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The Bulletin accepts research articles in the basic and clinical sciences, reviews of current topics, relevant surgery videos and extraordinary case reports for publication.

The main aim of the journal is to enable all physicians-especially urologists to access research findings from the urooncology field quickly and effectively. It also contributes to physicians' vocational training with specific numbers of reviews, surgery videos and case reports.

The Bulletin accepts manuscripts through an online submission system. Free access to full text versions is provided to members through the website and mobile applications.

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After online manuscript submission, leading reviewers from the relevant areas will evaluate the papers and send feedback to the authors within a short time mostly in one month duration.

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Instructions to Authors

1. General Information

The Bulletin of Urooncology is the official scientific publication of the Turkish Society of Urooncology. It is published quarterly (March, June, September, and December). Supplements are also published during the year if necessary. Accepted articles will be published in English online without a hard copy.

The Bulletin publishes basic and clinical research original articles, reviews, editorials, case reports, surgery videos (Video-urooncology) and letters to the editor relevant to urooncology (prostate cancer, urothelial cancers, testis and kidney cancer, benign prostatic hyperplasia, and any aspect of urologic oncology).

The Bulletin of Urooncology is indexed by several well-known international databases including Emerging Sources Citation Index (ESCI), TUBITAK/ULAKBIM Turkish Medical Database, Directory of Open Access Journals (DOAJ), EBSCO, CINAHL Complete Database, Gale/Cengage Learning, ProQuest, Index Copernicus, and British Library.

All submitted manuscripts are committed to rigorous peer review.

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All manuscripts should comply with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" produced and updated by the International Committee of Medical Journals Editors (www.icmje.org).

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The Bulletin adheres to the principles set forth in the Declaration of Helsinki 2016 version (http://www.wma.net/en/30publications/10policies/b3/index.html) and holds that all reported research involving human beings is conducted in accordance with such principles. Reports describing data obtained from research conducted in human participants must contain a statement in the "Materials and Methods" section indicating approval by an ethics review committee and affirmation that informed consent was obtained from each participant.

All manuscripts dealing with animal subjects must contain a statement indicating that the study was performed in accordance with "The Guide for the Care and Use of Laboratory Animals" (http://oacu.od.nih.gov/regs/guide/guide.pdf) with the approval (including approval number) of the Institutional Ethic Review Board, in the "Materials and Methods" section.

Prospective clinical trials, surgery videos and case reports should be accompanied by informed consent and the identity of the patient should not be disclosed.

During the evaluation of the manuscript or even after publication, the research data and/or ethics committee approval form and/or patients' informed consent document can be requested from the authors if it is required by the editorial board.

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It is the authors' responsibility to ensure their manuscript meets full ethical criteria detailed at www.uroonkolojibulteni.com/Peer-Review-and-Ethic.

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All submissions must include "Copyright Transfer and Author Declaration Statement Form". All authors should sign this form declaring acceptance of full responsibility for the accuracy of all contents in accordance with the order of authors. They should also indicate whether there is a conflict of interest regarding manuscript. The names of the institutions, organizations, or pharmaceutical companies that funded or provided material support for the research work, even in the form of partial support, should be declared and acknowledged in the footnote of the article. Copyright Transfer and Author Declaration Statement Form must also indicate that "Patient Consent Statement" is obtained for human studies particularly prospective clinical trials, surgery videos (Video-urooncology) and case reports. All manuscripts submitted must also be accompanied by an "Acknowledgements Form" which is available at www.uroonkolojibulteni.com.

The ORCID (Open Researcher and Contributor ID) number of the all authors should be provided while sending the manuscript. Free registration can be done at http://orcid.org.

3. Peer-Review Process

The Bulletin of Urooncology is an independent international journal based on double-blind peer-review principles. All articles are subject to review by the editors and peer reviewers. All manuscripts are reviewed by the editor, associate editors, and at least two expert referees. The scientific board guiding the selection of papers to be published in the

Bulletin consists of elected experts of the Bulletin and if necessary, selected from national and international authorities. The editorial board has the right to not publish a manuscript that does not comply with the Instructions for Authors, and to request revisions or re-editing from the authors. The review process will be managed and decisions made by the Editor-in-chief, who will act independently.

The editor and editorial board is the sole authority regarding reviewer selection. The reviewers are mainly selected from a national and international advisory board. The editorial board may decide to send the manuscript to independent national or international reviewers according to the subject.

Authors of accepted manuscripts accept that the editor and associate editors can make corrections without changing the main text of the paper.

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It is the authors' responsibility to prepare a manuscript that meets scientific criteria. All persons designated as authors should have made substantial contributions to the following:

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(2) drafting the article or revising it critically for intellectual content,

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If the article includes any direct or indirect commercial links or if any institution provided material support to the study, authors must state in the "Copyright Transfer and Author Declaration Statement Form". They must state that they have no relationship with the commercial product, drug, pharmaceutical company, etc. concerned; or specify the type of relationship (consultant, other agreements), if any. This information should also be included in the "Acknowledgements Form".

In case of any suspicion or allegation regarding scientific shortcomings or ethical infringement, the Bulletin reserves the right to submit the manuscript to the supporting institutions or other authorities for investigation. The Bulletin accepts the responsibility of initiating action but does not undertake any responsibility for an actual investigation or any power of decision.

-Abbreviations:

Use only standard abbreviations. Avoid abbreviations in the title and abstract. The full term for an abbreviation should precede its first use in the text, unless it is a standard abbreviation. Abbreviations that are used should be defined in parenthesis where the full word is first mentioned.

-Units of Measurement:

Measurements should be reported using the metric system, according to the International System of Units (SI).

-Statistical Evaluation:

All retrospective, prospective, and experimental research articles must be evaluated in terms of biostatics and should be stated together with an appropriate plan, analysis, and report. P values must be given clearly in the manuscripts (e.g., p=0.033). It is the authors' responsibility to prepare a manuscript that meets biostatistical rules.

-Language:

Accepted articles will be published in English online. It is the authors' responsibility to prepare a manuscript that meets spelling and grammar rules. Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English are encouraged to consult an expert. All spelling and grammar mistakes in the submitted articles are corrected by our redaction committee without changing the data presented.

5. Article Types

The Bulletin of Urooncology publishes articles prepared in compliance with the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals published by International Committee for Medical Journal Editors (ICMJE). Manuscripts that do not meet these requirements will be returned to the author for necessary revision prior to review.

The Bulletin requires that all submissions be submitted according to these guidelines: Manuscripts should be prepared as a word document (*.doc) or rich text format (*.rtf). Text should be double-spaced with 2.5 cm margins on both sides using 12-point type double spaced in Times Roman.

All manuscripts submitted must be accompanied by the "Copyright Transfer and Author Declaration Statement Form" (www.uroonkolojibulteni.com). The corresponding author must also provide a separate "Title Page" including full correspondence address including telephone, fax number, and e-mail address, list of all authors with The ORCID number. Contact information for the corresponding author is published in the Bulletin.

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Each section of the" Main Text" mentioned below should be started on a new page and be organized according to the following sequence:

1) First page: Title, abstract and keywords (without authors' credentials)

2) Manuscript text structured based on the article type (without authors' credentials)

3) References

4) Figure legends

5) Short Quiz for review articles.

Tables and figures should be uploaded separately.

Also, "Acknowledgements Form" should be uploaded separately.

A. Original Research Articles

Original prospective or retrospective studies of basic or clinical investigations in areas relevant to urologic oncology.

Content (Main text): Each part should start on a new page.

- First page: Title - Abstract (structured abstract limited to 300 words, containing the following sections: Objective, Materials and Methods, Results, Conclusions) - Keywords (List 3-5 keywords using Medical Subjects Headings [MeSH])

-Introduction

- Materials and Methods

- Results

- Discussion

- Study Limitations
- Conclusions
- References
- Figure Legends: These should be included on separate page after the references.

-Tables and figures should be uploaded separately.

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Preparation of research articles, systematic reviews, and meta-analyses must comply with study design guidelines: CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consortstatement.org/); PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/); STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.)(http://www.stard-statement.org/); STROBE statement, a checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/); MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

A word count for the original articles (excluding title page, acknowledgements, references , figure and table legends) should be provided not exceed 3000 words. Number of references should not exceed 30. Number of figure/tables is restricted to five for original articles.

