
<tr>
<td>2 hour</td>
<td>0.4±0.9</td>
<td>0.5±1.2</td>
<td>.94</td>
</tr>
<tr>
<td>3 hour</td>
<td>0.2±0.8</td>
<td>0.4±0.9</td>
<td>.22</td>
</tr>
<tr>
<td>4 hour</td>
<td>0.1±0.5</td>
<td>0.07±0.3</td>
<td>.57</td>
</tr>
</tbody>
</table>

Values: Mean ±SD, \(A\): Active Warming Group; \(C\): Control Group \(p^*p \leq 0.05\)

Shivering were observed in 44.8% of patients in the control group and 3.6% of patients in the active warming group; the difference between the groups was significant \((X^2:13.086; p \leq 0.001)\). It was determined that patients in the control group had a longer stay in the hospital \((Mann-Whitney U: 281000; p \leq 0.05)\) and the amount of time waking at the end of the anesthesia was shorter in the active warming group than the control group \((t: 2.832; p \leq 0.05)\).

**Discussion**

Hypothermia is an important complication during the perioperative period [15,16]. Body temperature should be followed very carefully in both the intraoperative and postoperative period [17]. Healthcare professionals should know the underlying causes of hypothermia and the associated risk factors and also know the strategies to prevent or treat hypothermia. Nowadays, there are many ways that clinicians may help reduce hypothermia in children. It is reported that the use of an active patient warming for children under anesthesia is an effective method to protect patients from...
unwanted perioperative hypothermia [18]. Tander states that children's body temperature drops within ten minutes after induction of anesthesia [9]. Continuous monitoring of core temperature is necessary to prevent hyperthermia.

Ying Pu et al. [19] reported that they warmed patients intraoperatively with warming blanket in gastrointestinal surgery; after the operation, heated patients were found to have lower pain than patients in the control group. Pain and delayed recovery are defined in relation to perioperative hypothermia [16]. Studies have reported that prevention from hypothermia reduced pain after surgery after the operation, especially in children, and this is confirmed by our study because the control group had higher pain scores in our study.

Gharavifard et al. [20] reported that awakening time of children from general anesthesia was 15.30±5.27 minutes after surgery and it was observed that the body temperatures of the children decreased during the surgery in their study. In our study, awakening time of children from general anesthesia was 10.7±3.0 minutes in control group. This value was found to be significantly higher than the awakening time of the warming group (8.4±3.0).

It was reported that normal thermoregulation should be provided in children, otherwise the hospitalization will be prolonged. In our study, it was seen that the control group stayed in the hospital longer than the warming patients.

Radiant heaters are not suitable for use in operating rooms. It also has its difficulties in when used for breastfeeding babies. The blanket or shield can be used for children [21]. The resistive heating system that was used in this study had a shield. Children can be transferred from one place to another using this system.

Studies have clearly shown the benefits of using the active warming systems in infants and children [22]. Sultan et al. [23] reported that the active warming after birth lowered the perioperative temperature and the incidence of hypothermia and shivering was decreased. In this study, postoperative shivering after intraoperative active warming was significantly lower than other patients.

Shen et al. reported that they found the incidence of postoperative hypothermia 5% in their study [24]. In this study, the postoperative hypothermia rate in the control group was 13.8%. No hypothermia was observed in children with the active warming group.

In the literature, force-air warming devices, radiant warmers, and circulating water mattresses are reported as a main method for perioperative hypothermia management [24]. Resistive heating method can be added to these active warming methods. It is also important that such materials can be cleaned due to the recent COVID 19 pandemic. In developing countries, the use of disposable covers cannot be applied due to cost. The resistive system with carbon fiber used in this study is recommended because it is a cost-effective method since it can be disinfected.

In addition to all these, if hypothermia develops, the children must be dressed and covered with a heated blanket and they should be heated by appropriate methods, such as active warming methods. Anesthesia team, who provide perioperative care, are responsible for ensuring and maintaining temperature control during the surgical process [25]. Children’ body temperature should be measured every 2 hours until it rises to more than 36.5 °C [26].

The limitations of this study are the fact that the body surface areas of the patients could not be
measured and the operating room temperature could not be fixed.

Conclusions
Consequently, active warming with a carbon fiber resistive system was found to be an effective method for the prevention of unplanned hypothermia in children. It was also determined that there was less shivering, the amount of time for waking the end of the anesthesia were faster and discharge times were shorter in active warming group.

Funding: There is no financial support and sponsorship
Conflict of Interest: The authors declare that they have no conflict of interest.
Ethical statement: The study was conducted in accordance with the ethical approval of the University Ethics Committee (Date and decision number: 2016/17-182).

ORCID iD of the author(s)
Ganime Esra Soysal / 0000-0002-8291-4310
Arzu Ilce / 0000-0001-8428-9865
Ummuhan Yigit / 0000-0001-6791-2299
Hulya Ozturk / 0000-0003-1719-4924
Murat Bilgi / 0000-0002-9001-2309

Copyrights: © 2021@ author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

References
Investigation of a healthcare-associated candida infections in a Turkish intensive care unit: risk factors, therapy and clinical outcome

Fatma Avcioglu¹ · Fatma Sirmatel² · Mustafa Behcet¹ · Ogulcan Ozarslan² · Hasan Tahsin Gozdas²
¹Department of Medical Microbiology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey
²Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey

ABSTRACT

Aim: Candida infections develop especially in intensive care unit (ICU) patients and increase the mortality rates. So, early and accurate diagnosis of Candida infections and determination of risk factors are very important. We aimed to retrospectively investigate Candida infections in terms of species and risk factors for candidemia caused by fungi.

Methods: Candida infections in critically ill patients hospitalized in the Intensive Care Unit of Bolu Abant Izzet Baysal University Training and Research Hospital between January 2014 and December 2018 were retrospectively analyzed. The isolated Candida species were evaluated according to the sample types. The cases were investigated in terms of mortality due to candidiasis, previously used antibiotics and isolated Candida species.

Results: 34 Candida species isolated from fungal cultures in ICUs were included in the study. Candida albicans (73.5%) was the most prevalent species isolated (NAC 26.5%). Patients with Candida isolated in their urine samples (76%) had a higher mortality rate than patients with Candida from other regions.

Conclusion: The results of our study suggest that the highest rate of candidiasis (88%) is in patients who received beta-lactam antibiotic treatment. In addition, we think that when Candida species are detected in the urine samples of critically ill patients in the ICU, care should be taken in terms of candidiasis.

Keywords: Candida, health care, intensive care unit, candidiasis, mortality.

Introduction

Healthcare-associated infections (HAIs) are the infections that occur while receiving healthcare, that are developed in a hospital or another health center. These infections are seen within the first 48 hours or in longer period after hospitalization or that occur within 30 days after receiving healthcare. Center for Disease Control and Prevention (CDC) reported that 1.7 million hospitalized patients acquire HAIs every year while receiving a treatment for other health problems and more than 98,000 patients (one out of 17) die annually due to HAIs [1]. Candida species are opportunistic pathogenic microorganisms that are normally seen in human flora and may be the cause of an infection when the host’s immune system weakens [2]. As a result of improving
healthcare services, many diseases are no longer a threat to people and their life expectancy is prolonged. However, various opportunistic infections occur due to prolonged hospital stays and antibiotics used. With the increase of awareness, the number of reported opportunistic fungal infections started to increase. In HAIs, besides patient-related factors, procedures applied to patients, long-term antibiotic use and candida colonization cause invasive candida infections with high mortality [3]. Candida species are the fourth most common pathogens causing nosocomial bloodstream infections [4]. The incidence of both Candida albicans (C. albicans) and non-albicans Candida (NAC) species is increasing [5]. Determination of risk factors in patients with Candida infections will be useful for the prevention of mortality and morbidity in the future. Hence, this study aimed to determine risk factors for candida infections due to C. albicans and NAC species and compare the patients infected by these two species.

Materials and Methods
The study protocol was approved by the Bolu Abant İzzet Baysal University Research Ethics Committee (Date and decision number: 2019/237). After getting approval from the local ethics committee, we retrospectively investigated candida infections in critically ill (A patient who needs monitoring to live because of organ/system failures or dysfunction) intensive care unit (ICU) patients in our institution between January 2014 and December 2018. Candida isolates were obtained from different clinical specimens such as urine, blood, sputum, wound, pleural fluid, and peritoneal fluid. Diagnosis of HAI candidiasis infection was made according to CDC criteria [6]. Only the first episode of candidiasis was considered in each patient, recurrent episodes were excluded. Patients were followed up by an infectious diseases specialist and infection control nurse of our hospital on daily basis and required antibiotic and antifungal treatments were managed according to the guidelines.

Microbiologic sample identification
Samples sent to our microbiology laboratory were cultivated into Sabouraud Dextrose Agar (SDA) (Oxoid, England) medium and incubated at 37°C for 24-48 hours. After incubation, yeast isolates in which growth was found were identified at the level of species by using germ tube test and VITEK 2 Compact (BioMérieux, France) fully automated identification system. Isolated candida species were evaluated according to the sample types. Cases were statistically compared in terms of mortality, previously used antibiotics and isolated candida species. Results were shown as the mean ± standard deviation for continuous variables, and the number of cases and percentage (%) for nominal variables. Categorical variables were evaluated using Pearson's Chi-square test and Fisher's exact test. Differences were considered statistically significant if the P value was less than 0.05.

Results
A total of 34 candida species were isolated from fungal cultures of 34 patients (19 female and 15 male). Cultures were obtained from different clinical specimens. Among the isolates, 25 were C. albicans and 9 were NAC species. 17 isolates were from blood samples, 13 from urine samples and 4 from other types of samples (pleural fluid, peritoneal fluid, wound and sputum). When isolated candida strains were distinguished as C. albicans and NAC, the highest rate of mortality was seen in patients in
whom *C. albicans* was isolated. When the clinical course of the patients was evaluated in terms of discharge and mortality, the highest mortality rate was found in patients in whom *C. albicans* were isolated from their urine samples. Mortality rates according to sample types of *C. albicans* and NAC were also given in Table 1.

When the patients were classified according to the antibiotics (Beta-lactam, Carbapenem, Quinolone, anti-anaerobic antibiotics, etc.) they used before *C. albicans* and NAC were isolated, the highest rate of candidiasis (88%) was seen in patients who received beta-lactam antibiotic treatment (Table 2).

**Table 1.** Previously used antibiotics of the cases.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Male (n/%)</th>
<th>Female (n/%)</th>
<th>Total (n/%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Lactam</td>
<td>17 / 89.50</td>
<td>13 / 86.70</td>
<td>30 / 88.20</td>
<td>0.801</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>3 / 15.80</td>
<td>4 / 26.70</td>
<td>7 / 20.60</td>
<td>0.436</td>
</tr>
<tr>
<td>Quinolone</td>
<td>5 / 26.30</td>
<td>3 / 20</td>
<td>8 / 23.50</td>
<td>0.666</td>
</tr>
<tr>
<td>Anti-anaerobic</td>
<td>3 / 15.80</td>
<td>2 / 13.30</td>
<td>5 / 14.70</td>
<td>0.841</td>
</tr>
<tr>
<td>Anti-staphylococcal</td>
<td>3 / 15.80</td>
<td>5 / 33.30</td>
<td>8 / 23.50</td>
<td>0.231</td>
</tr>
<tr>
<td>Other</td>
<td>6 / 31.60</td>
<td>3 / 20</td>
<td>9 / 26.50</td>
<td>0.447</td>
</tr>
</tbody>
</table>

**Table 2.** Discharge and mortality rates of the cases according to culture type.

<table>
<thead>
<tr>
<th>Culture type</th>
<th>Pathogen</th>
<th>Total (n/%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Candida albicans</em> (n/%)</td>
<td><em>Non-albicans Candida</em> (n/%)</td>
<td>Discharge</td>
<td>Exitus</td>
<td>Discharge</td>
</tr>
<tr>
<td>Urine</td>
<td>3 / 30</td>
<td>7 / 70</td>
<td>0 / 0</td>
<td>3 / 100</td>
<td>3 / 23.10</td>
</tr>
<tr>
<td>Blood</td>
<td>8 / 61.50</td>
<td>5 / 38.5</td>
<td>1 / 25</td>
<td>3 / 75</td>
<td>9 / 52.90</td>
</tr>
<tr>
<td>Sputum</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1 / 100</td>
<td>0 / 0</td>
<td>1 / 100</td>
</tr>
<tr>
<td>Wound</td>
<td>1 / 100</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1 / 100</td>
</tr>
<tr>
<td>Pleural Fluid</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1 / 100</td>
<td>0 / 0</td>
</tr>
<tr>
<td>Peritoneal Fluid</td>
<td>1 / 100</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1 / 100</td>
</tr>
<tr>
<td>Total</td>
<td>13 / 52</td>
<td>12 / 48</td>
<td>2 / 22.20</td>
<td>7 / 77.80</td>
<td>15 / 44.10</td>
</tr>
<tr>
<td>p</td>
<td>0.235</td>
<td>0.198</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion
Candida infections are important health problems in critically ill patients in ICUs. These infections occur as a result of complicated surgical procedures, use of invasive medical devices and long term and broad-spectrum antibiotic treatment [5]. Thus, determining the risk factors for Candida infections will contribute to the decrease in occurrence of these types of infections. Recently, distribution of Candida species has shown an increase in favour of NAC rather than *C. albicans* [7]. Less response to antifungal treatment in NAC infections contribute to the increase in mortality rates [8-10]. In studies conducted in Turkey, *C. albicans* (CA) was generally isolated more often and the incidence rates of CA differs between 45.8% and 75.6% (Table 3) [11-16].

In this study, the rate of CA among isolated Candida infections that were found in critically ill ICU patients was 73.5%. In our study, we also found the incidence of CA infections to be more than NAC infections. Compared with other studies, it was thought that the limited number of patient groups included in this study (critically ill) may lead to a higher incidence of CA.

Candida infections are more commonly seen in patients hospitalized in ICUs due to many different reasons and also because of their weak immune system. In these kinds of patients, interventional procedures such as urinary catheter and intravenous catheter applications increase the susceptibility of patients against these infections. Candida infections are

### Table 3. Previous studies of health care associated candidiasis infections from Turkey.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Institution</th>
<th>Subjects</th>
<th>Duration</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Çagan et al.(^\text{11})</td>
<td>Marmara University Faculty of Medicine</td>
<td>29 pediatric patients</td>
<td>2011-2013</td>
<td>55.1% <em>C. albicans</em></td>
</tr>
<tr>
<td>Karalti(^\text{12})</td>
<td>Yeditepe University Hospital</td>
<td>249 urine specimens</td>
<td>2006-2016</td>
<td>54.2% <em>C. albicans</em></td>
</tr>
<tr>
<td>Kilincel et al.(^\text{13})</td>
<td>Duzce University, Research and Training Hospital</td>
<td>81 blood cultures</td>
<td>2012-2018</td>
<td>55% <em>C. albicans</em></td>
</tr>
<tr>
<td>Savci et al.(^\text{14})</td>
<td>Bozok University Research and Application Hospital</td>
<td>42 clinical specimens</td>
<td>2014-2016</td>
<td>66.7% <em>C. albicans</em></td>
</tr>
<tr>
<td>Yapar et al.(^\text{15})</td>
<td>Tertiary-care educational hospitals in Turkey</td>
<td>83 Blood cultures</td>
<td>2008-2009</td>
<td>45.8% <em>C. albicans</em></td>
</tr>
<tr>
<td>Sav et al.(^\text{16})</td>
<td>Erciyes University Hospital</td>
<td>3905 clinical specimens</td>
<td>2011-2012</td>
<td>75.6% <em>C. albicans</em></td>
</tr>
<tr>
<td>Present study</td>
<td>Bolu Abant İzzet Baysal University Hospital</td>
<td>34 critically ill patients</td>
<td>2014-2018</td>
<td>73.5% <em>C. albicans</em></td>
</tr>
</tbody>
</table>
characterized with high rate of mortality [17]. The most common types of infections seen in ICUs are bloodstream infections, catheter-associated infections and urinary tract infections [18]. In this study, the highest rate of Candida was isolated from blood (50%) and urine (38%) samples. Mortality rate was higher in patients in whom candida was isolated from urine samples (76%) compared to the patients in whom candida was isolated from other sites. It should be considered that Candida isolated especially from urine samples of critically ill patients in ICUs may increase mortality.

Multiple risk factors were attributed to Candida infections. One of the most important predisposing factors is long-term and broad-spectrum antibiotic treatment [19]. In this study, when the patients were classified according to the types of antibiotics used before the detection of Candida infection, beta lactam antibiotics (88.2%) was found to be the most commonly used antibiotic group. Studies on the predisposing factors causing Candida infections are not sufficient in Turkey. However, Yapar et al. [15] and Büyüktuna et al. [19], revealed a relationship between long term antibiotic use and Candida infection.

Our study has some limitations. Patients in ICUs who were included in the study were only analyzed according to the types of antibiotics used in terms of predisposing factors. Predisposing conditions such as hematopoietic stem sell or solid organ transplantation, diabetes mellitus, malignancy, trauma and renal failure were not questioned in patients. Moreover, the presence of urinary catheter and intravenous catheter was not investigated. In addition, several factors other than candida infection might have contributed to mortality. Therefore, we cannot definitely say that Candida infections were the only reason of mortality rates.