B. Case Reports

Case reports should include cases which are rarely seen and distinctive in diagnosis and treatment. These can include brief descriptions of a previously undocumented disease process, a unique unreported manifestation or treatment of a known disease process, or unique unreported complications of treatment regimens, and should contribute to our present knowledge.

Content (Main text): Each part should start on a new page.

- First page: Title - Abstract (limited to 150 words, unstructured - Keywords (List 3-5 key words using Medical Subjects Headings [MeSH])

-Introduction

-Case Presentation

-Discussion

-References

- Figure Legends: These should be included on separate page after the references.

-Tables and figures should be uploaded separately.

-Also, "Acknowledgements Form" should be uploaded separately.

A word count for the case reports (excluding title page, acknowledgements, references, figure and table legends) should be provided not exceeding 1500 words. Number of references should not exceed 15. Number of figure/tables is restricted to three for case reports.

C. Review Article

These are manuscripts which are prepared on current subjects by experts who have extensive experience and knowledge of a certain subject and who have achieved a high number of publications and citations. Reviews are usually submitted directly or by invitation of the editorial board. Submitted reviews within the scope of the journal will be taken into consideration by the editors. The content of the manuscript should include the latest achievements in an area and information and comments that would lead to future studies in that area. Number of authors should be limited to three.

Content (Main text): Each part should start on a new page.

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-Introduction

- Text: This part should present detailed information based on current literature about the subject of the review. The author(s) should organize the manuscript into appropriate headings and subheadings to facilitate reading.

-Conclusions

-References

- Figure Legends: These should be included on separate page after the references.

-Short Quiz (a list of 3-5 questions about the context of article for CME credit). The editorial board and Urooncology Association of Turkey executive committee will evaluate the answers and members submitting correct answers may receive education grants).

-Tables and figures should be uploaded separately.

-Also, "Acknowledgements Form" should be uploaded separately.

Number of figure/tables is restricted to five for review articles. Number of references should not exceed 100.

D. Literature Review

These short reviews are solicited by the editor, will go through the peer review process, and will cover recently published selected articles in the field of urologic oncology. It is a mini-review article that highlights the importance of a particular topic and provides recently published supporting data. The guidelines stated above for review articles are applicable. Word count should not exceed 1500 and references are limited to 10.

E. Editorial Commentary

These short comments are solicited by the editor and should not be submitted without prior invitation. An original research article is evaluated by specialists in the area (not including the authors of the research article) and this is published at the end of the related article. Word count should not exceed 500 words and number of references is limited to 5.

F. Letters to the Editor

These are letters that include different views, experiments, and questions from readers about the manuscripts published in the Bulletin within the last year and should be no more that 500 words with maximum of 5 references. There should be no title or abstract. Submitted letters should indicate the article being referenced (with issue number and date) and the name, affiliation, and address of the author(s). If the authors of the original article or the editors respond to the letter, it will also be published in the Bulletin.

G. Surgery Videos on Urooncology (Video-urooncology)

These videos are solicited by the editor. The videos are prepared on urooncological surgeries by experts who have extensive experience and knowledge of certain advanced surgical techniques. This section is also intended to enable urologists to learn, evaluate, and apply new or complex surgical principles in their surgical practice. The videos can describe current sophisticated or new surgical techniques or modification of current techniques. The surgery video must be high quality material.

Videos are only submitted by the invitation of the editorial board. Submitted videos are also evaluated based on double-blind peer-review principles.

The Bulletin of Urooncology publishes original videos containing material that has not been reported elsewhere as a video manuscript, except in the form of an abstract. The authors should describe prior abstract publications in the "Acknowledgements Form". Published videos become the sole property of The Bulletin of Urooncology.

Video-urooncology submission should include:

1) Copyright Transfer and Author Declaration Statement Form: This form must indicate that "Patients' Informed Consent Statement" is obtained.

2) Title Page

3) Summary: Summary should point out critical steps in the surgery up to 500 worlds. This part was published as an abstract to summarize the significance of the video and surgical techniques. The author(s) may add references if it is required.

5) Video: Please upload your video to www.uroonkolojibulteni.com using online submission system. Accepted video formats are Windows Media Video (WMV), AVI, or MPEG (MPG, MPEG, MP4). High-Definition (HD) video is preferred.

6) "Acknowledgements From" should be uploaded separately.

Videos should be up to 30 minutes in duration. The video must include audio narration explaining the procedure. All text and audio in the video must be in English. Audio must include narration in clear, grammatically correct English. Videos must be clear, in focus, and without excessive camera movement. Radiographs and other material must not contain any patient-identifiable information. Limited number of slides incorporated into video may be included to provide details of patient history, clinical and laboratory findings.

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Manuscripts should be prepared following sequence according to article type:

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All manuscripts submitted must be accompanied by this form which is available at www.uroonkolojibulteni.com. All of the authors must sign this form. This form must indicate that "Patient Consent Statement" is obtained for prospective trials, surgery videos (Video-oncology) and case reports. By signing this form the authors declare that they obtained the Ethic Committee approval document regarding all experimental, clinical and drug human studies. By signing this form authors also state that the work has not been published nor is under evaluation process for other journals, and they accept the scientific contributions and responsibilities. No author will be added or the order of authors will be changed after this stage. Any funding and/or potential conflict of interest must be declared in this form.

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The title page should include the following:

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-Running title

-Authors' names and institutions

-The ORCID (Open Researcher and Contributor ID) number of all authors should be provided

-Corresponding author's e-mail and postal address, telephone, and fax numbers

C. Main Text (without authors' credentials)

Each section of the main text should be started on a new page and abide to the following sequence according to article type:

-First page: Title, Abstract and Keywords: Abstracts should be prepared in accordance with the specific instructions for the different article types. Only for original articles, a structured abstract should be provided using the following headings: Objective, Materials and Methods, Results, and Conclusions. Provide 3-5 keywords. English keywords should be provided from Medical Subject Headings (http://www.nlm.nih.gov/mesh).

-Introduction: Introduction should include brief explanation of the topic, the objective of the study, and supporting information from the literature.

-Materials and Methods: This section should describe the study plan, indicating whether the study was randomized or nonrandomized, retrospective or prospective, the number of trials, the characteristics, and statistical methods used. If applicable, it should be indicated that the results should be scrutinized.

-Results: This part should summarize the results of the study, with tables and figures presented in numerical order; results should be indicated in accordance with statistical analysis methods used.

-Discussion: The positive and negative aspects of the study data should be discussed and compared with literature.

-Study Limitations: Limitations of the study should be discussed for only original articles. In addition, an evaluation of the implications of the obtained findings/results for future research should be outlined.

-Conclusions: The conclusion of the manuscript should be highlighted.

- References: The author is responsible for the accuracy of references. Cite references in the text with numbers in parentheses. All authors should be listed if four or fewer, otherwise list the first three authors and add et al. Number references consecutively according to the order in which they first appear in the text. Journal titles should be abbreviated according to the style used in Index Medicus (consult List of Journals Indexed in Index Medicus).

Examples for writing references:

Format for journal articles: initials of author's names and surnames. title of article. journal name date; volume: inclusive pages.

Example:

Journal: Soukup V, Dušková J, Pešl M, et al. The prognostic value of t1 bladder cancer substaging: a single institution retrospective study. Urol Int 2014;92:150-156.

Format for books: initials of author's names and surnames. chapter title. In: editor's name, Eds. Book title. Edition, City: Publisher; Year. p. pages.

Example:

Book Chapters: Lang TF, Duryea J. Peripheral Bone Mineral Assessment of the Axial Skeleton: Technical Aspects. In: Orwoll ES, Bliziotes M, eds. Osteoporosis: Pathophysiology and Clinical Management. New Jersey, Humana Pres Inc, 2003;83-104.Books: Greenspan A. Orthopaedic

Radiology a Practical Approach. 3rd ed. Philadelphia: Lippincott Williams Wilkins; 2000. p. 295-330.

-Figure legends: These should be included in main text on a separate page after the references.

-Short Quiz: A list of 3-5 questions as the last page about the context of article for CME credit only for review articles.

D. Tables and Figures

If you use data from another published or unpublished source, obtain permission and fully acknowledge that source. Number of figure/tables is restricted to five for original article and reviews and three for case reports. Authors should contact the editor prior to submission regarding any manuscript exceeding these figure/table limitations.

Direct quotations, tables, or illustrations taken from copyrighted material must be accompanied by written permission for their use from the copyright owner and authors.

Tables: Supply each table in a separate file. Number tables according to the order in which they appear in the text, and supply a brief caption for each. Give each column a short or abbreviated heading. Write explanatory statistical measures of variation, such as standard deviation or standard error of mean. Be sure that each table is cited in the text.

Figures: Supply each figure in a separate file. Authors should number figures according to the order in which they appear in the text. Figures include graphs, charts, photographs, and illustrations. Each figure should be accompanied by a legend. Figures should be submitted as separate files, not in the text file. Image files must be cropped as close to the actual image as possible. Pictures/photographs must be in color, clear and with appropriate contrast to distinguish details. Figures, pictures/photographs must be uploaded as separate .jpg or .gif files (approximately 500x400 pixels, 8 cm in width and scanned at 300 resolution). Figure legends should be included in main text on a separate page after the references.

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If the manuscript presented as an abstract previously; the name, date, and place of the meeting should be mentioned.

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The Bulletin of Urooncology only accepts electronic manuscript submission at the web site www.uroonkolojibulteni.com.

Manuscripts should be prepared as a word document (*.doc) or rich text format (*.rtf). Text should be double-spaced with 2.5 cm margins on both sides using 12-point type double spaced in Times Roman.

Submissions must include according to the following sequence:

A-Original Article

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3) Main text (without authors' credentials): Each part should start on a new page.

First page (Title- structured abstract – keywords), Introduction, Materials and Methods, Results, Discussion, Study Limitations, Conclusions, References, Figure legends

4) Table(s)

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B. Case Reports

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First page (Title- abstract – keywords), Introduction, Case Presentation, Discussion, References, Figure legends

4) Table(s)

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3) Main text (without authors' credentials): Each part should start on a new page.

First page (Title- abstract – keywords), Introduction, Text (appropriate headings and subheadings), Conclusions, References, Figure legends, Short Quiz

4) Table(s)

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6) Acknowledgements Form

D. Literature Review

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3) Main text (without authors' credentials): Each part should start on a new page.

First page (Title- abstract – keywords), Introduction, Text (Appropriate headings and subheadings), Conclusions, References, Figure legends

4) Table(s)

5) Figure(s)

6) Acknowledgements Form

E. Editorial Commentary

1) Copyright Transfer and Author Declaration Statement Form

2) Title Page

3) Main text (Text, References)

4) Acknowledgements Form

F. Letters to the Editor

1) Copyright Transfer and Author Declaration Statement Form

2) Title Page (The title is "Letter to Editor about.....")
3) Main text (Text, References)
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G. Surgery Videos (Video-urooncology)

1) Copyright Transfer and Author Declaration Statement Form
2) Title Page
3) Summary (without authors' credentials)
4) Video
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Contents

Review

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Evaluation of Current Status of Urine and Serum Biomarkers in the Diagnosis and Follow-up of Bladder Cancer: Review

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Abstract

Bladder cancer is a very common, aggressive malignant tumor. It is associated with high recurrence and mortality rates. Early detection of bladder cancer and recurrences is very vital to ensure long survival. The main purpose of screening methods is to detect the disease at an early stage. Diagnosis and follow-up of these patients are currently based on endoscopic approaches, which is expensive and invasive, and urinary cytology with low sensitivity. In order to reduce the burden of cystoscopic evaluation, which is the standard approach used in the follow-up, urine strip tests have been used in order to evaluate the presence of hematuria, and cost effective and relatively high-performance molecular markers, respectively. However, there is still no safe biomarker to replace the conventional approach. Bladder tumors exhibit a wide heterogeneity with various molecular differences associated with different morphological symptoms and disease phenotypes. Therefore, the introduction of biomarkers that evaluate the aggressiveness of the disease, the risk of progression, the likelihood of recurrence and prognosis will improve patient management. In addition, integrating the use of molecular biomarkers with conventional pathological evaluation will allow us to make clinical decisions, such as the selection of adjuvant and salvage treatment.

Keywords: Bladder cancer, urinary biomarkers, molecular markers

Introduction

Bladder cancer is the second most common cancer in the United States among genitourinary cancers (1). In the world, it is the 11th most common cancer among all cancers (2). The worldwide standardized incidence for age was 8.9 for males (100,000 person-year) and 2.2 for females (2008 data), while it was 27 for males and six for females in Europe (2). The incidence is generally increasing in developed countries. Transitional epithelial cell cancers are the most common histological type in 90% of cases, but squamous cell cancers and adenocarcinomas are other types of cancer seen in the bladder (3).

Diagnosis of bladder cancer is made by cystoscopy, cytology and pathological evaluation of the excised material. If bladder cancer was detected or urinary cytology was found positive, diagnostic cystoscopy is omitted and biopsies from suspected areas or tumor resection are performed under anesthesia (4). However, despite initial treatment, tumor recurrence occurs after endoscopic resections in 30-85% of the cases, and 16-25% of them develop higher grade tumors (3). Approximately 10% of tumors without muscle invasion develop invasive or metastatic cancer during follow-up.

After the diagnosis of bladder cancer, the follow-up procedures for the disease are as important as the diagnosis of the disease. Cystoscopy and cytological evaluation of urine are recommended routinely. However, cystoscopy being an invasive procedure and low sensitivity of cytological evaluation of urine have revealed the necessity of identifying new markers.

In this review, bladder tumor biomarkers that are still in use or are under development in diagnosis and/or follow-up are reviewed.

Urine Cytology

Urinary cytological evaluation, which starts with the identification of abnormal cells in the urine sample, is a non-invasive urine marker commonly used in the diagnosis of bladder tumor. Non-invasive collection of urine and the tendency of cancerous cells

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to shed as a result of loss of cohesion make this examination easy and specific to high-grade lesions. For better evaluation, the method of urine collection, detailed clinical history, treatment and catheter insertion should be reported.

The urothelium consists of superficial cells with large cytoplasm and sometimes mucin, intermediate cells with less cytoplasm under it, and basal cells at the bottom. Cytological samples include mostly superficial and intermediate cells. Infection, catheter application, stones and various treatments may lead to reactive changes. Reactive changes are difficult to distinguish from papilloma, papillary urothelial neoplasia with low malignant potential and low-grade papillary urothelial carcinoma.

Patients with bladder cancer are classified as low or high-grade tumors according to their cytological features. Sensitivity and specificity are very high in high-grade papillary cancers and *in situ* cancers. In the literature, the diagnostic accuracy of cytological examination in low-grade cancers was reported as 17%-70% for spontaneous urine samples in original studies. This rate is 95%-100% in high-grade papillary and *in situ* cancers (5).

High sensitivity and specificity are important, as *in situ* cancer area may not be recognized by cystoscopy and *in situ* lesions often accompany invasive, high-grade, multifocal tumors (6,7). According to a recent study, sensitivity and specificity rates of urine cytology was 38% and 98.3%, respectively. There was no significant difference between spontaneous urine and washing (8,9). In order to better visualize cancer cells, urine samples to be used in cytology should be taken from patients who are well hydrated. The urine sample to be examined should be taken from the first morning urine. Positivity of urine cytology findings, even if upper urinary tract and bladder imaging results are negative, may indicate cancer elsewhere in the urinary tract such as calyxes or ureters. Furthermore, a negative result of urinary cytology does not necessarily indicate the absence of a low-grade bladder tumor (10). All of these causes have encouraged the search for more reliable urine tests for the diagnosis of urothelial malignancy.