In conclusion, it was concluded in this study that the highest rate of Candida infections in ICU patients belonged to *C. albicans* species. It should be considered that Candida species isolated from candida infections may affect the prognosis of patients. Early diagnosis and treatment in Candida infections will help to decrease mortality rates. In this study, the highest rate of mortality was seen in *C. albicans* infections strains isolated from urine samples. Therefore, we think that when Candida species are detected in the urine samples of critically ill patients in the ICU, care should be taken in terms of candidiasis.

**Acknowledgement**

This study was presented as an oral presentation in "Turkey 7th International EKMUD Congress 8-13 May 2018"

**Funding:** There is no financial support and sponsorship

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

**Ethical statement:** The study was conducted in accordance with the ethical approval of the University Ethics Committee (Date and decision number: 2019/237).

**ORCID iD of the author(s)**

Fatma Avcioglu / 0000-0002-6011-7775
Fatma Sirmatel / 0000-0003-0442-5981
Mustafa Behcet / 0000-0002-5676-6983
Ogulcan Ozarslan / 0000-0002-2635-7011
Hasan Tahsin Gozdas / 0000-0003-3857-685X

**Copyrights:** © 2021 author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s)
and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

References
[17] Pelit S, Uzun M. Investigation of species distribution and antifungal susceptibility of candida species isolated from various


Changes in levothyroxine pharmacokinetics following bariatric surgery in obese hypothyroid patients

Feyzi Gokosmanoglu¹ · Attila Onmez²
¹Department of Endocrinology, Medical Park Hospital, Ordu, Turkey
²Department of Internal Medicine, Medical Faculty, Duzce University, Duzce, Turkey

ABSTRACT

Aim: Levothyroxine (L-T4) requirements in obese hypothyroid patients receiving L-T4 therapy decrease following bariatric surgery. L-T4 absorption is also thought to decrease after surgery. The purpose of this study was to evaluate L-T4 requirements in hypothyroidism cases before and after weight loss through bariatric surgery.

Methods: Seventy-six cases of hypothyroidism receiving L-T4 replacement therapy and with a body mass index over 40 kg/m² were included in the study. Patients losing at least 10% of basal body weight following bariatric surgery were assessed in terms of thyroid hormone levels and L-T4 requirements over follow-up of at least one year. The L-T4 requirements of patients in whom euthyroidism was achieved were compared in terms of bariatric surgery procedures and hypothyroidism etiology.

Results: Seventy-six patients (56 women, 20 men) with a mean age of 38 years (18-51) were included in the study. Mean weight before bariatric surgery was 121.6 ± 6.8 kg, and mean body mass index was 49.5 ± 1.6 kg/m². Euthyroidism was confirmed at pre- and post-bariatric surgery evaluation. No statistically significant postoperative changes were determined in thyroid-stimulating hormone or free thyroxine-3 and -4 ($p > 0.05$). A statistically insignificant decrease was observed in L-T4 dosages after surgery in cases of Hashimoto’s thyroiditis ($p = 0.064$). A statistically significant decrease was determined in L-T4 dosages in cases of non-Hashimoto hypothyroidism ($p = 0.001$). L-T4 requirements decreased in both surgical procedures ($p = 0.001$)

Conclusion: Postoperative L-T4 requirements decrease with weight loss. In addition, no decrease appeared to occur in L-T4 absorption following the surgical procedures in this study.

Keywords: Obesity, hypothyroidism, bariatric surgery, levothyroxine.
Drug absorption may be deficient in association with the type of bariatric surgery performed. Drug absorption deficiency can lead to an increased L-T4 requirement in hypothyroid patients. However, the data concerning this are inconsistent [3]. Research has shown that obese hypothyroid patients require higher L-T4 dosages than normal weight individuals [4]. L-T4 dosages decrease following weight loss [5]. However, it may be necessary to increase the dosages due to the possibility of resection-related reduced drug absorption. Decreased L-T4 requirements have recently been shown in the majority of hypothyroid patients in line with weight loss achieved with modern bariatric surgery. The purpose of the present study was to investigate the effect of weight loss following bariatric surgery performed using mini-gastric bypass and sleeve gastrectomy procedures on L-T4 requirements in hypothyroid patients.

**Materials and Methods**

This study was performed in compliance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medicana International Samsun Hospital, Turkey (12.08.2020-9/7109). The research concerned 76 patients. The study group occurred of adult (over 18 years) hypothyroid patients (Hashimoto’s thyroiditis, thyroidectomy, radioactive iodine ablation therapy, congenital hypothyroidism, central hypothyroidism, post-subacute thyroiditis, or infiltrative causes) receiving L-T4 replacement therapy under follow-up in our endocrinology and obesity surgery department, with body mass index exceeding 40 kg/m2 (class 3 obesity), and undergoing bariatric surgery. Patients were assessed using thyroid gland ultrasonography in order to avoid diagnostic confusion.

Patients with known thyrotoxicosis during screening, with primary endocrine/systemic disease, any inflammatory disease other than Hashimoto’s thyroiditis, celiac disease and malabsorption, inflammatory gastrointestinal diseases, cancer, history of use of other medications reducing drug absorption, or receiving radiotherapy to the neck and head region were excluded. In the light of the study aims, patients achieving weight loss of at least 10% from baseline following bariatric surgery were evaluated in terms of thyroid hormone levels and LT-4 requirements initially and over a 12-month follow-up period at intervals of 2-3 months. Patients were given detailed instruction concerning L-T4 use and asked to take the drug as described one hour before breakfast. The importance of the timing of L-T4 use was emphasized, particularly in cases using multivitamins and proton pump inhibitors in the postoperative period. Cases using the drug irregularly or frequently forgetting to take L-T4 were placed under close monitoring, and thyroid hormones were evaluated during periods when L-T4 was used regularly. If TSH values were below 2.5 mIU/L in cases losing more than 10% of body weight following bariatric surgery, the L-T4 dosage was reduced by 25%. The dosage was decreased by 30% in cases with TSH below 1 mIU/L at postoperative follow-up.

Hormonal data for TSH (normal range 0.35-4.94 mIU/L), free thyroxine-4 (fT4; normal range: 9.01-19.05 pmol/L), free triiodothyronine (fT3; normal range: 3.5-8.0 pmol/L), albumin (normal range: 3.5-5.5 g/dL), thyroglobulin antibody (TgAb; normal range: 0-4.11 IU/mL), and thyroid peroxidase antibody (TPOAb; normal range: 0-5.61 IU/mL) levels were investigated using automated immune chemiluminometric assay.
Thyroid ultrasonography was applied using a high resolution device (Philips Medical system, USA) equipped with a 5–12 MHz broadband linear array probe. All procedures were performed by an experienced operative.

**Statistical analysis**

Data analysis was performed on SPSS version 21.0 software (SPSS Inc., Chicago, USA). Quantitative parametric data were expressed as mean plus standard deviation (SD), and quantitative non-parametric data as median values with minimum and maximum. The Kolmogorov Smirnov and Shapiro-Wilk test was used to analyze the distribution of variables. For non-parametric data, comparisons between different groups were performed using the Mann-Whitney U test, while the independent-t test was used to compare parametric data between the groups.

**Results**

Seventy-six patients (56 women, 20 men) with a median age of 38 years (18-51) were included in the research. Mini-gastric bypass was performed in 52 cases, and sleeve gastrectomy in 24. Postoperative follow-up time was 2.4 (1.7-3.6) years. Hypothyroidism associated with the surgical procedure was present in 28 cases, Hashimoto’s thyroiditis in 32, and hypothyroidism associated with other causes (radioactive iodine [RAI] intake, congenital hypothyroidism, post-subacute thyroiditis, and infiltrative causes) in 16. Patients’ mean weight before bariatric surgery was 121.6±6.8 kg, and mean body mass index was 49.5±1.6 kg/m². Mean weight after bariatric surgery decreased to 94.6±5.6 kg and mean body mass index to 33.3±1.4 kg/m². Cases’ pre- and postoperative characteristics are shown in Table 1.

All bariatric surgery procedures were performed using standard techniques by an experienced bariatric surgeon. Euthyroidism was confirmed at pre- and post-bariatric surgery checks. No statistical postoperative changes were determined in levels of the thyroid hormones TSH, fT4 or fT3 ($p>0.05$). We found a statistically significant decrease in L-T4 dosages in parallel with weight loss after bariatric surgery in cases diagnosed with hypothyroidism developing after bariatric surgery and other hypothyroidism cases (RAI intake, congenital hypothyroidism, post-subacute thyroiditis, and infiltrative causes) ($p=0.001$). We also determined a non-significant decrease in L-T4 dosages in cases of Hashimoto’s thyroiditis ($p=0.064$). The decrease in L-T4 dosages was highly significant in both surgical procedures ($p=0.001$). The findings are shown in Table 2.

**Table 1. Preoperative characteristics of the patients.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n=76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median(min-max)</td>
<td>38 (18-51)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>56 (73)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>20 (27)</td>
</tr>
<tr>
<td>Weight (kg), mean±SD</td>
<td>121.6±6.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>49.5±1.6</td>
</tr>
<tr>
<td>Etiology of hypothyroidism</td>
<td></td>
</tr>
<tr>
<td>Post-thyroidectomy</td>
<td>28</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>32</td>
</tr>
<tr>
<td>Other1</td>
<td>16</td>
</tr>
<tr>
<td>Type of bariatric surgery</td>
<td></td>
</tr>
<tr>
<td>Mini-gastric bypass</td>
<td>52</td>
</tr>
<tr>
<td>Sleeve gastrectomy</td>
<td>24</td>
</tr>
</tbody>
</table>

*BMI: Body Mass Index, SD: Standard Deviation

$^1$RAI intake, congenital hypothyroidism, post-subacute thyroiditis, infiltrative causes.
Discussion
L-T4 dosage requirements decrease in parallel with weight loss in obese cases diagnosed with hypothyroidism. Loss of body mass eliminates anxiety concerning a decrease in L-T4 absorption resulting from the operation. Newly evidence [5] and our own study data show that thyroid hormone requirements may decrease with weight loss in obesity. A few studies have determined significant decreases in TSH and fT3 levels with weight loss after obesity surgery. Weight loss also reduces thyroid hormone requirements [5]. Similarly in the present study, we found a statistically significant decrease in L-T4 requirements in association with weight loss. L-

Table 2. Changes in thyroid function and L-thyroxine dosages following bariatric surgery.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before bariatric surgery</th>
<th>After bariatric surgery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg) (mean±SD)</td>
<td>121.6±6.8</td>
<td>94.6±5.6</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>49.5±1.6</td>
<td>33.3±1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>2.1±0.5</td>
<td>1.9±0.4</td>
<td>0.652</td>
</tr>
<tr>
<td>fT4 (pmol/L)</td>
<td>13.1 ± 0.8</td>
<td>14.9 ± 0.6</td>
<td>0.860</td>
</tr>
<tr>
<td>fT3 (pmol/L)</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.8</td>
<td>0.972</td>
</tr>
<tr>
<td>L-thyroxine, mcg/day, dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical hypothyroidism</td>
<td>149.3±46.7</td>
<td>112.5±32.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>152.5±52.3</td>
<td>145.9±36.8</td>
<td>0.064</td>
</tr>
<tr>
<td>Other hypothyroidism¹</td>
<td>140.5±42.5</td>
<td>115.6±31.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.7±0.6</td>
<td>4.2±0.8</td>
<td>0.043</td>
</tr>
<tr>
<td>Type of bariatric surgery, L-thyroxine, mcg/day, dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mini-gastric bypass</td>
<td>148.4±49.2</td>
<td>125.2±35.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Sleeve gastrectomy</td>
<td>150.6±51.5</td>
<td>118.9±38.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TSH, thyroid-stimulating hormone; fT4, free-T4; fT3, free-T3; RAI, radioactive iodine; SD: Standard Deviation. ¹RAI intake, congenital hypothyroidism, post-subacute thyroiditis, infiltrative causes.

T4 dosage requirements decrease in parallel with weight loss to yield similar TSH levels in both types of surgery. In our previous study we observed that TSH levels decreased in correlation with insulin resistance following bariatric surgery [6]. Juiz-Valiña et al. [7] also reported similar findings. We therefore think that L-T4 requirements decreased through a similar pathway to the decrease in insulin resistance occurring with weight loss in association with thyroid hormone resistance in obese patients. It possibly concluded that weight loss restores thyroid hormone homeostasis. Research has shown that severely obese individuals may require higher L-T4 suppressing or replacement therapy than
normal weight subjects in association with impairment of the L-T4 pharmacokinetic parameters [8]. Decreased thyroid gene expression (particularly TSH-receptors) has been reported in visceral adipose tissue and subcutaneous in obese patients [9]. The decrease in adipose tissue in parallel with weight loss following obesity surgery results in corresponding changes in serum TSH and thyroid hormone levels. These changes show that adipocytes are involved in the regulation of thyroid hormones. Our research revealed that L-T4 dose requirements decreased in parallel with weight loss in cases of surgical hypothyroidism and other hypothyroidism cases. This finding suggested that the level of adverse effects on drug absorption caused the decrease in gastric acid in the sleeve gastrectomy group can be overlooked. In the mini gastric bypass cases, however, approximately 1/3 of the length of the intestine was bypassed. This method may have permitted sufficient drug absorption through the non-bypass intestine.

Research has shown that L-T4 requirements decrease in the postoperative period. The principal correlation shown in research is between weight loss and dosage [1]. The principal L-T4 fluid formulation has been shown to be capable of preventing the problem of malabsorption after bariatric surgery in malabsorptive operations (Roux-en-Y gastric bypasses and biliary pancreatic diversions) [10]. Our findings showed no L-T4 malabsorption in sleeve gastrectomy and mini gastric bypasses. Loss of both adipose and non-adipose mass following these operations may result in a decrease in postoperative L-T4 requirements by preventing absorptive effects resulting from surgery [11]. These studies have shown no decrease in L-T4 absorption in Roux-en-Y bypasses. Small but significantly delayed L-T4 absorption has been observed after surgery in these cases [12].

Research involving sleeve gastrectomy and mini gastric bypasses has shown no significant L-T4 absorption in the gut, duodenum, or upper part of the jejunum [13]. These are bypassed or removed in bariatric surgery. These studies offer powerful support for our own findings. L-T4 dosage requirements also decrease in parallel with weight loss. In contrast, although we observed a decrease in drug requirements in both types of operation in hypothyroidism cases associated with Hashimoto’s thyroiditis, this was not statistically significant. This variation may be due to an increase dose requirement deriving from the chronic progressive course of Hashimoto’s thyroiditis. Alternatively, these operations may have resulted in additional absorption insufficiency due to Hashimoto’s thyroiditis being frequently accompanied by celiac disease, inflammatory bowel disease, and autoimmune gastric diseases. We think that these factors may account for the decreased L-T4 dosage requirements determined in other cases not being observed in Hashimoto’s thyroiditis cases.

**Conclusion**

Both adipose and non-adipose body mass decrease with significant weight loss achieved with bariatric surgery. Marked weight loss results in a decrease in postoperative L-T4 requirements. This decrease is more powerful than the effect of resection on absorption. This study shows that sleeve gastrectomy and mini gastric bypasses do not result in L-T4 malabsorption. This study adds new findings to the actual evidence regarding changes caused by weight loss in thyroid morphology.

**Funding:** There is no financial support and sponsorship
Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical statement: This study was performed in compliance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medicana International Samsun Hospital, Turkey (Date and decision number: 12.08.2020-9/7109).

ORCID iD of the author(s)
Feyzi Gokosmanoglu / 0000-0002-6432-8668
Atilla Onmez / 0000-0002-7188-7388

Copyrights: © 2021@ author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

References

Optimization and screening of solid lipid nanoparticle production for gene delivery by factorial design and response surface methodology

Hasan Akbaba · Melike Ozder
Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Ege University, Izmir, Turkey

ABSTRACT

Aim: A successful gene therapy requires a delivery system for overcoming various biological barriers. For this, we adapted the factorial design and response surface methodology to the cationic solid lipid nanoparticle production process.

Methods: Screening and optimization of formulations were carried out with factorial design with 3 factors and 3 levels using Box-Behnken Design. Then, solid lipid nanoparticles were physicochemically characterized. Furthermore, optimal SLN formulation is examined in terms of complex formation with plasmid DNA, its protection potential against nucleases, cytotoxicity profile, and storage stability.

Results: Response-surface analyses demonstrated that the selected quadratic model holds significance for particle size and zeta potential. The interaction of independent variables was statistically determined. Optimization and prediction were performed using obtained second-order polynomial equations. Optimal formulation and complexes were found to be nanosized, positively charged and their polydispersity-index values below 0.3 as an indicator of being monodispersed. Cytotoxicity of the optimal formulation is compatible for further studies and no significant increase was observed in particle size until day 21 and until day 60 for polydispersity-index.

Conclusion: Optimal formulation provides a good basis as a gene delivery system was produced with developed systematic. Briefly, this methodology could be used to obtain SLNs with desired conditions.

Keywords: Solid lipid nanoparticle, factorial design, response surface method, gene delivery.