Microscopic Hematuria

Hematuria is the most common sign of bladder cancer. It is usually detected using a dipstick test. The test is easy to use and cost-effective. Sensitivity and specificity for microscopic hematuria have been reported as 91% and 99%, respectively (11). Hematuria is a common finding in the general population and is not only associated with bladder cancer. For population-based screening tests, bladder cancer was reported in 16-24% of men with hematuria who were older than 50 years; however, it was not detected in 32% of bladder cancers (12). Hematuria is intermittent in most patients (13). Recurrence increases the sensitivity of the hemoglobin dipstick test and the number of patients with false-positive findings that may lead to cystoscopy (reducing specificity). Patient compliance to home-based daily repeated tests is generally good (97.7%) (14). Weekly hematuria tests are useless, and researchers recommend daily testing for 10-14 days every 6-12 months (11). It is not recommended to use first urine in the morning, and urine following excessive physical activity or sex. High false-positive rates are associated with other genitourinary cancers, colic pain, anticoagulant treatments (Warfarin, salicylates, NSAIDs), and other pathologies (glomerulonephritis, urinary stones) that cause hematuria (11,12,13,14,15).

Urine Tumor Markers

The most common presenting symptom of bladder cancer patients is asymptomatic microscopic hematuria or painless macroscopic hematuria. The rate of symptomatic patients is often difficult to say because the symptoms are intermittent and cannot be shown in screening. However, the use of urinary markers, which have recently been developed for early detection of urinary malignancies, gives hope for early diagnosis. The use of these markers can reduce the number of regular cystoscopies performed to control non-muscle-invasive bladder cancer relapses and ultimately result in significant cost savings. Likewise, prediction of the patient population that will show progression may lead to an increase in disease-related survival (16).

Urine is in a continuous relationship with the urothelium in the renal pelvis, ureter and bladder starting from the calyces. Therefore, the use of urine samples seems to be more rational in the investigation of a marker for urothelial cancers (17).

An ideal marker:

- Should be non-invasive and easy to apply technically,
- Should be cheap,
- Should be reliable and repeatable,
- Should have high specificity and sensitivity,
- Should ensure early detection of high-risk tumors (e.g. carcinoma *in situ*) and ensure that the chance of curative treatment is not missed (18).

A. Molecular Markers in Urine

1. Bladder Tumor Antigen Test

It is based on the detection of human complement factor H-associated protein in urine that is produced in bladder cancer cell. In cell cultures, normal cells were unable to express H-associated proteins. The bladder tumor antigen test (BTA)-Stat assay is a qualitative immunoassay using two different monoclonal antibodies. BTA Stat is an immunochromographic, qualitative, NMP22-like test, approved by the United States Food and Drug Administration (FDA) for follow-up, not for screening or diagnosis. In many studies, the sensitivity varies between 36-89% (19). Specificity is high in healthy individuals (97%). However, in benign genitourinary diseases (hematuria, benign prostatic hyperplasia, BCG use, urinary diversions, urinary stone, cystitis, nephritis), it decreases to 46%. This leads to false positive results by binding of complement factor H, which is already present in the serum at a constant amount of 0.5 mg, to erythrocytes in the urine. BTA-stat is superior to urinary cytology in detecting low-grade cancers. In a large, multicenter study, 95% specificity was demonstrated in healthy volunteers without genitourinary disease. However, the specificity shows a significant decrease in patients with additional disease; it is around 88% in patients with benign prostatic hyperplasia and

50% in patients with urolithiasis. Specificity decreases to 33% after interventions to the bladder or prostate (20,21,22). The BTA-stat test should be interpreted with caution in patients with microscopic hematuria. The test is not safe as urine leads to false positivity when it is very bloody (23).

BTA-Trak test is a quantitative ELISA (Enzyme Linked Immunosorbent Assay) test and two monoclonal antibodies against complement factor H and complement factor H dependent protein are used. The overall sensitivity and specificity of the BTA-Trak test are 66% and 69%, respectively. The accuracy of the BTA-Trak test in low-grade tumors is better than the BTA-Stat test. The most important factor limiting the use of this test is high false-positive rates (24).

2. Nuclear Matrix Protein Test

The nuclear matrix protein is part of the internal framework of the cell nucleus and plays a role in DNA replication, transcription and transfer to RNA and possibly regulation of gene expression. This protein is associated with spindle bodies formed during mitosis and may be responsible for the proper and regular distribution of chromatids in daughter cells. When inappropriate distribution of chromatids occurs during mitosis, there is a 25-fold increase in nuclear mitotic apparatus proteins in tumor cells compared with normal bladder epithelial cells, such as in bladder tumors (25). Compared with normal tissue and transitional cells, there is at least a 10-fold increase in nuclear mitotic apparatus proteins in cancer tissue. There are two different nuclear matrix protein tests (NMP22) used to detect NMP22 in urine. The original NMP22 bladder cancer test kit is a laboratory-based, quantitative, sandwich-type enzyme immunoassay. The second test is NMP22 BladderChek, which contains a qualitative, NMP22-detecting antibody. Both tests were approved by the FDA for follow-up of bladder tumors. In addition, the NMP22 BladderChek test can be used for screening patients at risk for bladder tumors.

The sensitivity and specificity of NMP22 ELISA from various studies vary between 19-100% and 55-92%, respectively. This variability arises from the use of different predictive values in various studies and the diagnosis of recurrent tumor versus primary tumor diagnosis.

NMP22 has high false-positive rates in patients with inflammatory status, renal and bladder stones, foreign bodies in the body, intestinal interposition, other genitourinary cancer, and proteinuria (26). The BladderChek test has recently been studied by Grossman et al. (27) in a prospective study of a total of 1331 patients with lower urinary tract symptoms such as hematuria and dysuria, and with a history of smoking. In this study, NMP22 was compared with urinary cytology. The test has been shown to have 55.7% sensitivity and 85.7% specificity in the diagnosis of bladder tumors. For urine cytology, these values were reported as 15.8% and 99.2%, respectively.

3. NMP52

It is a 52-kilodalton nuclear matrix protein. It measures by using ELISA with polyclonal rabbit antibody. In a study of 149 patients with bladder cancer, the sensitivity of NMP52 test was 92% in the diagnosis of squamous cell carcinoma, 98% in the diagnosis of variable epithelial cell carcinoma, and 100% in the diagnosis of adenocarcinoma of the bladder. The specificity of the test was found to be 94% (28).

4. BLCA-1 and BCLA-4

BLCA-1 and BLCA-4 are nuclear transcription factors in bladder tumors. BLCA-1 is not released from non-malignant urothelium. BLCA-4 is a factor released from both tumor and benign areas adjacent to tumor, but not from non-malignant bladder (29,30). BLCA-4 is measured in urine by ELISA. It has been reported that its sensitivity reaches 89-96% and its specificity reaches 100% (31,32). This protein increases IL-1 α, thrombomodulin and IL-8 levels and affects the pathogenesis of bladder tumor (33).

In a study of 25 bladder cancer cases and 46 controls, BLCA-1 ELISA was shown to have a sensitivity of 80% and a specificity of 87% (34). BLCA-4 test has been shown to have sensitivity and specificity of more than 90% in two separate studies (35,36,37,38). In a study conducted by Van Le et al. (39) in 75 patients with primary bladder cancer, the sensitivity and specificity were 89% and 95%, respectively. BLCA-4 is a potential useful marker for bladder cancer screening because of its high sensitivity and specificity. However, it has not been sufficiently tested for survival.

5. Survivin

It is an antiapoptotic protein. It is an inhibitor of the apoptosis family (40). Survivin is found in 10-30% of bladder tumor tissues. The sensitivity of urine survivin levels measured using a bio-dot measuring apparatus is between 42.5-100% (41,42,43). High levels of survivin increase the risk of bladder cancer and the likelihood of having a higher-grade tumor (44). Clinical use in bladder cancer has been questioned because of its low specificity.