Introduction

According to the U.S. Food and Drug Administration (FDA), gene therapy purpose to change or control the expression of a gene or to make adjustments to the biological features of living cells for therapeutic use [1]. Successful gene therapy requires a gene delivery system for overcoming various biological barriers and the effects of the environment. The gene delivery system must protect the genetic material from lysosomal degradation when taken into cell cytoplasm with endocytosis and then be able to transcend the nuclear membrane as a biological barrier. Genetic material also should be protected against harsh environmental conditions such as pH and temperature change, redox reactions [2–5].
Various gene transfer techniques are applied. They are divided into viral and non-viral techniques. As viral techniques; adenoviruses, adeno-associated viruses, lentiviruses, and retroviruses are often used for gene delivery. Non-viral techniques contain; inorganic particles (calcium phosphate, silica, gold, magnetic), synthetic or natural biodegradable particles (polymeric, cationic lipid, peptide-based vectors) and physical methods (needle injection, ballistic DNA injection, electroporation, sonoporation, photoporation, magnetofection, hydroporation) [6–8].

Although viral techniques are mostly used in clinical trials, some of their difficulties caused alternative techniques to be considered. These difficulties are biosafety problems (high immunogenicity and potential insertional mutagenesis) and limited gene package capacity [9–11].

One of the non-viral methods, solid lipid nanoparticles (SLN); are non-toxic carrier systems stabilized by surfactants and made from solid lipids for controlled and targeted delivery. Their advantageous features are, biocompatibility, assembling of hydrophilic and hydrophobic drugs, maintain the release of the drug and avoid photochemical, chemical, or oxidative degradation [12]. These systems have several advantages such as improved transfection, reduced immunogenicity, large gene package size, ease of manufacture, protection from degradation, prolong circulation of the nanoparticles (NPs), reduce side effects, realize controlled release of the contents, and enhanced targeted delivery [13]. SLNs are preferred as gene delivery systems due to their large surface areas, small dimensions, ability to transfect genetic material, biocompatibility, and suitability for large-scale production [9,14]. SLNs could be produced by various methods; high shear homogenization and ultrasound, high-pressure homogenization (hot and cold), solvent emulsification/evaporation, microemulsion based SLN preparations [15–19].

For the production of SLNs, the selection of components, their proportions, and the differences depending on the preparation method lead to the presence of a wide alternative sample space. Therefore, a method that allows a systematic analysis should be applied prior to experimental investigation. In a factorial design, the appropriate number of samples for various factors and levels are determined for the analysis of independent variables and dependent variables. The main goal is to achieve the highest level of output with the least number of data in order to reduce the labor force and cost while increasing efficiency. Box-Behnken designs are used to generate higher-order response surfaces using fewer required runs with factorial design technique. Box-Behnken design can also perform statistical analysis of the model, response optimization, and prediction of the formulations that meet the desired conditions [20,21]. In this study, we used a factorial design for scanning microemulsion components and optimizing SLN formulation. We examined the Design of Experiments (DOE), which we propose to be used in the development of formulations that are potential as gene delivery systems using Response Surface Methodology (RSM). Moreover, we characterized the complex formed by the optimal SLN formulation with plasmid DNA and examined it in terms of its protection potential against nucleases, cytotoxicity profile, and storage stability.

**Materials and Methods**

This study has been projected by the Scientific and Technological Research Council of Turkey.
(TÜBİTAK) under code TÜBİTAK-SBAG-2185682. EGFP encoding plasmid pcDNA3-EGFP was a gift from Doug Golenbock (Addgene plasmid # 13031; http://n2t.net/addgene:13031; RRID: Addgene_13031). Behenoyl polyoxyl-8 glycérides (Compritol® HD5 ATO) (C-HD5) was kindly donated by Gatetfosse, France. Macrogolglycerol ricinoleat (Kolliphor® ELP) (K-ELP) was kindly donated by BASF, Germany. Mouse fibroblast cell line (L929) was purchased from ATCC, USA. Ethanol (EtOH), Sodium dodecyl sulfate (SDS) were provided from Merck-Co., Germany. Dimethyldioctadecylammonium bromide (DDAB) was purchased from Sigma-Aldrich Co., USA. Alamar Blue cell viability assay kit was purchased from Thermo Fisher Scientific, USA. All other chemicals were of analytical grade and used as received. Ultrapure water was used in all stages needed.

**Nanoparticle preparation**

Cationic SLNs were produced with slight modifications to the previously described melt-emulsification technique [22,23]. As formulation components, C-HD5 and DDAB were used as lipid phase; K-ELP was used surfactant (S); EtOH was used as co-surfactant (CoS), and UPH₂O was used as an aqueous phase. In the first step of production, microemulsion contents were weighed in a sealed cap glass vial as determined by factorial design based on preliminary laboratory studies. Then, the vial and its content were kept in a water bath at 10 degrees above the lipid melting point (75°C) until transparent microemulsion was self-established. Obtained oil in water (o/w) microemulsion was dispersed in cold ultra-pure distilled water (0-4 °C) at a ratio of 1:10 (w/v) under vigorous stirring. SLNs were obtained by sudden freezing of microemulsion droplets in cold water.

**Experimental design**

Screening and optimization of SLN formulations were carried out with factorial design with 3 factors and 3 levels. The amount of lipid (3%, 4%, and 5%) and the total amount of surfactant and co-surfactant (18%, 24%, and 30%) were selected as two variables. These variables are important since they form two main corners of the pseudo-ternary phase

<table>
<thead>
<tr>
<th>Variables</th>
<th>Level of variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variables</td>
<td>Low Level (-1)</td>
</tr>
<tr>
<td>A</td>
<td>Lipid (%)</td>
</tr>
<tr>
<td>B</td>
<td>Surfactant + Co-Surfactant (1:2, w/w) (%)</td>
</tr>
<tr>
<td>C</td>
<td>Lipid to cationic lipid ratio (w/w)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Goals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>Particle Size (nm)</td>
</tr>
<tr>
<td>Y2</td>
<td>Polydispersity Index</td>
</tr>
<tr>
<td>Y3</td>
<td>Zeta Potential (mV)</td>
</tr>
</tbody>
</table>
diagram, which is commonly used in the production of the microemulsion [24,25]. The presence and amount of cationic lipids, which are essential for cationic SLN production, were aimed to be investigated as a third variable. For this, the lipid to cationic lipid ratio (2:1, 1:1, 1:2, w/w) was determined as the third variable. Dependent on the preliminary studies and according to the literature, the factorial design matrix was created using the Minitab 19 software (Minitab LLC, USA) using the variables represented in Table 1. Totally 15 different compositions were determined as 12 alternative microemulsion contents and 3 midpoint replicates. Dynamic light scattering (DLS) analysis is widely used for the characterization of SLNs. Particle size, PDI, and zeta potential measurements obtained by DLS analysis were selected as dependent variables. Response optimization was determined to aim the smallest particle size and the most homogeneous particle size distribution in the manner of the lowest PDI value. Zeta potential was set as a target response value and determined as 35 mV. The designed variables with codes and actual responses in terms of DLS measurement results are given in Table 2. In order to provide screening in the sample space and according to the analysis of variance (ANOVA), the quadratic model was chosen for a Box-Behnken design. With the RSM analysis, P-Value, F-Value, coefficient determinant ($R^2$),

Table 2. Designed formulation parameters and measured responses using Box-Behnken Design for SLN production.

<table>
<thead>
<tr>
<th>No</th>
<th>Formulation Code</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Y1</th>
<th>Y2</th>
<th>Y3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipid (%)</td>
<td>S + CoS (%)</td>
<td>L/Cat.L (w/w)</td>
<td>Particle Size (nm) ± S.D.</td>
<td>PDI ± S.D.</td>
<td>Zeta Potential (mV) ± S.D.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C-HD5-SLN 1</td>
<td>3</td>
<td>24</td>
<td>2:1</td>
<td>3155 ±116</td>
<td>0.756 ±0.060</td>
<td>33.8 ±7.87</td>
</tr>
<tr>
<td>2</td>
<td>C-HD5-SLN 2</td>
<td>3</td>
<td>24</td>
<td>1:2</td>
<td>200.4 ±29.37</td>
<td>0.341 ±0.070</td>
<td>34.6 ±2.67</td>
</tr>
<tr>
<td>3</td>
<td>C-HD5-SLN 3</td>
<td>5</td>
<td>24</td>
<td>2:1</td>
<td>3513 ±117.1</td>
<td>0.983 ±0.046</td>
<td>64.6 ±4.09</td>
</tr>
<tr>
<td>4</td>
<td>C-HD5-SLN 4</td>
<td>5</td>
<td>24</td>
<td>1:2</td>
<td>146.1 ±15.6</td>
<td>0.239 ±0.042</td>
<td>41.4 ±8.25</td>
</tr>
<tr>
<td>5</td>
<td>C-HD5-SLN 5</td>
<td>4</td>
<td>18</td>
<td>2:1</td>
<td>4024 ±470.2</td>
<td>0.858 ±0.202</td>
<td>59.8 ±0.96</td>
</tr>
<tr>
<td>6</td>
<td>C-HD5-SLN 6</td>
<td>4</td>
<td>18</td>
<td>1:2</td>
<td>65.31 ±2.578</td>
<td>0.628 ±0.038</td>
<td>40.0 ±3.12</td>
</tr>
<tr>
<td>7</td>
<td>C-HD5-SLN 7</td>
<td>4</td>
<td>30</td>
<td>2:1</td>
<td>690.2 ±172.4</td>
<td>0.740 ±0.150</td>
<td>35.3 ±14.4</td>
</tr>
<tr>
<td>8</td>
<td>C-HD5-SLN 8</td>
<td>4</td>
<td>30</td>
<td>1:2</td>
<td>45.86 ±16.63</td>
<td>0.468 ±0.224</td>
<td>40.6 ±3.96</td>
</tr>
<tr>
<td>9</td>
<td>C-HD5-SLN 9</td>
<td>3</td>
<td>18</td>
<td>1:1</td>
<td>514.3 ±62.17</td>
<td>0.531 ±0.082</td>
<td>61.5 ±2.40</td>
</tr>
<tr>
<td>10</td>
<td>C-HD5-SLN 10</td>
<td>5</td>
<td>18</td>
<td>1:1</td>
<td>708.4 ±118.2</td>
<td>0.561 ±0.108</td>
<td>60.8 ±1.68</td>
</tr>
<tr>
<td>11</td>
<td>C-HD5-SLN 11</td>
<td>3</td>
<td>30</td>
<td>1:1</td>
<td>592.7 ±92.47</td>
<td>0.596 ±0.076</td>
<td>36.3 ±2.85</td>
</tr>
<tr>
<td>12</td>
<td>C-HD5-SLN 12</td>
<td>5</td>
<td>30</td>
<td>1:1</td>
<td>222.1 ±11.95</td>
<td>0.459 ±0.122</td>
<td>39.9 ±3.13</td>
</tr>
<tr>
<td>13</td>
<td>C-HD5-SLN 13</td>
<td>4</td>
<td>24</td>
<td>1:1</td>
<td>399.3 ±19.44</td>
<td>0.516 ±0.291</td>
<td>34.9 ±2.73</td>
</tr>
<tr>
<td>14</td>
<td>C-HD5-SLN 14</td>
<td>4</td>
<td>24</td>
<td>1:1</td>
<td>353.1 ±62.41</td>
<td>0.573 ±0.081</td>
<td>33.5 ±3.48</td>
</tr>
<tr>
<td>15</td>
<td>C-HD5-SLN 15</td>
<td>4</td>
<td>24</td>
<td>1:1</td>
<td>424.5 ±152.4</td>
<td>0.573 ±0.006</td>
<td>32.8 ±1.66</td>
</tr>
</tbody>
</table>
and adjusted coefficient determinant (R$^2$ adj) data were evaluated statistically. Actual data of the formulation determined by response optimization were measured and the statistically produced predicted values were calculated. The fit of the model and second-order polynomial equations were determined. Then, the 3D-surface and contour plots were analyzed.

**Agarose gel retardation assay, SDS-induced release, and DNase I protection studies**

Agarose gel retardation assay was performed to determine the optimal complex formation ratio of C-HD5-SLN and pcDNA3-EGFP. Complexes with various ratios were formed by mixing the constant amount of pcDNA3-EGFP (100 ng/µL) with the increasing amount of C-HD5-SLN under gentle shaking for 30 min at room temperature, which allows the formation of electrostatic interactions between the positive charges of SLNs and the negative charges of pcDNA3-EGFP. The resultant complexes were characterized by agarose gel retardation assay [26–28].

In order to show the release of pcDNA3-EGFP, SDS induced release study was performed. To evaluate the capacity of the C-HD5-SLN to protect pcDNA3-EGFP, complexes were incubated with DNase I (0.4 IU DNase I/1 µg pDNA) at 37°C for 30 minutes, then decomplexed in presence of SDS 1% (w/v) and further subjected agarose gel retardation assay [29]. The bands were observed by a UV transilluminator with a digital imaging system (Vilber Lourmat, France).

**Physicochemical characterization for freshly prepared SLNs and stability studies**

The particle size, polydispersity index, and zeta potential values of C-HD5-SLN and C-HD5-SLN:pcDNA3-EGFP complex were measured by DLS (Zeta sizer Nano ZS, Malvern Instruments Ltd., UK) method using non-invasive backscattering mode with the detector positioned at 173°. DLS measurements were reported as averaged intensity weighted distribution for particle size measurements. The refractive index of the Compritol (1.456) as a main ingredient of the lipid matrix was used for DLS calculations [30]. The triplicate measurements were performed using disposable polystyrene microcuvettes.

The morphology of C-HD5-SLN and C-HD5-SLN:pcDNA3-EGFP complex were visualized by using Scanning Electron Microscope (SEM, Carl Zeiss 300VP, Germany). Sample preparation was done by drying the nanoparticles on metal plates and then coating with 100 Å thick gold in the brand coating device (Quorum Q150 Res, UK).

The physicochemical stability of the formulation was followed up to 2 months. SLNs were stored at 4 °C and measured at days 0, 7, 14, 30, and 60 to evaluate the stability.

**Cytotoxicity analysis**

The in vitro cytotoxicity profile of the C-HD5-SLN and C-HD5-SLN:pcDNA3-EGFP complex was evaluated on the fibroblast cell line (L929). One day before the experiment, L929 cells were plated in 96-well plates at a density of 5 × 10$^3$ cells per well in 100 µL. After overnight incubation, cells were treated with C-HD5-SLN and C-HD5-SLN:pcDNA3-EGFP complex at increasing formulation volumes (3, 6, 9, 12, 15 µL/well), with respect to SLN volume for 24 h. The percentage of living cells was investigated by the Alamar Blue proliferation kit (Thermo Fisher Scientific, USA) according to the kit’s manual.

**Statistical analysis**

Except for the experimental design and RSM, the data analysis was performed with Prism 6
(GraphPad Software, Inc., USA) software. The statistical analysis between different groups has been assessed by a non-paired t-test and Two-way ANOVA followed by multiple comparison tests. Differences were considered statistically significant if the P value was less than 0.05. The results of all experiments were reported as mean ± S.D.

Results and Discussion

Microemulsion content is the main factor determining the physicochemical properties of SLNs obtained by the microemulsion dilution method. With this method, the first step in the production of SLN is to obtain clear transparent microemulsions. One of the most common methods to obtain clear microemulsions is to obtain pseudo-ternary phase diagrams and determine the microemulsion formation region on this diagram [24,25,31]. Then, SLNs are produced by selecting an initial microemulsion formulation based on the central point or several different points of this area. However, there can be an infinite number of alternative microemulsion formulation, regardless of the size of the area. A reliable statistical evaluation is required to decide which point to choose. In order to make this statistical evaluation, dependent and independent variables and related responses should be determined. Particle size is the most widely used variable for SLN evaluation. While encapsulation efficiency is an eligible parameter for SLNs loaded with drugs, the zeta potential value is an important parameter for cationic SLNs that will interact with nucleic acids via electrostatic forces on the surface.

On the pseudo-ternary phase diagrams used in the conventional determination of the content ratios of the microemulsion, each corner has one of the main components of the formulation. In most cases, these are the lipid phase, the aqueous phase, and the total of surfactant and co-surfactant amounts in percentages. Among these factors, the lipid phase and total of surfactant and co-surfactant were selected as formulation independent variables (Table 1). The aqueous phase was not chosen as a variable because determining the percentage of lipid and the percentage of total surfactant and co-surfactant ensures that the percentage of water is also determined since the total of the system

<table>
<thead>
<tr>
<th>Response</th>
<th>Regression analysis of variance</th>
<th>Regression equation in coded units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle Size</strong></td>
<td>R² 94.19%  R² adj 83.73%  F-Value 376.83  P-Value 0.003</td>
<td>Y1 = 1824 - 4268 C - 2077 A + 303 C + 1029 C<em>C + 332 A</em>A - 5.98 B<em>B - 103 C</em>A + 138.1 C<em>B - 23.5 A</em>B</td>
</tr>
<tr>
<td><strong>PDI</strong></td>
<td>R² 88.70%  R² adj 68.37%  F-Value 6.16  P-Value 0.143</td>
<td>Y2 = -0.39 + 0.251 C + 0.567 A - 0.0061 B + 0.1015 C<em>C - 0.0377 A</em>A + 0.00086 B<em>B - 0.0822 C</em>A - 0.00592 C<em>B - 0.01154 A</em>B</td>
</tr>
<tr>
<td><strong>Zeta Potential</strong></td>
<td>R² 88.62%  R² adj 78.14%  F-Value 64.12  P-Value 0.015</td>
<td>Y3 = 319.9 - 5.7 C - 61.5 A - 12.99 B + 2.08 C<em>C + 7.78 A</em>A + 0.2252 B<em>B - 6.00 C</em>A + 1.046 C<em>B + 0.179 A</em>B</td>
</tr>
</tbody>
</table>

Table 3. The fit of the model and second order polynomial equations of the responses
will be equal to one hundred percent. Therefore, the solid lipid/cationic lipid ratio in the lipid phase was chosen as the 3rd variable. The cationic lipid plays an important role in the charge of the formulation, hence its stability and its electrostatic interaction with negatively charged nucleic acids.