***6.* Urinary Bladder Cancer Test**

It is an ELISA test that investigates the presence of fragments of cytokeratin 8 and 18 in urine. Cytokeratin 8 and 18 are normal structural components of the cell. Increased expression has been observed in transitional epithelial cell cancers, especially in high-grade ones (45). In a study by Babjuk et al. (46) in which recurrent tumor formation is monitored in 88 patients with non-muscle invasive bladder cancer, sensitivity was 54% and specificity was 97%. They concluded that it could not be used to reduce the number of cystoscopies in routine urology practice due to its low sensitivity. Mungan et al. (47) evaluated the diagnostic value of urinary bladder cancer test (UBC) in 100 patients with non-muscle invasive bladder tumors and reported sensitivity, specificity, positive and negative predictive values as 20.7%, 79.2%, 28.6% and 71.3%, respectively. With these results, they concluded that UBC test was insufficient in the follow-up of patients with bladder tumors.

7. CYFRA 21.1 Test

It is an ELISA based assay that allows the detection of cytokeratin 19 fragments by means of two monoclonal antibodies (BM19.21 and KS19.1). In a study by Fernandez-Gomez et al. (48) and colleagues in 446 patients with Ta and T1 bladder cancer, 125 patients had recurrence, and the sensitivity and specificity of the test were found to be 43% and 68%, respectively. Cut-off

value was accepted as 4 ng/mL and 12 patients with Ta tumor could not be determined. When the cut-off value was accepted as 1.5 ng/mL, the rate of detecting Ta recurrences increased by 73%, but its specificity decreased to 43%. They emphasized that this test was not a suitable marker for follow-up in patients with bladder cancer.

8. Fibrinogen Degradation Products, ACCU-DX

Cancer cells produce an angiogenic factor that induces vascular endothelium, called vascular endothelial growth factor. This factor increases vascular permeability in tumor tissue. This leads to the passage of blood and plasma proteins, such as plasminogen, fibrinogen, coagulation factors, into the extravascular space. Fibrinogen is converted to fibrin and binds to plasminogen and converts to plasmin. Plasmin, a potent proteolytic enzyme, breaks down fibrinogen and fibrin into Fibrinogen degradation products (FDP). FDP circulates and is also found in urine in patients with bladder cancer. Urine FDP can be measured by latex agglutination test, monoclonal antibody-based ELISA and monoclonal antibody immunassay methods (49,50,51,52,53). The ACCU-DX test is a qualitative test using murine monoclonal antibodies specific for FDP. However, the availability of this test is low in the presence of hematuria because these antibodies also interact with intact fibrinogen, which is typically found in human serum (54). FDPs are either absent or extremely low in the urine of healthy individuals. In some inflammatory events, FDP may be detected in the urine, but the detection of FDP in the urine is generally interpreted in favor of transitional epithelial cell cancer. FDP levels in urine increase as tumor stage and grade increase. When used together with cytology, its sensitivity increases to 75-80% (20).

9. Hyaluronic Acid, Hyaluronidase Test

Hyaluronic acid (HA) forms the extracellular glycosaminoglycan layer that protects tumor cells from the control of the immune system. Adhesion and migration of tumor cells is also facilitated by this layer. The HA-Hyaluronidase (HAase) assay is a combination of two similar ELISA assays. It measures the urinary HA and HAase levels. HA test can detect bladder cancer regardless of tumor grade and HAase test detects high-grade tumors. The combination of HA test-HAase test has a sensitivity of 83-94% in detecting both primary and recurrent tumors (41,42). It has sensitivity between 75% and 100% in both low-grade/stage and high-grade/stage tumors (55,56). In addition, in the follow-up of bladder cancer recurrence with the HA-HAase test, even a false-positive value indicates an increased risk of recurrence by 4 to 10 times within 5 months (57). The specificity of HA-HAase test between normal individuals and patients with benign urological conditions is 80% (41,42).

A. Tests with Shed Cells into the Bladder Lumen

1. ImmunoCyt

In this test, urine specimens containing tumor cells shred into the lumen of the bladder are used in patients with bladder tumors. It is an immunohistochemical test that detects sulfated mucin glycoproteins and carcinoembryonic antigen on the surface of bladder tumor cells. Assay is performed using three fluorescent stained monoclonal antibodies. These fluorescent monoclonal antibodies are 19A211, M344 and LDQ10. The main advantage of this test is the high rate of detecting low grade and well-differentiated tumors. The disadvantage is that false positive and negative rates are high and an experienced cytopathologist is needed. The detection of a single fluorescent cell in one sample represents the positivity of the assay. In the study of Lodde et al. (58) in 216 patients, sensitivity was 84% and specificity was 78%.

2. Lewis X antigen

It is based on the detection of a blood group antigen that is not normally found in urinary transitional epithelial cells using monoclonal antibodies. This blood group antigen is synthesized by all cancerous cells, regardless of tumor grade and stage. In the study of spot urine samples of 260 patients, Pode et al. (59) found sensitivity as 79.8% and specificity as 86.4%, and Cis was found to be 100%. With the evaluation of two different urine specimens, Golijanin et al. (60) stated that the sensitivity of Lewis X antigen test increased from 81.2% to 97%, and the specificity increased to 85.5%.

3. DD23

DD23 is a monoclonal antibody that detects a protein dimer released from bladder cancer cells. It is an immunohistochemical test with alkaline phosphatase bound to monitor tumor cells in urine. In two controlled trials, DD23 showed high sensitivity between 70-80% (61,62). This marker has high sensitivity in both low-grade tumors (approximately 70%) and high-grade tumors (approximately 87%). Its sensitivity slightly increases when used in combination with urinary cytology (78-85%). However, the specificity of DD23 is about 60% and the specificity of the samples obtained by washing is lower (61,62).

4. Cytokeratin 20

Cytokeratins are intermediate filament proteins of epithelial cells. Cytokeratin 20 is specifically expressed by the bladder and gastrointestinal epithelium. It can also be detected in normal cells in scans but has been upregulated in carcinoma patients. In two studies, the mean sensitivity and specificity of cytokeratin 20 were found to be 85% and 76%, respectively (63,64). Studies comparing cytokeratin 20 with other markers other than urine cytology have not yet been performed.

5. Telomerase

Most cells in the body can proliferate in a limited number before losing their ability to divide. Chromosome terminations are called telomere. The ability to continuously proliferate is acquired by expression of the telomerase gene. Telomerase is normally expressed in cells such as stem cells or gametes, which must be divided into an unlimited number. Telomerase immortalizes the chromosome or telomeres by maintaining the ends normally shortened in each division. Although active telomerase is important for survival in malignant cells and for long survival in normal cells, telomerase itself has no effect of inducing a malignant phenotype. However, the presence of oncogene and/or tumor suppressor gene inactivation in

addition to telomerase may cause malignant transformation. Genitourinary cancers have a high level of telomerase activity as in all cancers (65,66,67). Two different methods have been developed to evaluate telomerase activity in tissue. First, TRAP (telomeric repeat amplification protocol) is based on the telomeric amplification protocol by ELISA or RT-PCR. Second, hTERT (human telomerase reverse transcriptase) is based on the measurement of mRNA levels by RT-PCR method.

Increased telomerase levels by 90% especially in high-grade and staged cancers made it an important tumor marker (68). In their study of 200 bladder cancer patients (primary or recurrent distinction), Eissa et al. (69) recruited 85 benign bladder lesions and 30 healthy patients as the control group. They found the sensitivity to detect bladder cancer as 96%.

Telomerase activity was evaluated by a polymerase chain reaction test using TRAP. In the detection of bladder tumors, the sensitivity of the telomerase test is between 7% and 100%, usually 70-86%, and the specificity ranges from 24% to 90%, usually 60-70% (70,71,72,73). In a study conducted to determine the TRAP threshold range in 2005, a sensitivity of 90% and a specificity of 88% when the threshold value was taken as 50 enzyme units (74).

6. UroVysion Fluorescence *İn Situ* Hybridization (UroVysion FISH)

It is a multi-targeted, multicolour fluorescent *in situ* hybridization (FISH) test that involves staining urine cells with 4 denatured centromeric chromosome enumeration probes. It detects chromosome 3 (spectrum red), chromosome 7 (spectrum green), chromosome 17 (spectrum aqua) and locus-specific probe 9p21 (spectrum gold). Cells are examined under fluorescence microscopy. Diagnostic criteria for bladder cancer in the UroVysion test are: five and/or more cells with polysomy of two or more chromosomes, isolated gain of a single chromosome in 10% or greater cells, and homozygous deletion of 9p21 in 20 or more cells. Four types of genetic abnormalities (polysomy, tetrasomy, trisomy, and homozygous 9p21 deletion) were observed in the UroVysion examination of patients with bladder cancer. In various studies, the sensitivity of UroVysion test ranges from 69% to 87% (75,76). This test has been approved by the FDA for the follow-up of patients with bladder tumors and for the detection of bladder tumors in patients with hematuria. UroVysion test has a very good sensitivity rate (83-100%) in detecting Tis and high grade/high stage tumors. However, sensitivity is not good in detecting low-grade/low-grade tumors (76,77).