With this information, as indicated in Table 1, lipid percentage, total surfactant and co-surfactant percentage, and solid lipid cationic lipid ratio were determined as three independent variables. For the analysis of the response related to these variables, particle size, PDI, and zeta potential values were examined. Box-Behnken Design allows these variables to be evaluated with fewer samples. While it is necessary to test $3^3 = 27$ different microemulsions in 3-factor 3-level factorial design, with Box-Behnken Design optimization, this factorial design can be completed with only 15 experiments with 3 replicate center points. The contents of the microemulsion presented accordingly are given in Table 2 together with the response characterization values.

Response surface regression analysis was performed using the values in Table 2. The second order polynomial equations and statistical evaluations obtained accordingly are summarized in Table 3. According to the RSM analysis, the F value for the particle size was determined as 376.83 ($p = 0.003 < 0.05$). The F value demonstrated that the selected quadratic model holds significance [20,21]. The coefficient determinant ($R^2$) value was found 94.19% value indicates the reliability of the model together with adjusted $R^2$ value. Overall, it can be predicted that particle size is affected by all variables. The same interference is valid for zeta potential depending on the statistical data presented in Table 3. However, PDI value does not fit the model while F value was found to be 6.16 and the P-value is 0.145 >0.05.

When we investigate the surface and contour plots of response surface regression analysis in terms of particle size, PDI, and zeta potential, we observed that the surface and contour plots lines are curved. If the model showed no interaction, the 3D surface plots would be planar [32,33]. Curved structures are the sign of interaction between variables. Likewise, in contour plots, this situation can be demonstrated by the presence of elliptical lines. These inclined plots are indicative of a second-order polynomial equation for the model (Figure 1,2,3). In the polynomial equation, the negative sign coefficient value indicates the antagonist effect and positive values indicate the agonist or synergist effect [20,34]. Regression Equation in Coded Units was given in Table 3. For particle size, $A$, $C * C$, $A * A$, and $C * B$ values have positive coefficient values while $B$, $C$, $B * B$, $C * A$, and $A * B$ values have negative coefficient values. In terms of PDI, $A$, $C$, $C*C$, and $B*B$ values have positive coefficient values while $B$, $A*A$, $C*A$, $C*B$, and $A*B$ values have negative coefficient values. And for Zeta potential, $C*C$, $A*A$, $B*B$, $C*B$, and $A*B$ values have positive coefficient values while $A$, $B$, $C$, and $C*A$ values have negative coefficient values.

RSM helps in the prediction of the theoretical optimum conditions for desired responses [20,21,32]. Mathematically offered formulation components were experimentally obtained. Optimal desired formulation component ratios and, predicted and actual experimental values were shown in Table 4. The obtained data successfully predicts the responses in parallel significance with P values. The particle size and zeta potential values of this formulation, coded as C-HD5-Opt-SLN, are very close to the predicted value. The PDI value was relatively
Figure 1. 3D surface response plots and contour plots showing the interference of the variables on particle size.

Figure 2. 3D surface response plots and contour plots showing the interference of the variables on PDI.
far from the predicted value, as it was shown in the regression analysis that the PDI value was not fit for the model (P>0.05). Following the determination of consistency in optimized formulation values with the predicted values, the potential of this optimal SLN formulation was investigated in terms of the gene delivery system requirements.

RSM helps in the prediction of the theoretical optimum conditions for desired responses [20,21,32]. Mathematically offered formulation components were experimentally obtained. Optimal desired formulation component ratios and, predicted and actual experimental values were shown in Table 4. The obtained data successfully predicts the responses in parallel

![Figure 3](image)

**Figure 3.** 3D surface response plots and contour plots showing the interference of the variables on zeta potential.

**Table 4.** Observed and predicted responses of the optimal formulation.

<table>
<thead>
<tr>
<th>C-HD5-SLN-Opt</th>
<th>Optimized variables</th>
<th>Actual and predicted responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Predicted Values</strong></td>
<td>% 4.45</td>
<td>% 28.3</td>
</tr>
<tr>
<td><strong>Actual Values</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
significance with P values. The particle size and zeta potential values of this formulation, coded as C-HD5-Opt-SLN, are very close to the predicted value. The PDI value was relatively far from the predicted value, as it was shown in the regression analysis that the PDI value was not fit for the model (P>0.05). Following the determination of consistency in optimized formulation values with the predicted values, the potential of this optimal SLN formulation was investigated in terms of the gene delivery system requirements.

Cationic SLNs developed as gene delivery systems form complexes with plasmid DNA via electrostatic interactions. However, the ratio of this complex should be determined to find optimal C-HD5-SLN-Opt: pcDNA3-EGFP ratio. For this, electrophoretic mobility assays were performed for the pcDNA3-EGFP plasmid. Samples were prepared by forming complexes with increasing amounts of C-HD5-SLN-Opt, against a fixed amount of pcDNA3-EGFP (100 ng) with vigorous shaking at room temperature. When the agarose gel electrophoresis images were examined, it was observed that the electrophoretic mobility completely stopped at the ratio of 1:1 (v/v) as seen in the fourth well. (Figure 4a). The same ratios investigated for the SDS-induced release study and at all ratios, SLNs were released the pcDNA3-EGFP for the total concentration of 1% SDS (w/v) solution (Figure 4b). In Figure 4c, all complex ratios performed in electrophoretic mobility assay were prepared freshly and then treated with DNase I (0.4 IU DNase I/1 µg pDNA) enzyme to determine the protection capacity of the formulation [27,35]. After incubation at 37°C for 30 minutes, SDS-induced release of pcDNA3-EGFP was performed on both samples and further subjected by agarose gel electrophoresis to compare the condition of the released plasmid. Naked pcDNA3-EGFP was treated with the same amount of DNase I as control of enzyme activity (Lane C). As shown in Figure 2c, the best protection was provided in starting 1:1 (v/v) ratio, which is seen in lane 4 and determined as optimal according to the electrophoretic mobility assay.

Next, the physicochemical properties of the complex (C-HD5-SLN-Opt: pcDNA3-EGFP, 1:1, v/v) were characterized. Particle size was
again found nanosized and below 80 nm. This size range is adequate for traverse into the cells [36]. Moreover, the charge of the system remained cationic. This is a factor that increases the entry of particles and genetic material into the cell by electrostatic interaction with the negatively charged cell membrane (Table 5). One of the precursors of an optimal and compact complex formation ratio is the PDI value. The PDI values below 0.4 indicate that the nanoparticle system is considered as monodispersed for drug delivery and PDI values over 0.7 indicate that the nanoparticle system has a broad size distribution for being a drug delivery system [23,37]. Another important parameter for the characterization of nanoparticles is the particle size distribution plot. For both C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP (1:1, v/v) complex, a single peak was observed (Figure 5). If the structure of the complex was not adequately compact, more than one peaks would have been observed representing the excessive amount of pcDNA3-EGFP or bare C-HD5-SLN-Opt.

Increasing the amount of C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP (1:1, v/v) complex was evaluated for cytotoxicity test (Figure 6). In cytotoxicity studies, C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP (1:1, v/v) complex containing an equivalent amount of nanoparticle were tested in L929 cells. Cytotoxicity profiles are parallel to each other for the nanoparticle and the complex. Although there is dose-dependent cytotoxicity in both cell lines, the cell viability for the required amounts for transfection studies has not fallen below 70%. Delgado et al. used 2.5 µg / mL plasmid DNA for transfection. This corresponds to the use of the C-HD5-SLN-Opt: pcDNA3-EGFP complex of less than 3 µL sample volume used in the cytotoxicity study [38].

Table 5. Physicochemical characterization of optimal complex.

<table>
<thead>
<tr>
<th>Characterization of Complex</th>
<th>Particle Size (nm) ±S.D.</th>
<th>PDI ± S.D.</th>
<th>Zeta Potential (mV) ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-HD5-SLN-Opt: pcDNA3-EGFP (1:1, v/v)</td>
<td>73.76 ± 0.093</td>
<td>0.253 ±0.007</td>
<td>30.1 ± 1.48</td>
</tr>
</tbody>
</table>

Figure 5. Size distribution plot of C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP, (1:1, v/v) complex. n=3.
The physicochemical stability of the developed C-HD5-SLN-Opt vector system was also evaluated. For this purpose, C-HD5-SLN-Opt were stored at 4 °C and particle size was measured at days 0, 7, 14, 30, and 60 (Figure 7). No significant increase was observed in particle size until day 21 (p>0.05). PDI value did not change significantly during the duration of stability monitoring.

Representative SEM photographs show that of C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP, (1:1, v/v) complex are in the global structure and their size is correlated with the DLS measurement (Figure 8). For the advanced characterization of C-HD5-SLN-Opt and C-HD5-SLN-Opt: pcDNA3-EGFP, (1:1, v/v) complex statistical evaluations can be enriched with various methods such as differential scanning calorimetry (DSC) analysis, permeability studies, and pharmacokinetic studies [19].

**Conclusion**

The cationic solid lipid nanoparticles were successfully prepared using factorial design. For this purpose, Box Behnken design was used to optimize and analyze the formulation parameters. Response optimization was performed via software to find out the optimum conditions for desired SLN formulation. Optimum solid lipid nanoparticle formulation was investigated in terms of its protection potential against nucleases, cytotoxicity profile, and storage stability. In this regard, the obtained C-HD5-SLN-Opt coded solid lipid nanoparticle formulation provides a good basis as a gene delivery system. We showed that Box-Behnken design is a useful model to reduce labor force and cost especially for compounds with low quantities and high prices. With this method, it
is possible to produce solid lipid nanoparticles with the desired feature by differing the variables and responses. Moreover, transfection efficiency can also be analyzed and incorporated into the Box-Behnken Design as a dependent variable and its efficiency can be optimized in the further studies.

Acknowledgment
This study has been financially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under grant code TÜBİTAK-SBAG-218S682.

Conflict of Interest: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.
No Competing interests are at stake and there is No Conflict of Interest” with other people or organizations that could inappropriately influence or bias the content of the paper.

ORCID iD of the author(s)
Hasan Akbaba / 0000-0001-9273-6346
Melike Ozder / 0000-0001-6150-7444

Copyrights: © 2021@ author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice.
No use, distribution or reproduction is permitted which does not comply with these terms.

References


Evaluation of risk factors associated with pancreatic adenocarcinoma in Black Sea region, Turkey

Murat Derebey¹ · Kagan Karabulut¹ · Saim Savas Yuruker¹ · Ilhan Karabicak² · Necati Ozen¹
¹Department of General Surgery, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey
²Department of General Surgery, VM Medical Park Hospital, Samsun, Turkey

ABSTRACT

Aim: To evaluate the risk factors in patients with pancreatic adenocarcinoma in Turkey's Black Sea region and to determine groups at high risk for pancreatic cancer.

Methods: 106 newly diagnosed pancreatic adenocarcinoma patients living in Black Sea region of Turkey who applied to our clinic between January 2015 and December 2016 were included in this study. In the same period a control group was formed with 92 patients of similar age. Both groups were asked to fill out a form that questioned the pancreatic cancer risk factors. Data were analyzed.

Results: Pancreatic cancer risk was 3.5 times higher in people over 65 years of age. Patients with deficient level of serum vitamin D level (<20 ng/ml) had 10.2 times more risk of pancreatic cancer than patients with normal level of Vitamin D (≥30 ng/ml). While newly diagnosed type 2 diabetics had 19.5 times higher risk of pancreatic cancer, long term type 2 diabetics had 1.2 times higher risk than normal individuals. Those with pancreatic cancer in family had 4.3 times higher risk than those who did not. It was observed that people with rhesus (Rh) antigen negative blood group has 70% less risk of pancreatic cancer.

Conclusion: Determination of pancreatic cancer risk factors, organization of imaging and screening programs for high-risk people can provide early diagnosis of the disease and prolong survival.

Keywords: Pancreatic adenocarcinoma, risk factors, pancreatic neoplasms, etiology, epidemiology.

Introduction

Although pancreatic cancer is the 11th most common cancer type, it ranks as the third leading cause of cancer-related death in the USA [2]. Pancreatic cancer is predicted to be the second most common cause of cancer-related deaths in coming 20 years [3]. In a study conducted by Parkin et al. in 2002, pancreatic cancer accounts for 3% of all cancer types worldwide, however, it is among the top in cancer-related deaths due to its poor diagnosis [1]. In many patients, when pancreatic cancer is detected, the disease is advanced and the tumor is not operable. Median survival rate is less than a year, and mortality rate is approximately 99%. Only less than 10% of pancreatic tumors can be detected at an early phase and resected. Usually the disease starts to develop two years before its diagnosis [4]. Most patients initially have a non-specific pain in epigastrium. As the disease develops in time, the pain spreads over to the back, upper thoracic and lumbar region. Upon progress of the disease, most patients consult doctors with complaints of pain, jaundice, and
loss of weight. It is often the case that when patient seeks medical advice with these complaints, the disease has already reached the advanced stage [1-5].

There are numerous studies conducted to identify causes of pancreatic cancer. In etiology, environmental and genetic causes are considered to play a joint role. Risk factors are divided into two as changeable and unchangeable risk factors. Unchangeable risk factors are age, gender, race, genetic factors, family history, atopy/allergy history, blood groups, surgery history, diabetes and chronic pancreatitis. Changeable risk factors, on the other hand, can be listed as smoking, obesity, use of alcohol, dietary, socio-economic conditions, infections, levels of vitamin D and folic acid. Analysis of risk factors and identification of factors that significantly increases the risk are of high importance [1-10]. Identifying populations of high-risk, establishing imaging and screening programs for these groups can support early diagnosis of the disease and increase survival period. Therefore, in this study, we aimed to identify high-risk groups together with risk factors in patients with pancreatic adenocarcinoma in Turkey's Black Sea region.

**Materials and Methods**

Ethical approval for this study was obtained from the local ethics committee (IRB approval number 144/2015). Necessary permissions were obtained from the hospital and families. 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.

106 newly diagnosed pancreatic adenocarcinoma patients living in Black Sea Region of Turkey who applied to our clinic between January 2015 and December 2016 were included in this study. In the same period, a control group was also formed with 92 patients of similar age group who visited our clinic for another disease and had not been diagnosed with cancer. Both groups were asked to fill out a form that questioned the risk factors. The factors to be investigated in this study, conducted as a case-controlled and cross-sectional study, are defined as follows: age (≤50, 51-65, >65); gender (female, male); family history of pancreatic cancer (yes, no); atopy/allergy (yes, no); blood type (A,B,O,AB; rhesus (Rh) antigen negative, Rh antigen positive); cholecystectomy (yes, no); gastrectomy (yes, no); type 2 diabetes (yes, no); duration of diabetes - newly diagnosed type 2 diabetes (<2 years), long term type 2 diabetes (≥2 years); smoking (never smoked, still smoking or stopped smoking); body mass index (BMI) normal weight (18.5-24.99), pre-obesity (25-29.99), obesity (≥30); alcohol consumption (yes, no); place of residence (rural, urban); helicobacter pylori history (yes, no); level of vitamin D normal (>30, 80 ng/ml), insufficient (20, ≤30 ng/ml), deficient (<20 ng/ml); level of folic acid low (<4.6 ng/ml), normal (4.6-18.7 ng/ml), high (>18.7 ng/ml).

**Statistical analysis**

The collected data were analyzed with IBM SPSS V23. In the evaluation of the study data, besides the descriptive statistical methods (mean, standard deviation), in the comparison of qualitative data; Pearson's chi-squared test were used. The p-value <0.05 was significantly considered. Independent Sample T-test was applied in the analysis of the significance of the difference between the averages obtained.

**Results**

There was no significant difference across groups concerning age and gender (p>0.05)
Pancreatic cancer risk was found 3.5 times higher in patients over 65 years of age compared to the control group ($p<0.05$). Gender was not associated with increased pancreatic cancer risk ($p>0.05$). The risk of pancreatic cancer was observed 3.4 times higher in people with insufficient serum vitamin D level. For people with deficient level of serum vitamin D, the risk increased 10.2 times more ($p<0.05$). When non-smokers were taken as reference, there was no increase in the risk of pancreatic cancer in individuals who quit smoking or who are smoking ($p>0.05$). The risk of pancreatic cancer was detected to increase 1.2-fold in diabetic patients ($p<0.05$). When diabetic patients were classified as newly diagnosed type 2 diabetes (<2 years) and long-term type 2 diabetes (≥2 years), the risk of pancreatic cancer increased by 19.5-fold in newly diagnosed type 2 diabetic patients ($p<0.05$). There was no association between BMI, alcohol use, chronic pancreatitis and pancreatic cancer risk ($p>0.05$). Compared to the control group, the risk of pancreatic cancer was observed to increase 4.3-fold in patients with pancreatic cancer history in the family ($p<0.05$).

There was no significant difference in terms of pancreatic cancer risk between those living in urban areas and rural areas ($p>0.05$). Similarly, we observed no relation between the level of serum folic acid, distribution of ABO blood types, helicobacter pylori, atopy/allergy, gastrectomy, cholecystectomy, and the risk of pancreatic cancer ($p>0.05$). However, the risk of pancreatic cancer was found less patients with Rh antigen negative blood group ($p<0.05$) (See table 2).

There was no significant difference across groups concerning age and gender ($p>0.05$) (See Table 1). Pancreatic cancer risk was found 3.5 times higher in patients over 65 years of age compared to the control group ($p<0.05$). Gender was not associated with increased pancreatic cancer risk ($p>0.05$). The risk of pancreatic cancer was observed 3.4 times higher in people with insufficient serum vitamin D level. For people with deficient level of serum vitamin D, the risk increased 10.2 times more ($p<0.05$). When non-smokers were taken as reference, there was no increase in the risk of pancreatic cancer in individuals who quit smoking or who are smoking ($p>0.05$). The risk of pancreatic cancer was detected to increase 1.2-fold in diabetic patients ($p<0.05$). When diabetic patients were classified as newly diagnosed type 2 diabetes (<2 years) and long-term type 2 diabetes (≥2 years), the risk of pancreatic cancer increased by 19.5-fold in newly diagnosed type 2 diabetic patients ($p<0.05$). There was no association between BMI, alcohol use, chronic pancreatitis and pancreatic cancer risk ($p>0.05$). Compared to the control group, the risk of pancreatic cancer was observed to increase 4.3-fold in patients with pancreatic cancer history in the family ($p<0.05$).

**Table 1. Age and gender distribution.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pancreatic Cancer</th>
<th>Control Group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average age (Years)</strong></td>
<td>65 ±11</td>
<td>62 ±13</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>11 (10.4%)</td>
<td>19 (20.7%)</td>
<td>0.679</td>
</tr>
<tr>
<td>51-65</td>
<td>33 (31.1%)</td>
<td>33 (35.9%)</td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>62 (58.5%)</td>
<td>40 (43.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63 (59.4%)</td>
<td>52 (56.5%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43 (40.6%)</td>
<td>40 (43.5%)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Comparison of pancreatic cancer risk factors between groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pancreatic Cancer Group</th>
<th>Control Group</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>11 (10.4%)</td>
<td>19 (20.7%)</td>
<td>Reference</td>
<td>0.014</td>
</tr>
<tr>
<td>51-65</td>
<td>33 (31.1%)</td>
<td>33 (35.9%)</td>
<td>1.1 (0.4 – 4.1)</td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>62 (58.5%)</td>
<td>40 (43.5%)</td>
<td>3.5 (1.2 – 10.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63 (59.4%)</td>
<td>52 (56.5%)</td>
<td>Reference</td>
<td>0.616</td>
</tr>
<tr>
<td>Female</td>
<td>43 (40.6%)</td>
<td>40 (43.5%)</td>
<td>0.9 (0.4 – 2.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Serum vitamin D level (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Normal (&gt;30, 80)</td>
<td>3 (2.8%)</td>
<td>10 (10.9%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Insufficient (20, ≤30)</td>
<td>6 (5.7%)</td>
<td>15 (16.3%)</td>
<td>3.4 (0.5 – 25.4)</td>
<td></td>
</tr>
<tr>
<td>Deficient (&lt;20)</td>
<td>97 (91.5%)</td>
<td>67 (72.8%)</td>
<td>10.2 (2 – 52.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.242</td>
</tr>
<tr>
<td>Never smoked</td>
<td>51 (48.1%)</td>
<td>52 (56.5%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Still smoking or smoked before/quit smoking</td>
<td>55 (51.9%)</td>
<td>40 (43.5%)</td>
<td>1.6 (0.7 – 3.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.757</td>
</tr>
<tr>
<td>Normal (18.5-24.99)</td>
<td>22 (20.8%)</td>
<td>28 (30.4%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Pre-obesity (25-29.99)</td>
<td>44 (41.5%)</td>
<td>32 (34.8%)</td>
<td>1.7 (0.7 – 4.3)</td>
<td></td>
</tr>
<tr>
<td>Obesity (≥30)</td>
<td>40 (37.7%)</td>
<td>32 (34.8%)</td>
<td>1.3 (0.5 – 3.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Type 2 diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>65 (61.3%)</td>
<td>73 (79.3%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41 (38.7%)</td>
<td>19 (20.7%)</td>
<td>1.2 (0.4 – 3.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of type 2 diabetes (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>No</td>
<td>65 (61.3%)</td>
<td>73 (79.3%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Newly diagnosed type 2 diabetes (&lt;2 years)</td>
<td>23 (21.7%)</td>
<td>2 (2.2%)</td>
<td>19.5 (3 – 127.3)</td>
<td></td>
</tr>
<tr>
<td>Long term type 2 diabetes (≥2 years)</td>
<td>18 (17.0%)</td>
<td>17 (18.5%)</td>
<td>1.2 (0.6 – 2.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Family history of pancreatic cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.041</td>
</tr>
<tr>
<td>No</td>
<td>96 (90.6%)</td>
<td>89 (96.7%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (9.4%)</td>
<td>3 (3.3%)</td>
<td>4.3 (0.8 – 22.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.796</td>
</tr>
<tr>
<td>No</td>
<td>88 (83.0%)</td>
<td>77 (83.7%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (17.0%)</td>
<td>15 (16.3%)</td>
<td>0.9 (0.3 – 2.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.054</td>
</tr>
<tr>
<td>Rural</td>
<td>50 (47.2%)</td>
<td>27 (29.3%)</td>
<td>2 (0.9 – 4.3)</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>56 (52.8%)</td>
<td>65 (70.7%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic pancreatitis history</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.814</td>
</tr>
<tr>
<td>No</td>
<td>104 (98.1%)</td>
<td>91 (98.9%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (1.9%)</td>
<td>1 (1.1%)</td>
<td>0.7 (0.1 – 32.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Helicobacter pylori history</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.310</td>
</tr>
<tr>
<td>No</td>
<td>93 (87.7%)</td>
<td>85 (92.4%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (12.3%)</td>
<td>7 (7.6%)</td>
<td>1.5 (0.5 – 5.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Serum folic acid level (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.099</td>
</tr>
<tr>
<td>Low (0, ≤4.6)</td>
<td>9 (8.5%)</td>
<td>18 (19.6%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Normal (4.6-18.7)</td>
<td>91 (85.8%)</td>
<td>69 (75.0%)</td>
<td>3.7 (1.2 – 11.5)</td>
<td></td>
</tr>
<tr>
<td>High (&gt;18.7)</td>
<td>6 (5.7%)</td>
<td>5 (5.4%)</td>
<td>2.8 (0.5 – 15.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood groups</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.835</td>
</tr>
<tr>
<td>O</td>
<td>30 (28.3%)</td>
<td>32 (34.8%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>56 (52.8%)</td>
<td>36 (39.1%)</td>
<td>1.5 (0.7 – 3.4)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>14 (13.2%)</td>
<td>19 (20.7%)</td>
<td>0.6 (0.2 – 1.8)</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>6 (5.7%)</td>
<td>5 (5.4%)</td>
<td>1.1 (0.2 – 5.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Rh status</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>Rh antigen negative</td>
<td>91 (85.8%)</td>
<td>69 (75.0%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Rh antigen positive</td>
<td>15 (14.2%)</td>
<td>23 (25.0%)</td>
<td>0.3 (0.1 – 0.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Atopy/allergy</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.453</td>
</tr>
<tr>
<td>No</td>
<td>99 (93.4%)</td>
<td>82 (89.1%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (6.6%)</td>
<td>10 (10.9%)</td>
<td>0.6 (0.1 – 2.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Cholecystectomy</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.466</td>
</tr>
<tr>
<td>No</td>
<td>91 (85.8%)</td>
<td>83 (90.2%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (14.2%)</td>
<td>9 (9.8%)</td>
<td>1.6 (0.5 – 5.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Gastrectomy</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.926</td>
</tr>
<tr>
<td>No</td>
<td>102 (96.2%)</td>
<td>90 (97.8%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (3.8%)</td>
<td>2 (2.2%)</td>
<td>1.1 (0.2 – 8.2)</td>
<td></td>
</tr>
</tbody>
</table>
1.2-fold in diabetic patients \((p<0.05)\). When diabetic patients were classified as newly diagnosed type 2 diabetes \((<2\text{ years})\) and long-term type 2 diabetes \((\geq 2\text{ years})\), the risk of pancreatic cancer increased by 19.5-fold in newly diagnosed type 2 diabetic patients \((p<0.05)\). There was no association between BMI, alcohol use, chronic pancreatitis and pancreatic cancer risk \((p>0.05)\). Compared to the control group, the risk of pancreatic cancer was observed to increase 4.3-fold in patients with pancreatic cancer history in the family \((p<0.05)\). There was no significant difference in terms of pancreatic cancer risk between those living in urban areas and rural areas \((p>0.05)\). Similarly, we observed no relation between the level of serum folic acid, distribution of ABO blood types, helicobacter pylori, atopy/allergy, gastrectomy, cholecystectomy, and the risk of pancreatic cancer \((p>0.05)\). However, the risk of pancreatic cancer was found less patients with Rh antigen negative blood group \((p<0.05)\) (see Table 2).

**Discussion**

In the United States, 10% of pancreatic cancers are under the age of 50. It has been reported that the risk of cancer increases with age \([5-7]\). When people aged 50 and below are referenced, the risk of pancreatic cancer has increased 3.5-fold for those who are older than 65 \((p<0.05)\). Many studies on pancreatic cancer have not revealed a significant difference between genders \([8, 9]\). In our study, gender was not correlated to pancreatic cancer risk \((p>0.05)\). The best indicator of vitamin D level in serum is 25-hydroxyvitamin D3. This level is determined by dietary vitamin D and sun exposure. It is stated that serum vitamin D level may vary depending on age, race, BMI and diseases. Experimental studies suggest that the risk of pancreatic cancer may be reduced if the optimal level of serum vitamin D is achieved, depending on the diet or sun rays. However, clinical studies are inadequate; there are some conflicting data available, too. In one of these, it has been suggested that there may be an increased risk of pancreatic cancer with increased serum 25-hydroxyvitamin D3 levels. However, at the end of the study, the difference was considered to stem from the difference in patient-control group analysis of the studied population or the difference in the method used to measure the level of 25-hydroxyvitamin D3 \([10]\). In an experimental study conducted by Kasiappan et al. \([11]\) a group of mice with tumors were given a high-fat diet for 30 days; another group with a tumor was given a high-fat diet and the vitamin D analogue seacalcitol (EB1089). When the two groups were compared, tumor growth was more than 50% in mice that did not receive the vitamin D analogue seacalcitol. In our study, the risk of pancreatic cancer increased by 10.2-fold in people with deficient serum D level compared to individuals with normal vitamin D level \((p<0.05)\). Pre-clinical studies contain data which indicates that cancer risk will decrease with vitamin D support. Clinical studies cannot adequately express this. This relationship is maybe really weak; perhaps it may be present in certain cancers.

Smoking is the most important known risk factor associated with pancreatic cancer. In a study, the risk of pancreatic cancer was found to be two times higher in smokers than in nonsmokers. It was stated that this risk would increase in direct proportion with the amount of cigarettes used and the duration of time smoked \([12]\). According to our data, there was no correlation between smoking and the risk of pancreatic cancer \((p>0.05)\). Based on American Cancer Society studies, those with BMI \(\geq 30\) had a 2.08 times greater risk of pancreatic cancer.
cancer than those with BMI < 25 [13]. In the data obtained, there was no correlation BMI and pancreatic cancer risk \((p>0.05)\). Diabetes is a risk factor for pancreatic cancer; it can also be a sign of the emergence of pancreatic cancer or a condition that may occur after surgery. In a meta-analysis of 20 studies conducted by Everhart and Wright in 1995, the risk of pancreatic cancer in diabetic individuals was found to be 2 times more than in non-diabetic [1]. In the data obtained, the risk of pancreatic cancer in diabetic patients was found 1.2 times higher than in non-diabetic patients \((p<0.05)\). In a study conducted with 1428 pancreatic cancer and 1528 healthy control groups, the rate of diabetes was 27.6% in people with pancreatic cancer, while the rate of diabetes was 9.9% in people in the control group. Of the patients who have both pancreatic cancer and diabetes, 76.2% had newly diagnosed diabetes, which was significantly higher than the control group [14]. When non-diabetic patients were referenced, the risk of pancreatic cancer increased 19.5-fold in the presence of newly diagnosed type 2 diabetes (<2 years); those with long-term type 2 diabetes (≥2 years) had a 1.2-fold increased risk of pancreatic cancer \((p<0.05)\).

Familial pancreatic cancer accounts for 10% of all patients with pancreatic cancer. In a study, the risk of pancreatic cancer for people in whose immediate family (mother, father, and siblings) there are at least two with pancreatic cancer increases the risk ten times compared to those with no family history [15]. According to our data, the risk of cancer increased 4.3 times in patients with a family history of pancreatic cancer \((p<0.05)\). 1530 pancreatic cancer patients and 1530 healthy individuals from the Pancreatic Cancer Cohort Consortium were evaluated in a case-control study. There was no significant statistical difference between those who had >0 or <5 grams of alcohol daily and those who had ≥60 grams of alcohol daily [16]. In another study, it was reported that consuming 40 grams of alcohol daily increased the risk of pancreatic cancer 1.45 times, and that it could increase up to 1.62 times [17]. The results from these studies are contradictory. In our study, there was no correlation between alcohol use and the risk of pancreatic cancer \((p>0.05)\).

Research has shown that pancreatic cancer is more common in people living in urban areas with low socioeconomic condition. According to our data, no relationship was found between the place of residence and the risk of pancreatic cancer \((p>0.05)\). Four percent of patients with chronic pancreatitis are believed to develop pancreatic cancer [18]. If a person has a history of familial pancreatitis, the risk increases 50-60 times [19]. In our study, there was no correlation between the risk of chronic pancreatitis and pancreatic cancer \((p>0.05)\).

The International Agency for Research on Cancer classifies the helicobacter pylori as a proven carcinogen. In four contemporary studies conducted, it was iterated that there is a possible relationship between pancreatic cancer and helicobacter pylori, however further studies are needed [20]. According to our data, there was no correlation between helicobacter pylori and pancreatic cancer risk \((p>0.05)\). The International Agency for Research on Cancer has proposed that foods containing folate may be protective against pancreatic cancer [21]. In a study evaluating 14 prospective cohort studies that involves 319716 men and 542948 women, the relationship between folate use and pancreatic cancer was investigated. Over 7-20 years after the study, 2195 pancreatic cancers were identified. There was no correlation between folate intake and pancreatic cancer risk when the 15% quartile of the highest folate intake diet and 15% quartile of the lowest folate
intake diet were compared [22]. In the current literature, inconsistent findings have been observed between the amount of folate in the diet and the level of serum folate and the risk of pancreatic cancer. In the data obtained, no correlation between folic acid level and pancreatic cancer risk \((p>0.05)\) was found.

In a study conducted on blood types with 224 patients who have pancreatic cancer, the risk was 1.5 times higher in individuals with blood type B than in individuals with other blood types; the risk was lower in individuals with 0 type blood [23]. In our study, there was no correlation between ABO blood types and pancreatic cancer risk \((p>0.05)\). It has been investigated whether there is any difference in pancreatic cancer risk based on Rh status or not. According to the statistical analysis, the risk of pancreatic cancer in Rh antigen negative people was 70% less \((p<0.05)\). Given the differences in distribution of ABO and Rh blood types of people living all over the world and current studies concerning blood types, further studies are needed to determine whether there is a link between blood types and pancreatic cancer risk.

When 13 community-based studies were examined, the risk of pancreatic cancer in atopic/allergic individuals was 30% less [24]. Studies examining the relationship between pancreatic cancer and atopy/allergy suggest that there is need for further studies. According to our data, there was no significant association between atopy/allergy and pancreatic cancer risk \((p>0.05)\). A study shows that cholecystectomy increased the risk of pancreatic cancer by 23% and gastrectomy by 50% [25, 26]. In our study, there was no relation between the risk of pancreatic cancer and cholecystectomy and/or gastrectomy \((p>0.05)\).

In the current literature, the relationship between cholecystectomy or gastrectomy and the risk of pancreatic cancer is not clear enough.

The limitation of this study is that it is case-controlled and cross-sectional. Therefore, the results may suggest that vitamin D deficiency is more common in patients with pancreatic cancer, but we cannot clearly predict the increased risk of pancreatic cancer in cases of vitamin D deficiency. Smoking is a proven risk factor for pancreatic cancer, but this was not demonstrated in our study. On the other hand, a similar situation applies to the relationship between Rh status and pancreatic cancer risk. We cannot expressly conclude that the risk of pancreatic cancer is reduced in people with Rh antigen negative. A population-based follow-up study is required for more reliable results. This article may contribute to the following population-based studies.

The main problem with pancreatic cancer is that the disease cannot be detected early; when diagnosed, the disease is often at an advanced stage. Because the survival rate is low and that diagnosis cannot be made in the early stages of pancreatic cancer, primary prevention is important. Therefore, identification of risk factors and better elucidation of etiology is necessary.