Despite a normal cystoscopy, some of the patients with positive FISH test were suggested to develop urothelial cancer after a while. Many studies have reported positive bladder biopsy within 12 months in 85% to 89% of patients with positive FISH tests (77,78). However, some other studies have shown that the recurrence rate after positive FISH test and negative cystoscopy is <50% (79).

7. MicroRNA Markers

MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression after transcription (80). Because they are stable in urine and are more resistant to nuclease degradation due to their small size, they can be used as an ideal bladder marker (81). There are many nucleases in the urine, and a large number of tests that analyze mRNA expression are not successful due to target degradation. Recently, urinary miRNA expression has been reported and upregulation of miRs-126/182/199a has been found to differentiate between healthy and bladder tumor patients (82). In spite of the inability to distinguish the expression of these miRNAs in normal and malignant urothelium, the combination of mi-126 and 182 was found in 77% of bladder tumor cases (83). Further study of these markers is needed. In a study of 485 bladder cancer patients presenting with macroscopic hematuria in Australia, urine cytology, NMP22 and MicroRNA efficacy were evaluated. MicroRNA showed a higher specificity rate of 85% than urine cytology and NMP22. However, the presence of urinary stone causes false positive results. In addition, it was reported that specificity was affected at a low rate with gender, age and creatinine level (84). In a similar study conducted in the UK, 121 urine samples from 68 patients with bladder cancer and 53 non-bladder cancer samples were subjected to polymerase chain reaction using fifteen microRNA fragments. Results reported that urinary microRNAs were successful at a rate of 94% in detecting urothelial cancers (85).

8. Microsatellite Analysis

Microsatellite analysis has been mentioned in the literature since 1997 and its success in detecting low-grade bladder cancers has been evaluated. It is a polymerase chain reaction test that recognizes the tumor DNA. Recent studies have reported recurrence rates of 83% in those with positive microsatellite test and 22% in those with negative test (86,87).

C. Other Markers

1. Microtubule-associated Proteins

It is a microtubule-associated protein (MAP) localized in the Tau *17q21* gene. It regulates the cell cycle of stathmin, which is the protein disrupting microtubule stabilization. In case of mutation or deregulation, it leads to uncontrolled cell proliferation (88). High expression or activity of stathmin may be indicative of metastasis and may be associated with poor prognosis. In a recent study examining MAP levels in 32 patients with bladder cancer, high levels of tau and stathmin protein measured before intravesical taxane treatment were associated with decreased recurrence-free survival (88). Multivariate analysis showed that tau positivity was an independent risk factor for recurrence-free survival. Stathmin-positive patients had a recurrence-free survival of 16 months shorter than the negative ones. It is an experimental marker used to determine prognosis.

2. Mammary Serine Protease Inhibitor

Mammary serine protease inhibitor (Maspin) is a protein encoded by SerpinB5s localized in the *18q21.3* gene (89). The protein, a gene product, acts as a tumor suppressor gene and reduces the invasion and metastasis ability of cancer cells. Acikalin et al. (89) conducted a study to evaluate the effect of maspin by immunohistochemical method in 68 newly diagnosed T1 bladder cancer patients. They showed that

patients with maspin negative were 2.2 times more likely have recurrence than positive ones. Maspin negative patients were 4.3 times more at risk for progression than maspin positive patients. Decreased expression of maspine was found to be an independent risk factor for recurrence and progression.

3. Tumor Associated Trypsin Inhibitor

It is a low molecular weight (6 kilodalton) trypsin inhibitor used in bladder cancer screening. The role of trypsin in cancer pathogenesis is not yet known. The expression of tumor associated trypsin inhibitor (TATI) in urine was determined by an immunofluorometric method. In 80 patients with primary bladder cancer, Gkialas et al. (90) found a sensitivity of 85.7% and a specificity of 76% in a screening study using TATI. However, no further studies have been conducted to support this data.

Conclusion

Although many studies have been published in the literature to identify an ideal tumor marker in recent years, most of these tests have better sensitivity and lower specificity in the diagnosis of bladder cancer. Therefore, it is not uncommon to use unnecessary biopsy and imaging techniques because of false-positive results. Whether such tests can provide additional information in decision-making, treatment and prognosis in non-muscle invasive bladder cancer is not yet known due to the lack of multicenter prospective data on the subject. The combined use of current novel markers can result in higher performance, eliminating the drawbacks of one test by the advantages of another test. For a valuable test in detection of bladder cancer; sensitivity and negative predictive value are important. Sensitivity and negative predictive value should be above 90% in all tumors if they are to replace cystoscopy. It should be over 95% in "dangerous" high-grade tumors (55). Today, there are no tests that meet these criteria. The use of urinary tumor markers is optional in low and moderate risk disease in NCCN (National Comprehensive Cancer Network) 2015 guidelines and the degree of recommendation is 2B.

There is currently no scientific guideline to routinely recommend the use of either of these tests in urology practice, diagnosis, or follow-up. In summary, urinary cytology and cystoscopy are the gold standard in the diagnosis and follow-up of bladder tumors.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.Y., Design: M.Y., Data Collection or Processing: M.Y., Analysis or Interpretation: H.G., Literature Search: M.Y., Writing: M.Y.

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Original Article

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ABO Blood Types and Risk of Testicular Cancer in Turkish Population: Preliminary Results

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Abstract

Objective: ABO blood type has been reported to be associated with various types of cancer such as lung cancer, prostate cancer, bladder cancer and pancreatic adenocarcinoma. We aimed to determine whether there is a relationship between ABO blood subtypes and testicular cancer incidence in Turkish population.

Materials and Methods: We retrospectively analyzed the medical records of all patients who underwent inguinal orchiectomy in our department between 2008 and 2018.

Results: A total of 138 patients were included in the study. The age of the patients ranged from two to 67 years, with a mean age of 32.9±10.9 years. Of the 138 participants, 45 (32.6%) had blood type A, 27 (19.6%) had blood type B, 50 had blood type O (36.2%) and 16 (11.6%) had blood type AB. One hundred and twenty-five patients (90.6%) were Rhesus (+) and 13 patients (9.4%) were Rhesus (-). There was no statistically significant difference between ABO blood subtypes according to testicular pathology (p=0.713). Interestingly, the percentage of patients with AB blood subtype was the lowest.

Conclusion: These results suggest that Turkish men with O blood subtype and Rh (+) are prone to develop testicular cancer. However, these findings should be supported by further studies conducted with a large number of participants.

Keywords: Testicular cancer, blood group, ABO, rhesus

Introduction

ABO blood type was first discovered by Karl Landsteiner in 1900 and has been widely studied in various diseases and blood transfusion complications (1). ABO blood type was suggested to be associated with various types of cancer such as lung cancer (2), prostate cancer, bladder cancer (3) and pancreatic adenocarcinoma (4).

To the best of our knowledge, there is no study in the literature on the relationship between ABO blood types and testicular cancer. In the present study, we aimed to determine whether there is a relationship between ABO blood subtypes and testicular cancer incidence.

Materials and Methods

After obtaining approval from the Local Ethics Committee (2018/350), we retrospectively analyzed the medical records of all patients who underwent inguinal orchiectomy in our department between 2008 and 2018. The parameters examined included demographics, testicular cancer pathology results and ABO blood subtypes. All male patients diagnosed with testicular cancer without an age limitation were included the study. Patients with a final pathology result consistent with benign pathologies or other malignancies such as lymphoma and rhabdomyosarcoma were excluded from the study. The patients were divided into two groups according to the final pathology results; Group 1: seminoma and Group 2: non-seminomatous germ cell tumors (Figure 1,2).