**Conclusion**

Determination of pancreatic cancer risk factors, organization of imaging and screening programs for high-risk people can provide early diagnosis of the disease and prolong survival. Population-based studies are needed to reveal pancreatic cancer risk factors.

**Funding:** There is no financial support and sponsorship

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

**Ethical statement:** The study was conducted in accordance with the ethical approval of the
University Ethics Committee (Date and decision number: 144/2015).

ORCID iD of the author(s)
Murat Derebey / 0000-0002-0654-846X
Kagan Karabulut / 0000-0003-4723-5360
Saim Savas Yuruker / 0000-0002-6371-337X
Ilhan Karabicak / 0000-0002-3505-9534
Necati Ozen / 0000-0002-8072-9234

Copyrights: © 2021 author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

References


Effect of gallic acid on liver injury during obstructive cholestasis after bile duct ligation in rat

Erol Basuguy¹ · Mehmet Hanifi Okur¹ · Serkan Arslan¹ · Hikmet Zeytun¹ · Gulay Aydogdu² · Aysun Ekinci³

Department of Pediatric Surgery¹, Pathology² and Biochemistry³, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

ABSTRACT

Aim: To investigate the hepatoprotective, anti-inflammatory and antioxidant effects of gallic acid (GA) against obstructive cholestasis (OC) -induced liver damage in rats.

Methods: Thirty female Sprague-Dawley rats were divided into three groups. Group 1 (n=10) was the sham-operated group. In group 2 and group 3, hepatoduodenal ligament dissection was performed after laparotomy. Once the common bile duct was made apparent, it was ligated with 4/0 silk surgical suture and cut between both sutures. Group 2 (n=10) was the control group. Group 3 (n=10) was the GA group. GA (50 mg/kg) was administered by oral gavage daily for 10 days. At the end of the experiment on day 10, the rats were anesthetized. Fibrosis, inflammation, ductal proliferation and necrosis were evaluated histopathologically. Serum levels of AST, ALT, TBIL, DBIL, LDH and GGT levels were determined. In the serum and liver, TAS, TOS, MDA, TNF-α, IL-1, IL-6, and IL 10 levels were evaluated.

Results: When group 2 and group 3 were compared histopathologically, fibrosis and inflammation were significantly lower in group 3. In group 3, all LFTs (except DBIL), liver and serum IL-6, IL-1, TOS, MDA, and TNF-α levels were significantly lower than group 2, whereas IL-10 and TAS values were increased.

Conclusion: Findings of this research indicate that GA may be effective against OC-induced liver damage in a rat model. We presume that the beneficial effects of GA are closely associated with its antioxidant and anti-inflammatory activities. Therefore, we think that using GA can save us time before resorting to the surgical method.

Keywords: Obstructive cholestasis, bile ducts ligation, rats, gallic acid, liver, oxidative stress, fibrosis.

Introduction

Obstructive cholestasis (OC) is a common condition that requires surgery. Cholelithiasis is the most common cause of OC; other causes include biliary cyst stenosis, pancreatitis, parasitic infections, congenital bile duct anomalies, infectious cholangitis, cholelithiasis, biliary atresia, primary sclerosing cholangitis, abdominal traumas, and cholangitis associated with Langerhans cell histiocytosis [1,2]. The liver is the primary organ affected by OC. Retention of hydrophobic bile salts via membranes causes it to accumulate in hepatocytes, thereby disrupting membrane fluidity and function and causing cell damage [2,3]. Patients with OC experience many pathophysiological alterations that influence

---

Dr. Erol Basuguy,
Department of Pediatric Surgery, Dicle University,
Faculty of Medicine, Diyarbakır, Turkey
E-mail: erbas.80@hotmail.com

Received: 2020-10-14 / Revised: 2020-10-20
Accepted: 2020-11-19 / Published online: 2021-01-01
several organs and physiological processes. Bilirubin accumulates at high levels in patients with OC due to dysfunctional bile secretion. Thus, bile acid in hepatocytes causes acute liver damage as a result of oxidative stress [4]. Subsequently, hemostatic abnormalities, cardiac disorders, and multiple system disorders (e.g., renal failure) occur, which cause changes in patient immunity [5]. It remains unknown why OC causes multiple organ dysfunction; however, bilirubin breakdown and impaired bile acid homeostasis have been suggested [6]. Although the most common treatment for OC is surgery, percutaneous transhepatic biliary drainage and medical treatment are occasionally needed to stabilize the patient's condition and restore biliary flow before surgery [7]. Therefore, drugs are primarily used to reduce hepatic damage caused by inflammatory cells and elevated bilirubin levels, as well as to reduce elevated biliary pressure in ducts and portal veins. For prevention of intrahepatic cholestasis, novel drugs are needed to provide an alternative to surgical treatment of OC [8].

In numerous scientific reports, polyphenolic compounds were demonstrated to have different pharmacological activities that may be associated with the antioxidant system [9]. In 

\textit{in vitro} studies [10], the ethyl acetate fraction (EF) was shown to have an antifibrotic effect. Based on mass spectrometry, EF has two main components: gallic acid (GA) and ellagic acid [11]. Gallic acid is a natural phenolic antioxidant; it is found in green tea leaves, sumac, gallnuts, witch hazel, mulberries, blackberries, oak bark, raspberries, strawberries, and dragon fruit [12]. The antioxidant effects of GA are presumably due to the hepatoprotective activity of hydroxyl groups [13]. GA has attracted considerable attention due to its antioxidant effects and the cleaning properties of reactive oxygen species (ROS) [14,15].

In this study, the hepatoprotective effects of gallic acid (GA) against liver damage in obstructive cholestasis (OC) model caused by bile duct ligation in rats were investigated.

\section*{Materials and Methods}

\subsection*{Animals}

Thirty female Sprague-Dawley rats (230–270 g) were obtained from Dicle University Animal Experimental Care Unit (Diyarbakir, Turkey). All experiments were approved by the Dicle University Animal Experiments Local Ethics Committee (DUHADEK) (Decision No: 14-TF-149) and were performed in compliance with the DUHADEK guidelines. Rats were housed in cages and fed with rat diet and tap water ad libitum at a room temperature of 26±3°C, a relative humidity of %55-70, and a time period of 12 h light and 12 h dark.

\subsection*{Groups and surgical method}

Animals were divided into three groups. Each group was consisted of ten animals. After an overnight fasting, rats were anesthetized before the experimental procedure. The rats’ abdomens were shaved and cleaned with 10% povidone-iodine solution. Under sterile conditions, an incision was made at the midline of the abdomen (i.e., laparotomy was performed); the liver, diaphragm, and adjacent organs with apparent hepatoduodenal ligament were carefully dissected. 

\textbf{Group 1 (n=10)} was the sham group. After laparotomy, the hepatoduodenal ligament was dissected; the common bile duct was exposed, but no binding or cutting was conducted. Then, the animals were given standard feed and water for 10 days.

\textbf{Group 2 (n=10)} was the control group. After laparotomy, hepatoduodenal ligament
dissection was performed. Once the common bile duct was made apparent, then it was ligated with 4/0 silk surgical suture and cut between both sutures [16]. Animals were given standard feed and water for 10 days.

**Group 3 (n=10)** was the GA group, which underwent the same procedures as in group 2. In addition, 50 mg/kg GA dissolved in 1 mL saline solution (Gallic acid®, 97.5–102.5% (titration), Sigma-Aldrich, Shanghai, China) was administered by oral gavage daily for 10 days [17].

After surgical procedures had been completed, the abdominal incisions were sutured en bloc with 3/0 silk sutures. Postoperative feeding was allowed. The rats were kept in the preoperative room and fed as described above. On the 10th day, the rats were anesthetized. Then, an abdominal incision was made, liver samples were collected, and blood was drawn. Finally, the animals were sacrificed by exsanguination. Blood samples were centrifuged at 4°C and 4,000 rpm for 15 min. Plasma samples (200 μL) were obtained and stored at -80°C for biochemical parameter analysis.

Two rats (one in Group 2 and one in Group 3) died during the course of the experiment, prior to sample collection, and that replaced with new ones.

**Histopathological examination**
Dissected liver tissues were processed in 10% formalin solution, then embedded in paraffin wax in accordance with a routine embedding protocol. Next, 4-μm-thick sections were cut from paraffin blocks. In addition to hematoxylin and eosin staining, Masson’s trichrome (Biopica, Milan, Italy) staining was performed on liver slides to facilitate the evaluation of periportal fibrotic bands. Sections were evaluated using a Zeiss imager A2 microscope. Fibrosis, inflammation, and necrosis were evaluated histopathologically in accordance with the method described by Ishak et al. [18], ductal proliferation was evaluated in accordance with the method described by Ara et al. [19].

**Liver function tests (LFTs)**
For LFTs, sera were obtained by centrifuging blood samples at 4,000 g for 5 min. Subsequently, an automated biochemical analyzer (Abbott Laboratories, Chicago, IL, USA) was used, in accordance with the manufacturer's protocol, to determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct bilirubin (DB), total bilirubin (TB), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT).

**Assessment of oxidative stress and inflammatory cytokines in liver and serum**
The total oxidant status (TOS) and total antioxidant status (TAS) in serum samples were determined using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) and an autoanalyzer (Abbott Architect C16000, Abbott Diagnostics Laboratories), based on automatic colorimetric methods described by Erel et al. [20] TAS results are offered as micromolar Trolox equivalent per liter (μmol Trolox equiv/L). TOS results are shown as micromolar hydrogen peroxide per liter (μmol H₂O₂ equiv/L). Malondialdehyde (MDA) levels were detected by spectrophotometric measurement of the color produced between the reaction of thiobarbituric acid and MDA [21].

Serum levels of interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor-α (TNF-α) were measured using the ELISA method, in accordance with the manufacturer's instructions. Biochemical parameter results were recorded. Tissues weighing 0.13–0.25 g were washed with saline water, then dried using blotting paper. Next, the tissues were placed in Eppendorf tubes and
stored at -80°C until use. Before biochemical analysis, the samples were brought to -20°C, warmed to +4°C, and gradually thawed. Tissues were homogenized in phosphate-buffered saline (pH 7.4) on ice using a WiseTis Homogenizer. Tissues were centrifuged at 3,000 rpm for 10 min in a Nüve™ centrifuge and the supernatant was collected. Tissues were prepared for ELISA and washed in a BioTek ELX50 microplate washer; ELISA results were recorded at 450 nm (Triturus GRI FOLS™). TAS, TOS, MDA, TNF-α, IL-1, IL-6 and IL-10 levels were evaluated in liver tissues. The optical density values and standard (std) concentration obtained from the standard solution were used to plot the distributions of each inflammatory cytokine in Excel software. The formula calculated based on the curve was used to calculate the concentrations of the samples by means of their optical densities.

**Statistical analysis**
Statistical analyses were computed by SPSS Statistics, version 24.0; scores were shown as mean ± standard deviation (SD). Kruskal-Wallis and Mann-Whitney U tests were used for differences between group comparisons. When a p value was less than 0.05, the differences were considered as statistically significant.

**Results**

**Histopathological findings**
Ductal proliferation, inflammation, necrosis, and fibrosis were found to be significantly increased in the liver tissues of all groups with biliary obstruction compared to the Group 1. Although necrosis and ductal proliferation were improved in Group 3, no statistically significant difference was observed when compared with Group 2. However, when Groups 2 (Figures 1A, B and C) and 3 (Figure 2A, B and C) were compared, fibrosis and inflammation levels were statistically significantly lower in group 3 (p < 0.05; Table 1).

**Liver function tests (LFTs)**
All LFTs values studied in groups with biliary duct obstruction (Group 2 and Group 3) were significantly higher (p<0.05) compared to group 1 values. When group 2 and group 3 were compared; all LFTs except DBIL were significantly lower (p<0.05) in group 3 (Table 2) than group 2.

**Liver interleukin levels and oxidative stress parameters**
The liver IL-6, TOS, MDA, and TNF-α levels were significantly (p<0.05) increased in the biliary obstruction groups (Group 2 and group 3) compared to the Group 1. When groups 2 and 3 were compared, IL-6, IL-1, TOS, MDA, and TNF-α levels were significantly lower (p < 0.05) in the group 2, whereas IL-10 and TAS values were significantly higher in group 3. In addition, mean TAS values were significantly higher in group 3 than in group 1 (Table 3).

**Blood interleukin and oxidative stress parameters**
Significant increases in TNF-α levels, MDA, IL-6, IL-1, and TOS, as well as a significant decrease in IL-10 levels, were followed in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ductal proliferation</th>
<th>Fibrosis</th>
<th>Inflammation</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 (n=10)</td>
<td>0,01±0,01</td>
<td>0,01±0,01</td>
<td>0,42±0,20</td>
<td>0,42±0,29</td>
</tr>
<tr>
<td>G 2 (n=10)</td>
<td>2,28±0,28*</td>
<td>1,28±0,18*</td>
<td>1,71±0,18*</td>
<td>4,14±0,73*</td>
</tr>
<tr>
<td>G 3 (n=10)</td>
<td>1,57±0,29*</td>
<td>0,42±0,20*</td>
<td>0,85±0,14*</td>
<td>2,57±0,36*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. G1: Sham-operated group. G2: Control group. G3: GA group. * Group 1 vs Group 2 and Group 3 p<0.05; ▲ Group 2 vs Group 3 p<0.05.
group 2, compared with group 1 (p < 0.05). However, in group 3, the IL-6, IL-1, TOS, MDA, and TNF-α levels significantly decreased, while IL-10 and TAS levels significantly increased (p < 0.05), compared with group 2. In addition, no significant differences were followed between groups 1 and 3 for all parameters, except MDA and TNF-α; however, MDA and TNF-α values were higher in group 3 than in group 1 (p < 0.05; Table 4).

**Discussion**

Cholestasis, known as disruption of biliary flow, leads to the collection of bile acids and other toxins in the liver; this causes hepatotoxicity, thus damaging intracellular compartments and cell membranes. The main hypothesis regarding the mechanism of liver damage due to liver cholestasis is that hepatocellular apoptosis increases due to bile acid accumulation in hepatocytes and blood [22]. An alternative hypothesis is that excess

**Figure 1.** In control group (Group 2), fibrous expansion of some portal areas (A, B) (Masson's trichrome, x20); moderate portal inflammation (C) (Masson's trichrome, x20).

**Figure 2.** In gallic acid treated group (Group 3), mild portal fibrosis (A) (Masson's trichrome, x20); no portal inflammation (B) (Masson's trichrome, x20); no portal fibrosis (C) (Masson's trichrome, x20).

**Table 2.** Comparison of liver function tests of the groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH</th>
<th>GGT</th>
<th>AST</th>
<th>ALT</th>
<th>TBIL</th>
<th>DBIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 (n=10)</td>
<td>261±29,1</td>
<td>10,1±1,2</td>
<td>105±6,8</td>
<td>39±2,9</td>
<td>0,27±0,10</td>
<td>0,06±0,01</td>
</tr>
<tr>
<td>G 2 (n=10)</td>
<td>1426±82,3*</td>
<td>74,7±3,3*</td>
<td>883±104*</td>
<td>218±28*</td>
<td>11,9±1,20*</td>
<td>7,58±0,91*</td>
</tr>
<tr>
<td>G 3 (n=10)</td>
<td>768±50,4*▲</td>
<td>38±1*▲</td>
<td>407±40*▲</td>
<td>102±12*▲</td>
<td>7,30±0,54*▲</td>
<td>6,15±0,78*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. G1: Sham-operated group. G2: Control group. G3: GA group. * Group 1 vs Group 2 and Group 3 p<0.05; ▲ Group 2 vs Group 3 p<0.05. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DBIL: Direct bilirubin; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase; TBIL: Total bilirubin.
accumulation of bile acids occurs in the blood and liver (sterile inflammation); many studies have been performed regarding this issue. The toxic build-up of bile acids is an important mechanism for cell toxicity, including oxidative stress, apoptosis, and fibrosis [23]. Neutrophils are recruited to the area of injury approximately six hours after bile duct ligation; they peak at 48–72 hours, when liver damage is most commonly observed [24]. Neutrophil accumulation is directly associated with injury or inflammation. Neutrophils can be especially toxic to leukocytes when superoxides are generated by potent oxidant hypochlorous acid and nicotinamide adenine dinucleotide phosphate oxidase is generated by myeloperoxidase. This shows that hypochlorous acid can cause intracellular oxidant stress in hepatocytes and consequently cause cell damage [25]. Histopathological findings have been observed in the liver following bile duct ligation in various studies. In the study by Hongwei et al., livers with bile duct ligation showed elevated bile duct proliferation, parenchymal necrosis, and inflammatory cell infiltration. When Hongwei et al. treated the rats with retinoic acid and ursodeoxycholic acid, the pathological changes were reduced and liver tissues were restored to normal structure [26]. In another study by Zong et al., less collagen deposition was observed in the group subjected to both bile duct ligation and treatment with ethyl pyruvate. In addition, the liver fibrosis score was lower in rats treated with ethyl pyruvate. Thus, Zong et al. concluded that treatment with ethyl pyruvate delayed liver fibrosis [27].