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Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.21.0 (IBM Corp., Armonk, NY). Quantitative values were expressed as mean ± SD or median (range), whereas qualitative values as numbers and percentages. Shapiro-Wilk test was used to determine whether the values were normally distributed or not. Cross table analysis, chi-square test and Fisher's exact test were applied to compare the qualitative characteristics of the two groups. The level of statistical significance was set at $p<0.05$.

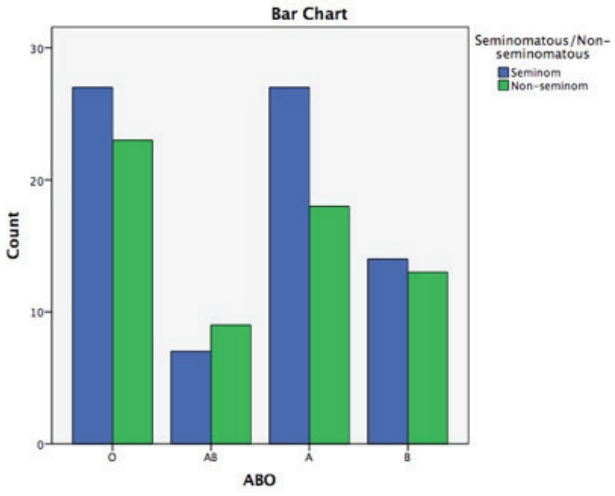

Figure 1. Distribution of ABO blood types according to pathology

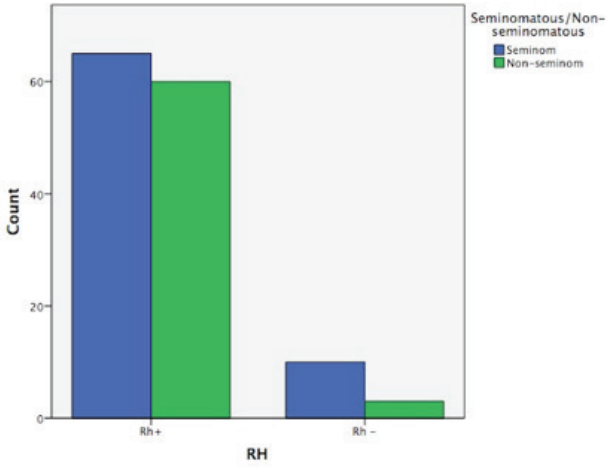

Figure 2. Distribution of Rhesus blood types according to pathology

Table 1. Distribution of patients according to blood group subtypes

Blood Subtypes	Rhesus	Seminomatous (n=77)	Non-seminomatous (n=63)	P value
Group A	Rh (+)	25 (33.3%)	17 (27%)	0.222*
	Rh (-)	2 (2.7%)	1 (1.6%)	
Group B	Rh (+)	11 (14.7%)	12 (19%)	
	Rh (-)	3 (4%)	1 (1.6%)	
Group AB	Rh (+)	6 (8%)	8 (12.7%)	0.713#
	Rh (-)	1 (1.3%)	1 (1.6%)	
Group O	Rh (+)	23 (30.7%)	23(36.5%)	
	Rh (-)	4 (5.3%)	0 (0%)	

#p value for ABO group subtypes
*p value for rhesus groups

Results

A total of 138 patients were included the study. The mean age of the patients was 32.9±10.9 years, ranging from 2 to 67 years. Of the 138 participants, 45 (32.6%) had blood type A, 27 (19.6%) had blood type B, 50 had blood type O (36.2%) and 16 (11.6%) had blood type AB. One hundred and twenty-five patients (90.6%) were Rhesus (+) and 13 (9.4%) were Rhesus (-). Final pathological examination revealed seminoma in 75 (54.3%) patients, malign mix germ cell tumor in 45 (32.6%) patients and germ cell tumors in 18 (13%) patients. There was no statistically significant difference between ABO blood subtypes according to testicular pathology (p=0.713). Interestingly, the percentage of patients with AB blood subtype was the lowest. There was no statistically significant difference in rhesus status of patients with seminomatous and non-seminomatous pathology (p=0.142) (Table 1).

Discussion

Testicular cancer accounts for 1-2% of all tumors in men and it is the most common cancer in young men between the ages of 15-34. There is a significant geographical variation in the age-standardized incidence of testicular cancer; such the incidence is 0.5/100.000 in Egypt and 9.6/100.000 in Norway (5). There are several risk factors for testicular cancer including cryptorchidism, ethnicity, racial differences and genetic causes, such as Down syndrome and testicular dysgenesis syndrome (6,7).

The ABO blood grouping system was described in the early 1900s. This grouping system is based on the expression of A and B blood cell surface antigens. The absence of both antigens results in the O phenotype. ABO blood group was found to be associated with numerous diseases and hemostatic complications such as increased risk of coronary artery disease and venous thromboembolism in patients with non-O blood type (8).

Several studies have been conducted to determine the possible relationship between ABO blood types and cancer (4,9). The frequently studied cancers include breast cancer (10), gastric cancer (11), lung cancer (2), and bladder, prostate and kidney cancers (3,12,13,14).

In a study by Meo et al. (10), blood group A was found to have the highest association with breast cancer compared to other blood groups. The incidences of breast cancer in blood groups A, B, O and AB were 45.88%, 16.6%, 31.69%, and 6.27%, respectively. They also reported that "Rhesus +" blood group had higher incidence of breast cancer compared to "Rhesus –" (87.31% vs 11.68%).

Xu et al. (11) studied the relationship between ABO blood types and gastric cancer prognosis in 1412 patients who were

diagnosed with gastric cancer and underwent surgery. They concluded that non-AB blood groups were associated with poor prognosis.

In another study, Stakisaitis et al. (3) aimed to reveal the association between ABO blood polymorphisms and various urological cancers including prostate, bladder and kidney. They found a significantly higher incidence of prostate cancer in patients with blood group B ($p<0.05$). The incidence of bladder cancer was also higher in men with blood group B ($p<0.04$). ABO blood subtype O was associated with a decreased risk of bladder cancer in women ($p<0.05$). No significant difference was observed in kidney cancer in terms of ABO blood subtypes.

To the best of our knowledge, there is no study in English literature examining the association between ABO blood subtypes and testicular cancer in men. In a unique study by Yildiz et al. (15), the authors investigated the effect of ABO blood subtype on prognosis in patients with non-seminomatous testicular cancer who were treated with high dose chemotherapy and autologous stem cell transplantation. ABO blood subtypes and Rhesus distribution of patients in this study were as follows: A subtype in 19 (29.7%), B subtype in seven (10.9%), O subtype in 34 (53.1%), AB subtype in four (6.3%), Rh (+) in 61 (95.3%) and Rh (-) in three (4.7%) patients. They found that overall survival of patients with O blood subtype was longer than other blood subtypes; however, it was not statistically significant ($p=0.071$). One-year progression-free survival was also higher in patients with O blood subtype compared to all other groups (79.1% vs 65.2%, $p=0.19$): however, it was also not statistically significant. In concordance with Yildiz et al. (15), most of the patients in our cohort had blood subtype O (36.2%) and the patients with AB blood subtype constituted the lowest percentage (11.6%). Also, 90.6% of the patients were Rhesus (+).

Conclusion

These results suggest that Turkish men with O blood subtype and Rhesus (+) are prone to develop testicular cancer. However, these findings should be supported by further studies conducted with large number of participants.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (no: 2018/350).

Informed Consent: There is no need informed consent form due to the study is retrospective.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: Ş.İ.G., E.G., Design: Ş.İ.G., E.G., Data Collection or Processing: Ş.İ.G., E.G., Analysis or Interpretation: Ş.İ.G., E.G., Literature Search: Ş.İ.G., E.G., Writing: Ş.İ.G., E.G.

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Publication: The results of the study were not published in full or in part in form of abstracts.

Contribution: There is not any other contributors who may not be listed as authors.