Table 3. Comparison of liver interleukin and oxidative stress parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-6</th>
<th>IL-1</th>
<th>IL-10</th>
<th>TAS</th>
<th>TOS</th>
<th>MDA</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 (n=10)</td>
<td>65±6,5</td>
<td>1282±118</td>
<td>151±20</td>
<td>0,81±0,04</td>
<td>15,2±1,01</td>
<td>12,3±0,94</td>
<td>250±115</td>
</tr>
<tr>
<td>G 2 (n=10)</td>
<td>182±11,5*</td>
<td>2034±175*</td>
<td>105±15,1</td>
<td>0,87±0,04</td>
<td>34,8±2,1*</td>
<td>42,2±1,67*</td>
<td>350±120*</td>
</tr>
<tr>
<td>G 3 (n=10)</td>
<td>111±8,5*▲</td>
<td>1273±94▲</td>
<td>217±26▲</td>
<td>1,48±0,86▲</td>
<td>23,8±2,1*▲</td>
<td>22,8±2,38*▲</td>
<td>275±120*▲</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. G1: Sham-operated group. G2: Control group. G3: GA group. * Group 1 vs Group 2 and Group 3 p<0.05; ▲ Group 2 vs Group 3 p<0.05; ¥ Group 1 vs Group 3 p<0.05. IL: interleukin; MDA: Malondialdehyde; TAS: Total antioxidant status; TNF-α: Tumor necrosis factor-α; TOS: Total oxidant status.

Table 4. Comparison of serum interleukin and oxidative stress parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-6</th>
<th>IL-1</th>
<th>IL-10</th>
<th>TAS</th>
<th>TOS</th>
<th>MDA</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 (n=10)</td>
<td>83±22*</td>
<td>1348±173*</td>
<td>202,1±41*</td>
<td>1,1±0,2*</td>
<td>18,7±6*</td>
<td>14±3,8**</td>
<td>251±9**</td>
</tr>
<tr>
<td>G 2 (n=10)</td>
<td>170,5±50▲*</td>
<td>1783±497▲*</td>
<td>106±28▲*</td>
<td>0,8±0,1▲*</td>
<td>28±3,8▲*</td>
<td>37±9▲*</td>
<td>338±35▲*</td>
</tr>
<tr>
<td>G 3 (n=10)</td>
<td>107±42▲*</td>
<td>1277±233▲*</td>
<td>176±47▲*</td>
<td>1,4±0,3▲*</td>
<td>21±6▲*</td>
<td>21,6±7▲*</td>
<td>275±15▲*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. G1: Sham-operated group. G2: Control group. G3: GA group. * G1, G2 vs G3 p<0.05; ▲ G2 vs G3 P<0.05; ¥ G1 vs G3 P<0.05. IL: Interleukin; MDA: Malondialdehyde; TAS: Total antioxidant status; TNF-α: Tumor necrosis factor-α; TOS: Total oxidant status.
histological analysis in both bile duct ligation groups (groups 2 and 3). This was an indicator of neutrophil collection after bile duct ligation. Furthermore, histological examination revealed a reduction in the parameters associated with liver fibrosis and inflammation in the group 3, which confirmed the above-mentioned morphological results. Gamma-glutamyl transferase, bilirubin, and LFTs (e.g., AST, ALT, and lactate dehydrogenase) are particularly sensitive determinants of liver damage and dysfunction in membrane transport, as well as in cell and mitochondrial membrane properties [28]. Zong et al. reported that the bile duct ligation group had significantly higher serum AST and ALT levels, compared with the sham group. Ethyl pyruvate treatment significantly reduced ALT and AST levels, compared with the bile duct ligation group [27].

In the study by Xie et al., the serum ALT, AST, and total bilirubin levels were significantly lower in the bile duct ligation + dexmedetomidine group than in the bile duct ligation group [29]. In the present study, significant increases in LFTs were observed in the biliary obstruction groups (Groups 2 and 3). However, a decrease in LFTs (except direct bilirubin) in the group 3 compared to Group 2 indicated that GA inhibits or reduces OC-induced liver membrane damage due to its antioxidant properties; this findings is consistent with the results of other studies [30,31].

The anti-inflammatory and antioxidant properties of GA may be associated with elevated liver superoxide dismutase and liver catalase activities, liver TNF-α expression, liver MDA, and serum protein carbonyl reduction [31]. MDA is an indicator of lipid peroxidation and a commonly used marker of oxidative stress [14]. In the study by Cao et al., elevated MDA was observed in liver tissue after bile duct ligation; rats in the GA-treated group showed reduced MDA levels [32]. In the study by Demirbilek et al., the MDA level was significantly increased in rats with bile duct ligation. However, decreased MDA levels were observed in rats treated with sulfasalazine and pyrrolidine dithiocarbomate [33]. In the present study, due to bile duct ligation-induced liver injury, MDA levels in liver and serum were significantly higher in Group 2 than in Group 1. Conversely, treatment with GA led to lower MDA levels in liver tissue and serum, which is consistent with the previously reported findings [34]. These effects may be, at least in part, due to the antioxidant properties of GA.

TAS and TOS measurements are valuable to evaluate oxidative stress. The TOS is an important indicator of oxidative stress [35], while TAS functions to preserve tissue integrity from oxidative injury. The TAS is a precise indicator of antioxidant level [36]. In the study by Coban et al., the TOS level increased and the TAS level decreased in rats in the bile duct ligation model; conversely, the TOS level decreased and the TAS level increased in the treatment group [37]. Similar results were observed in other experimental injury models [38]. In the present study, the TOS level was significantly higher in the Group 2 than in Group 1. In the Group 3, a significant decrease in TOS and a significant increase in TAS levels were observed (p < 0.05), presumably due to the antioxidant properties of GA.

IL-1 and IL-6 play crucial roles in inflammatory events and immunity [39,40]. In the liver, TNF-α production induces apoptosis and necrosis of hepatocytes, leading to liver inflammation [41]. In a study by Xie et al., dexmedetomidine treatment decreased serum IL-6 and TNF-α proinflammatory cytokine levels, compared with the bile duct ligation
In a study by Pan et al., following bile duct ligation, IL-1 levels were improved in the treated rats [42]. In the present study, significantly higher serum TNF-α, IL-1, and IL-6 levels were observed in Group 2 (p < 0.05). However, Group 3 showed significantly lower serum TNF-α, IL-1 and IL-6 levels, compared with Group 2 (p < 0.05). Thus, the observed reductions in TNF-α, IL-1, and IL-6 serum levels confirm that GA has hepatoprotective effects via its anti-inflammatory properties.

IL-10 is a Th2-type cytokine that exerts inflammatory effects by inhibiting the production of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1, IL-6, and IL-8). In addition, IL-10 plays a role in regulating monocyte production of soluble TNF-α and IL-1 receptor antagonist [43]. In the present study, IL-10 levels were lower in the group 2 than in the group 1, which is consistent with previously reported results. However, IL-10 levels were increased in Group 3 compared to Group 2 and Group 1 (p < 0.05).

**Conclusion**

Findings of this research indicate that GA may be effective against OC-induced liver damage in a rat model. We presume that the beneficial effects of GA are closely associated with its antioxidant and anti-inflammatory activities. Therefore, we think that using GA can save us time before resorting to a surgical method. However, further studies are needed to investigate the optimal dosage and duration of application.

**Abbreviations**

GA: Gallic acid  
OC: Obstructive cholestasis  
LFTs: Liver function tests  
ALT: Alanine aminotransferase  
AST: Aspartate aminotransferase  
TBIL: Total bilirubin  
DBIL: Direct bilirubin  
LDH: Lactate dehydrogenase  
GGT: Gamma-glutamyl transferase  
ROS: Reactive oxygen species  
ELISA: Enzyme-linked immunosorbent assays  
TAS: Total antioxidant status  
TOS: Total oxidant status  
MDA: Malondialdehyde  
IL: Interleukin  
TNF-α: Tumor necrosis factor-α  
EF: Ethyl acetate fraction

**Funding:** This study was supported by a grant from the Scientific Research and Project Coordinator (DUBAP, 14-TF-149) at Dicle University, Diyarbakır, Turkey.

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

**Ethical statement:** The study were confirmed by the Dicle University Animal Experiments Local Ethics Committee (DUHADEK) (Decision No: 14-TF-149)

**ORCID iD of the author(s)**

Erol Basuguy / 0000-0002-9217-423X  
Mehmet Hanifi Okur / 0000-0002-6720-1515  
Serkan Arslan / 0000-0002-5879-4478  
Hikmet Zeytun / 0000-0002-7159-952X  
Gulay Aydogdu / 0000-0002-3784-7622  
Aysun Ekinci / 0000-0002-0547-4139

**Copyrights:** © 2021@ author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice.
References


Aim: Multiple sclerosis (MS), which is inflammatory in its pathogenesis, damages the myelin sheath in the central nervous system (CNS) and causes axonal loss. Epicardial fat tissue (EFT), located between the myocardium and the visceral layer of the pericardium, surrounds the heart and several inflammatory cytokines is secreted from this tissue. In this study, we aimed to investigate EFT thickness in MS patients and compared with that of volunteer non-MS subjects.

Methods: A total of 154 subjects comprising 61 MS patients and 93 volunteers matched for gender and age were included in our study. Epicardial fat tissue thickness was measured by echocardiography. All values were compared between groups.

Results: Echocardiographic parameters were similar in both groups. However, the mean EFT thickness was significantly higher in the MS group than in the control group \( (p<0.001) \). Epicardial fat tissue thickness was also significantly correlated with the presence of MS \( (r=0.33, p<0.001) \).

Conclusion: The results of our study suggest that the increase in epicardial adipose tissue thickness in MS patients may be a predictive factor for cardiovascular disease. However, the clinical significance of this finding and its relevance to MS pathogenesis should be investigated in further studies.

Keywords: Multiple sclerosis, epicardial fat tissue, cardiovascular diseases, inflammation, biomarkers.
Inflammatory cytokines play a crucial role in the pathogenesis of MS [9]. MS, which is an autoimmune disease, progresses with demyelination in the CNS [10]. EFT thickness is associated with various autoimmune diseases [11,12], and there are various studies showing the release of various cytokines from EFT [13]. Hence, EFT thickness may be considered a significant parameter in MS patients.

In this study, we aimed to investigate echocardiographic EFT thickness, which may be an indicator of coronary artery disease, in MS patients and compared it with that of normal subjects.

**Materials and Methods**

The patients who were admitted to a university hospital between February 2019 and March 2020 were included in the study. The study was approved by Bolu Abant Izzet Baysal local ethics committee (Date: 30/01/2019; Decision number: 2019/27). Written and verbal consent were obtained from all patients.

A total of 154 subjects comprising 61 MS patients and 93 volunteers matched for gender and age were included in our study. The demographic characteristics of the participants were recorded. The laboratory parameters of the patients at the time of admission were recorded from the laboratory database. Echocardiography (echo) was performed on all participants.

**Exclusion criteria**

Patients who had a history of coronary, carotid, or peripheral artery atherosclerosis, valvular heart disease, heart failure, arrhythmias or conduction disorders, prosthetic heart valves, pacemaker implantation, significant renal failure, severe liver dysfunction, thyroid disorders, electrolyte imbalances, systemic inflammatory or infectious disease, and poor ultrasound image quality were excluded.

**Measurement of epicardial fat thickness**

EFT thickness was measured with a 4-Mhz Vivid S6 transducer (GE Vingmed, N-3191, Horten, Norway). A blinded cardiologist performed the echo under continuous electrocardiographic monitoring in the left lateral position. Measurements were recorded as the average of the values obtained over three cardiac cycles. EFT thickness was noted as an echo-free or hyperechoic area between the epicardium and the visceral layer of the pericardium and reflects visceral adipose tissue [14]. The assessment of EFT thickness was performed from the parasternal long- and short-axis views of the free wall of the right ventricle. The measurement was made at the level of the aortic annulus perpendicular to the free wall of the right ventricle. The measurements of maximum values were made at the end of diastole. The intraobserver variation for EFT was <5%.

**Statistical analysis**

For all statistical analyses, the 18.0 version of SPSS (Statistical Package for the Social Sciences) for Windows, (Chicago, Illinois, USA) was used. The numerical data were expressed as mean ± standard deviation. The difference between the study and the control groups was evaluated by Student t-test for data which were normally distributed. For the data which were not normally distributed Mann-Whitney’s U-test was used. The correlation between EFT and MS was evaluated using Spearman's correlation analysis. A p-value < 0.05 was considered as significant.

**Results**

The clinical risk factors and baseline characteristics of the patients are shown in Table 1. No significant difference was found between the groups in terms of baseline characteristics (Table 1).
Likewise, echocardiographic findings were similar in both MS and non-MS groups. However, the mean EFT thickness value was found significantly higher in MS group than in non-MS group (p<0.001) (Table 2). No significant difference was found between the groups in terms of laboratory parameters such as creatinine, triglyceride, and cholesterol values. The Spearman’s correlation test revealed that EFT thickness (r=0.33, p<0.001) was significantly correlated with the presence of MS (Table 2, Figure 1).

**Table 1.** Baseline characteristics of the study groups.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Control Groups (n=93)</th>
<th>MS Groups (n=61)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33±12</td>
<td>36±12</td>
<td>0.25</td>
</tr>
<tr>
<td>Sex (Male/female), n</td>
<td>46/47</td>
<td>22/39</td>
<td>0.08</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>116±14</td>
<td>111±12</td>
<td>0.05</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71±9</td>
<td>70±7</td>
<td>0.64</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25±4</td>
<td>25±3</td>
<td>0.17</td>
</tr>
<tr>
<td>Smoking</td>
<td>19 (20%)</td>
<td>17 (28%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>1 (1%)</td>
<td>3 (5%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2 (2%)</td>
<td>4 (7%)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**MS:** Multiple sclerosis, **DBP:** Diastolic Blood Pressure, **SD:** Standard Deviation, **SBP:** Systolic Blood Pressure.

**Table 2.** Echocardiographic and laboratory parameters of the study groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group (n=93)</th>
<th>MS Group (n=61)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>66±3</td>
<td>65±3</td>
<td>0.36</td>
</tr>
<tr>
<td>LVDD(cm)</td>
<td>4.6±0.4</td>
<td>4.5±0.3</td>
<td>0.28</td>
</tr>
<tr>
<td>LVSD(cm)</td>
<td>2.8±0.4</td>
<td>2.8±0.3</td>
<td>0.26</td>
</tr>
<tr>
<td>PW(cm)</td>
<td>0.86±0.12</td>
<td>0.88±0.12</td>
<td>0.33</td>
</tr>
<tr>
<td>IVST(cm)</td>
<td>0.88±0.14</td>
<td>0.93±0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Left atrium</td>
<td>2.9±0.5</td>
<td>3.0±0.4</td>
<td>0.69</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78±12</td>
<td>0.78±11</td>
<td>0.98</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>107±52</td>
<td>107±59</td>
<td>0.97</td>
</tr>
<tr>
<td>EFT(cm)</td>
<td>0.41±0.17</td>
<td>0.50±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>46 (8.8)</td>
<td>51 (17.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>101 (13.7)</td>
<td>102 (38.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>169 (21)</td>
<td>178 (34)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**EF:** Left ventricular ejection fraction, **LVDD:** Left ventricular end-diastolic diameter, **LVSD:** Left ventricular end-systolic diameter, **PW:** Posterior wall thickness, **IVST:** Interventricular septum thickness, **EFT:** Epicardial fat thickness, **SD:** Standard Deviation, **IQR:** Interquartile range.
Figure 1. EFT thickness is significantly higher MS group compared to non-MS group.

Discussion
In the present study it is found that EFT thickness was significantly higher in the MS group than in the non-MS group, and EFT thickness was significantly correlated with MS. There is no consensus on whether MS patients are at greater risk of CVD when compared to the general population [15-18]. While Roshanisefat et al. found no difference between MS and non-MS patients in terms of CVD incidence, Moccia et al. reported that the Framingham General Cardiovascular Disease Risk Score (FR) was associated with the severity, disability, and clinical course of MS [19-20]. In some studies, it has been reported that indirect markers of CVD, such as highsensitive C-reactive protein and carotid intima media thickness (CIMT), were related to MS [21]. Hence, the overall data suggest an increased CVD risk in patients with MS [22].

In the context of the reported studies, it is not clearly understood whether the increase in CVD is related to the presence of obesity, hypertension, hyperlipidemia, or diabetes mellitus in MS. Further studies are needed to solve this issue [22]. An association between EFT thickness, CVD, and metabolic syndrome has been observed in several studies [8,23]. Therefore, our finding of increased EFT thickness in MS patients may provide indirect evidence and a pathway for the association of MS with CVD.

In the early phase of relapsing-remitting MS, demyelination by inflammation predominates, while in the progressive phase, neuroaxonal damage by neurodegeneration predominates [24]. Frischer et al. reported a significant association between inflammation and axonal injury in MS. In older MS patients with longer disease duration, inflammatory infiltrates declined to levels similar to those found in age-matched controls [25]. Therefore, although it is not known whether the role of inflammation is causative or protective, there is a strong association between neurodegeneration and inflammation in MS.

Epicardial fat tissue is a paracrine and endocrine tissue and is a source of adiponectin, adrenomedullin, anti-inflammatory adipokines, and several proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and nerve growth factor (NGF) [13]. Mazurek et al. [7] examined epicardial and subcutaneous adipose tissues among elective coronary artery bypass grafting surgery patients. Significantly higher mRNA and protein levels of IL-1, IL-6, MCP-1, and TNF-α were detected in EFT when compared to subcutaneous adipose tissue. Coincidentally, IL-6, IL-1, and TNF-α levels are associated with MS, and these cytokines have been found to be related to axonal injury [26]. Additionally, Sharief et al. reported that TNF-α levels are correlated with MS disease progression and disability level [27].

The clinical significance of the inflammatory potential of EFT and its role in other diseases is unclear. Our finding of increased EFT thickness among MS patients may provide new insight into the association between inflammation and
MS. Nevertheless, future studies are needed to further explore this aspect.

There are some limitations not studied. This is a single-center study with a small sample size, which predisposes it to an inherent selection bias. The accuracy and reproducibility of EFT thickness measurements with MRI and CT are higher than that of echo. Therefore, the use of echo for the measurements of EFT thickness may reduce the reliability of our results. Nonetheless, echo was performed as it is simple and inexpensive. Lastly, since EFT has a three-dimensional distribution, echo, which produces a two-dimensional image, may not fully evaluate the total amount of epicardial adipose tissue.

Conclusions
The results of our study suggest that the increase in epicardial adipose tissue thickness in MS patients may be a predictive factor for cardiovascular disease. To our knowledge, this is the first report about epicardial adipose tissue in MS patients. The clinical significance of this finding and its relevance to MS pathogenesis should be explored in further studies.

Funding: There is no financial support and sponsorship

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

Ethical statement: The study was approved by Bolu Abant Izzet Baysal University local ethics committee (Date: 30/01/2019; Decision number: 2019/27).

ORCID iD of the author(s)
Mehmet Cosgunn / 0000-0002-6965-7444
Isa Sincer / 0000-0003-2399-9585
Yılmaz Gunes / 0000-0003-3817-851X
Zafer Kok / 0000-0001-7458-9135
Süle Aydın Turkoglu / 0000-0001-8616-832X

Copyrights: © 2021@ author(s).
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

References


[26] Ramirez-Ramirez V, Macias-Islas MA, Ortiz GG, et al. Efficacy of fish oil on serum of TNF α, IL-1 β, and IL-6 oxidative stress markers in multiple sclerosis treated with

Investigation of the presence of pregnancy rhinitis in the third trimester with rhinomanometry

Rustem Filiz1 · Ahmet Ural1 · Mehmet Ata Topcuoglu2 · Muharrem Dagli3

1Department of Otorhinolaryngology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey
2Department of Obstetrics & Gynecology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey
3Department of Otorhinolaryngology, Health Sciences University, Ankara Dişkapi Yıldırım Beyazıt Health Research Hospital, Ankara, Turkey

ABSTRACT

Aim: Pregnancy rhinitis (PR) is characterized with nasal symptoms during pregnancy without any signs of respiratory infection and it usually disappears within 2 weeks after delivery. We aimed to investigate the relationship between pregnancy rhinitis and findings derived from anterior rhinoscopy (AnR), anterior rhinomanometry (ARM) and subjective nasal obstruction score (SNOS).

Methods: This prospective, controlled study was performed in otorhinolaryngology and obstetrics and gynecology departments of our tertiary care center. A total of 30 pregnant women in the third trimester and 30 non-pregnant women were involved. All participants underwent otorhinolaryngology examination, as well as clinical evaluation for AnR, ARM and SNOS.

Results: Pregnancy rhinitis was detected in 66.7% of the pregnant women. The mean AnR was 3.60 ± 1.35 in pregnant women and 0.77 ± 0.73 in the control group. Total nasal inspiratory resistance (TNID) was 0.46±0.23 in pregnant women and 0.27±0.06 in the control group. The mean SNOS was 1.37±0.72 in pregnant women and 0.57±0.63 in the control group. AnR, ARM and SNOS findings were significantly higher in pregnant women (p<0.05). There is a low positive and significant correlation between AnR, ARM, and SNOS values in pregnant women (p<0.05).

Conclusion: Our data yielded that nasal obstruction and pregnancy rhinitis were common in pregnant women. Nasal symptoms and complaints must be carefully examined during pregnancy. Further prospective, controlled, randomized trials on larger series are warranted to elucidate the clinical and pathophysiological features of pregnancy rhinitis.

Keywords: Pregnancy rhinitis, subjective nasal obstruction score, anterior rhinoscopy, pregnancy rhinitis, anterior rhinomanometry.
and progesterone levels causes hyperemia and edema in the nasal mucosa. Also, placental growth hormone was found to be high in pregnancy rhinitis [3, 4]. Rhinitis does not directly affect pregnancy. However, uncontrolled rhinitis can increase stress by indirectly disturbing the nutritional and sleep status of the pregnant woman. Pregnancy hypertension, low Apgar score, preeclampsia and intrauterine growth retardation may be encountered due to rhinitis induced snoring [5, 6]. Control of rhinitis during pregnancy reduces the risk of additional medication by increasing the quality of life of pregnant women [7, 8].

We aimed to investigate the frequency of pregnancy rhinitis and seek whether there is a correlation between anterior rhinoscopy (AnR) findings, anterior rhinomanometry (ARM) measurements, and subjective nasal obstruction scores (SNOS).

**Materials and Methods**

This prospective study was carried out by the otorhinolaryngology and obstetrics & gynecology clinics of our institution. The study was approved by the local ethics committee and consent was obtained from all the participants (2017/73). A total of 30 pregnant women aged between 19-35 years who were in the third trimester of pregnancy were recruited. The control group included 30 non-pregnant women. The participant women had no history of allergic rhinitis (AR), septal deviation, and signs of acute or chronic upper respiratory tract infections, non-allergic rhinitis, nasal polyps, and sinonasal tumors.

**Outcome parameters**

A complete otorhinolaryngologic examination and decongestant-free active anterior rhinomanometry (ARM) were performed routinely for all participants. All examinations and tests were performed between 27th and 40th weeks of pregnancy. In the history, pregnant women were asked to score the level of nasal congestion as for SNOS (none=0, mild=1, moderate=2, severe=3, fully blocked=4).

In the first examination, nulliparous women were asked whether they had PRs in the first and second trimesters. Multiparous women were asked whether they had PR in the previous pregnancies or during the current pregnancy. The gender of the baby was noted and pregnant women were asked to describe the nasal obstruction during pregnancy as either “increased”, “decreased” or “unchanged”.

In endoscopic examination, turbinate hypertrophy in a nasal cavity was scored by 1-point, the mild congestion of the mucosa was scored as 1 point and severe congestion was scored as 2-points. Therefore, the highest score a pregnant can get in anterior rhinoscopic examination was 6 points [9].

Decongestant-free ARM was performed on all pregnant women by using Rhinostream SRE 2000 (Interacoustics A/S, Drejervaenget 8, DK-5610 Assens, and Denmark) rhinomanometry device. The mask covered both the mouth and the nose. The pressure probe passed through the mask was placed in a nostril and the nasal flow probe in the other nostril. During placement of the probes, attention was paid not to deform the nostrils and to prevent the air leakage. Pregnant women were asked to keep their mouths closed and breathe through their noses. The values were read at 150 Pa. After expiration and inspiration, nasal resistance was recorded in Pa/cm³ and the individual resistances of each nostril and the total inspiratory nasal resistance were calculated. Patients who had nose clearance before ARM had rested for 20 minutes in a room with a temperature of 20±3 °C, the humidity was 50%, and the illumination
was moderate. The patients were instructed about the test. They had not exercised for 2 hours preceding the test and they were asked not to have tea, coffee, and cigarettes. Results from the pregnant group were compared with 30 non-pregnant women in the control group who were non-smokers and did not have any signs of acute upper respiratory tract infections, or chronic sinonasal diseases. Thus, AnR, ARM and SNOS scores were recorded under same conditions.

**Statistical analysis**

Statistical analysis was performed by using SPSS 24.0 software (*Statistical Package for Social Sciences Inc., Chicago, IL, USA*). In terms of descriptive statistical methods, Mann Whitney U test for difference tests and Spearman Brown test for correlation were used since the data displayed non-parametric features. The results were evaluated with a confidence interval of 95% and a p-value less than 0.05 was considered as statistically significant.

**Results**

The mean ages in the study and control groups were 30.43±3.65 and 30.37±4.07, respectively. The gestational age during examination ranged between 27 and 39 weeks (mean: 34.2±3.71; median: 36). Of the 30 pregnant women, 9 (30%) were nulliparous and 21 (70%) were multiparous. A total of 16 newborns were male (53.3%) and 14 (46.7%) were female.

None of the pregnant women reported any relief of nasal congestion during pregnancy. The complaints did not change in 11 women (36.7%) and were increased in 19 women (63.3%). Fifteen of 21 multiparous women (71.4%) reported absence of PR, while 6 (28.6%) of them reported presence of PR in their previous pregnancies. In their current pregnancies, no PR were diagnosed in 10 pregnant women (33.3%), while PR was diagnosed in 20 pregnant women (66.7%) (Table 1).

**Table 1.** Gestational features and rhinitis findings in pregnant women.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pregnancy weeks</td>
<td>34.2±3.71</td>
</tr>
<tr>
<td>Median pregnancy weeks</td>
<td>36</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>30% (n=9)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>70% (n=21)</td>
</tr>
<tr>
<td>Nasal congestion decreased</td>
<td>0% (n=0)</td>
</tr>
<tr>
<td>Nasal congestion unchanged</td>
<td>36.7% (n=11)</td>
</tr>
<tr>
<td>Nasal congestion increased</td>
<td>63.3% (n=19)</td>
</tr>
<tr>
<td>Presence of rhinitis</td>
<td>66.7% (n=20)</td>
</tr>
<tr>
<td>Absence of rhinitis</td>
<td>33.3% (n=10)</td>
</tr>
</tbody>
</table>

The mean ANR values in the study and control groups were 3.60±1.35 and 0.77±0.73, respectively. Therefore, pregnant women exhibited higher AnR scores compared to non-pregnant women.

Regarding ARM findings, the mean total nasal inspiratory resistance (TNID) was 0.46±0.23 in the study group and 0.27±0.06 in the control group. The minimum and mode TNID scores were similar between 2 groups (0.19 and 0.18; 0.24 and 0.23). However, the maximum TNID was 0.39 in the control group and 1.07 in the study group. Therefore, the average TNID score was higher in pregnant women. Moreover, TNID values were grouped according to 0.12-0.33 Pa/ml/s, which was accepted as the normal value range in rhinomanometric measurements. With reference to this threshold, 9 (30%) of pregnant women in the study group and 25 (83.3%) of the non-pregnant women in the control group were within this range.
On the other hand, the mean SNOS in the study and control groups were 1.37±0.72 and 0.57±0.63, respectively. We noted that SNOS averages of pregnant women were significantly higher than non-pregnant women (Table 2). The analysis of descriptive findings yielded that AnR, ARM and SNOS displayed significant differences between study and control groups (p<0.05). AnR, ARM and SNOS findings of in pregnant women were significantly higher than the control group (Table 3).

In the study group, the correlation between AnR, ARM and SNOS was sought and the correlation between AnR and ARM was 0.176. The correlation between AnR and SNOS was 0.295 and the correlation between ARM and SNOS was 0.197. These correlations were found to be low and positive, but they were significant (p<0.05).

As for these results, if any of AnR, ARM and SNOS values of the pregnant women increased, the others also tended to increase mildly. The

| Table 2. AnR, ARM and SNOS results in the study and control groups. |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Groups              | Minimum | Maximum | Average               | Median   | Mode      |
| Study group         |          |          |                       |          |          |
| AnR                 | 0        | 6        | 3.60±1.35             | 4        | 4         |
| ARM                 | 0.19     | 1.07     | 0.46±0.23             | 0.39     | 0.24      |
| SNOS                | 0-none   | 3-severe | 1.37±0.72             | 1-mild   | 1-mild    |
| Control group       |          |          |                       |          |          |
| AnR                 | 0        | 2        | 0.77±0.73             | 1        | 1         |
| ARM                 | 0.18     | 0.39     | 0.27±0.06             | 0.25     | 0.23      |
| SNOS                | 0-none   | 2-moderate | 0.57±0.63          | 0.50-mild | 0-none    |

[Abbreviations: *Anterior rhinoscopy (AnR), anterior rhinomanometry (ARM) and subjective nasal obstruction score (SNOS)].

| Table 3. Comparison of AnR, ARM and SNOS results in the study and control groups. |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Groups               | Groups              | Minimum | Maximum | Average | Median | Mode | U | Z | p-value* |
| (n=30)               | (n=30)              |          |          |         |        |      |   |   |          |
| AnR                  | Study               | 3.60     | 44.20    | 1326.00 | 39,000 | -6.200 | <0.00 |
|                      | Control             | 0.77     | 16.80    | 504.00  |         |        |     |   |          |
| ARM                  | Study               | 0.46     | 40       | 1200.00 | 165,000 | -4.219 | <0.00 |
|                      | Control             | 0.27     | 21       | 630.00  |         |        |     |   |          |
| SNOS                 | Study               | 1.37     | 38.88    | 1166.50 | 198,500 | -4.042 | <0.00 |
|                      | Control             | 0.57     | 22.12    | 663.50  |         |        |     |   |          |

* Mann-Whitney U Test. [Abbreviations: **Anterior rhinoscopy (AnR), anterior rhinomanometry (ARM) and subjective nasal obstruction score (SNOS)].
determination coefficient (explained variance=$r^2$) was calculated to determine the impact of changes of AnR, ARM and SNOS measurements on the other. As a result, $r^2$ value between AnR and ARM was found to be 3.1. This value was 8.7 between AnR and SNOS; and it was 3.8 between ARM and SNOS. Any changes in AnR or ARM constitutes an alteration of 3.1%. In the other parameters, these percentages were found to be 8.7% and 3.8%.

Furthermore, the regression equation between AnR and SNOS was $y=0.6+0.21x$. It was $y=1.16+0.46x$ between ARM and SNOS, and $y=0.39+0.02x$ between AnR and ARM.

In the control group, the correlation was 0.134 between AnR and ARM, 0.131 between AnR and SNOS, and -0.36 between ARM and SNOS. Therefore, the correlations between AnR-ARM and AnR-SNOS were low and positive. However, the relationship between ARM and SNOS was very low and negative.

As for these results, $r^2$ between AnR and ARM was 1.8, $r^2$ between AnR and SNOS was 1.7, and $r^2$ between ARM and SNOS was 0.1. These findings may be interpreted as any change in AnR or ARM constitutes 1.8% change. In the other parameters, these percentages were 1.7% and 0.1%.

**Discussion**

In the present study, the prevalence of PR was 66.7% and it was reported as 40% in previous publications [10]. The nasal obstruction symptom evaluation (NOSE) scores in the study and control groups displayed no significant differences ($p = 0.866$). Minimal cross sectional areas at internal nasal valve and at the level of the head of inferior turbinate decreased significantly between the first and third trimesters (2). There was no difference between the trimesters as for total nasal resistance. The correlation analysis between the NOSE score, total volume and minimal cross sectional area at internal nasal valve demonstrated no significant differences [11].

In Sweden, the prevalence of PR was found to be 22% in a series of 599 patients [12]. Thus, it is possible to say that the global prevalence is considerably higher than the prevalence in Turkey. However, relatively larger samples should be studied for achievement of more objective outcomes.

We noted that nasal obstruction and PR were common during pregnancy. The previous PR was found in 28.6% of multiparous pregnancies. The presence of rhinitis in the current pregnancy was detected as 66.7%. The mean AnR score was 3.60±1.35. Our results were close to the value of 3.47 reported by Philpott et al. [13]. The utilities of AnR and ARM has been recommended for evaluation of rhinitis and nasal physiological changes during pregnancy [13]. Subjective nasal obstruction scale (SNOS) has been validated as a reliable tool of nasal congestion in previous studies [14].

Ülkümen et al. [15] reported that an incidence of 38.89% for PR. Nasal congestion was significantly associated with body-mass index and gestational weeks. Patients should be informed about adverse fetal and maternal outcomes of pregnancy related nasal congestion which may be aggravated by obesity and excessive weight gain during pregnancy.

In our study, the mean ARM was 0.46±0.23 and the mean SNOS was 1.37±0.72. The correlations between AnR-ARM, ARM-SNOS and AnR-SNOS were 0.176, 0.195 and 0.295, respectively.

The nasal obstruction associated with PR may lead to with diminution of the quality of life and sleep [16]. Gilbey et al. [17] suggested that
rhinosinusitis-specific quality of life was lower in the third trimester compared to that of the second trimester and that of patients without rhinosinusitis. Thus, increased awareness is important to improve the quality of life in pregnant women, prevent sleep apnea and related morbidity, and positively affect the gestational outcomes. There is controversy on the influence of atopy on the pregnancy outcomes since some authors postulated that atopy may be associated with more favorable results [17]; whereas some publications indicated that there was no link between atopy and recurrent abortions [18,19].

The main limitations of our study include relatively small sample size, subjectivity of detection of SNOS and data limited to the experience of a single center. On the other hand, prospective design and integrity of specific data constituted the strengths of our study.

Conclusions

To conclude, nasal obstruction and PR are common conditions in pregnant women. As reflected in higher scores of AnR, ARM and SNOS values, nasal symptoms and complaints must be questioned during follow-up in pregnancy. A low positive, but significant correlation was noted between AnR, ARM and SNOS measurements in pregnant women. The implementation of further multicentric trials on larger series may aid in accomplishment of more accurate results.

Funding: There is no financial support and sponsorship

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

Ethical statement: The study was approved by Bolu Abant Izzet Baysal University local ethics committee (Decision number: 2017/73).