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Original Article

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Researching Predictive Value of White Blood Cell Rates for Diagnosis of Prostate Cancer in the Patients Undergoing Prostate Biopsy: A Pilot Study

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Abstract

Objective: The aim of this study was to assess the usefulness of neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR) and neutrophil-to-monocyte (NMR) as biomarkers in men who had a prostate-specific antigen (PSA) level of 4 to 10 ng/mL and who subsequently underwent prostate biopsy.

Materials and Methods: We retrospectively analyzed the records of 546 patients who underwent multicore (≥12) TRUS-guided biopsy at our institution between April 2010 and November 2017. Age, PSA level, f/t PSA, NLR, PLR, LMR, NMR, Gleason score in patients with prostate cancer (PCa) and biopsy results were collected. Histological results were categorized into three groups as benign prostatic hyperplasia, prostatitis and PCa.

Results: The median age of patients was 64 years. The mean total PSA level and f/t PSA ratio were 6.52±1.76 and 0.2±0.09, respectively. The mean NLR, LMR, PLR and NMR were 2.46±1.46, 3.94±2.07, 120.69±60.73 and 8.52±7.97, respectively. The f/t PSA ratio in the PCa group was significantly lower compared to the other two groups ($p<0.001$). There was no statistically difference in NLR, LMR, PLR and NMR values ($p=0.293$, $p=0.066$, $p=0.189$ and $p=0.334$, respectively). Multivariate logistic regression analysis showed that age, PLR and f/t PSA were more likely to detect PCa. ($p<0.001$, $p=0.018$ and $p<0.001$, respectively)

Conclusion: Several studies have been published with controversial results trying to specify the predictive value of the ratios of white blood cells in diagnosis of PCa. In this study, univariate and multivariate analyses showed that PLR value would be promising for future studies. Prospective studies are needed to find biomarkers for PCa detection.

Keywords: Prostate biopsy, prostate cancer, neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, platelet-to-lymphocyte ratio, neutrophil-to-monocyte ratio

Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer mortality among men (1). Despite the increasing incidence, PCa-related mortality rate decreases. This can be explained by prostate-specific antigen (PSA) screening and improved biopsy techniques. Serum PSA level is a useful tool for detecting PCa. After detecting elevated PSA levels, transrectal ultrasound (TRUS) guided prostate biopsy is required for the histological diagnosis of PCa. However, non-malignant conditions, especially benign prostatic hyperplasia (BPH) and prostatitis, often cause an increase in serum PSA levels. PSA lacks sufficient sensitivity and specificity for detecting PCa (2). Relevant to this issue, several studies have investigated the usefulness of free/total (f/t) PSA, PSA density, velocity and prostate cancer antigen-3 (PCA-3) for differentiating between benign conditions and PCa, especially in gray-zone patients with a PSA level of 4-10 ng/mL. Simple and inexpensive additional biomarkers with high specificity and sensitivity are needed to prevent unnecessary biopsies and to avoid possible complications of biopsy.

A number of studies have shown that systemic inflammation plays an important role in the development and progression of various cancers (3). Neutrophil-to-lymphocyte ratio (NLR),

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lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR) and neutrophil-to-monocyte (NMR) can be easily calculated from routine complete blood counts (CBC), and they were found to be independent prognostic factors in patients with gastric cancer (4), breast cancer (5) and non-small cell lung cancer (6). NLR is one of the most common markers of inflammation in cancer patients and it was reported to have prognostic value in PCa (7,8). The role of white blood cells rates in diagnosing PCa prior to prostate biopsy was investigated (9,10,11,12,13,14) and controversial results emerged.

In this study, we aimed to assess the usefulness of NLR, LMR, PLR and NMR as a biomarker in men who had PSA levels of 4 to 10 ng/mL and who subsequently underwent prostate biopsy.

Materials and Methods

We retrospectively analyzed the records of 2123 patients who underwent multicore (≥12) TRUS-guided biopsy at our institution between April 2010 and November 2017. Puncture indications were as follows: elevated PSA levels, abnormal digital rectal examinations, or hypoechoic lesions detected by TRUS. In all men, the prostate was routinely biopsied by transrectal route under local anesthesia following preoperative administration of a single dose antibiotic prophylaxis and gastrointestinal system cleaning. Patients with a history of autoimmune or inflammatory disease or symptomatic prostatitis or urinary tract infection or anti-inflammatory drug use were excluded.

We further investigated the records of 984 patients with PSA levels between 4-10 ng/mL. Our study was in accordance with the Helsinki Declaration and it did not require ethics committee permission as it included retrospective data. NLR was calculated by dividing neutrophil count by lymphocyte count, PLR was calculated by dividing platelet count by lymphocyte count, LMR was calculated by dividing lymphocyte count by monocyte count, and NMR was calculated by dividing neutrophil count by monocyte count. For each patient, age, PSA level, f/t PSA, NLR, PLR, LMR, NMR, Gleason score (GS) in patients with diagnosed PCa, and biopsy result were collected. Histological results were categorized into three groups as BPH, prostatitis and PCa.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY). Fisher's exact test and Pearson chi-square analysis were performed for categorical variables. Normality assumptions were checked by Shapiro-Wilk test. The differences between two groups were evaluated by Student's t-test for normally distributed data or Mann-Whitney U test for non-normally distributed data. Kruskal-Wallis test was used for comparison of non-parametric variables between groups and Bonferroni-Dunn test was used as a post-hoc test for significant cases. One-Way ANOVA with post-hoc Tukey HSD test was used for parametric variables. The receiver operating characteristic (ROC) curve analysis was applied to evaluate predictive performance of NLR, LMR, PLR, NMR and f/t PSA on determining PCa and non-PCa patients. Area under the curve (AUC), sensitivity, specificity, negative and positive predictive values (NPV-PPV) were calculated and reported at a %95 confidence interval. Youden's index was calculated to determine the optimal cut-off values. For the assessment of correlations between parameters, Spearman correlation analysis was used. Univariate and multivariate logistic regression analyses were performed to determine the association between study parameters and PCa detection. Data were expressed as n (%), mean ± standard deviation or median (min-max), where appropriate. $P<0.05$ was considered statistically significant.

Results

The study included 984 patients with PSA ranged from 4 to 10 ng/mL. Of these, 318 did not meet the inclusion criterion of available complete blood count results. Besides, 21 patients with autoimmune and inflammatory diseases, and nine patients with a history of anti-inflammatory drug use were excluded. Ninety patients who had high-grade intraepithelial neoplasia or atypical small acinar proliferation in the pathology report were also excluded.

The median age of the 546 men analyzed in the present study was 64 years. The mean total PSA (tPSA) level and f/t PSA ratio were 6.52±1.76 and 0.2±0.09, respectively. Mean NLR, LMR, PLR and NMR were 2.46±1.46, 3.94±2.07, 120.69±60.73 and 8.52±7.97, respectively. Among all patients, PCa was detected in 186 (34.1%) and GS was 6 in 138 patients. There were 360 patients in the benign category. Out of these 360 patients, 300 had BPH and 60 had prostatitis. The characteristics of the patients are summarized in Table 1.

The patients were first classified as BPH, prostatitis and PCa. We found that f/t PSA ratio in the PCa group was significantly lower compared to the other two groups ($p<0.001$). There was no statistically significant difference in NLR, LMR, PLR and NMR values ($p=0.293$, $p=0.066$, $p=0.189$ and $p=0.334$, respectively). When the patients were grouped with regard to having PCa, a statistically significant difference was detected between groups in terms of f/t PSA ratio ($p<0.001$) (Table 2). There was a significant difference in f/t PSA when the cut-off value was taken as 0.15 in routine practice of our clinic. Although it was not statistically significant, the median NLR in PCa group was higher than in non-PCa group ($p=0.681$). Median PLR values with and without cancer were 102.75 (34.5-345.55) and 110 (33.57-833), respectively, and there was no significant difference ($p=0.073$). Multivariate logistic regression analysis showed that age, PLR and f/t PSA were more likely to detect PCa. ($p<0.001$, $p=0.018$ and $p<0.001$, respectively) (Table 3).

ROC analysis was performed to assess the sensitivity and specificity of the study parameters in PCa detection (Table 4). AUC value for f/t PSA was 0.660 (95% CI, 0.619-0.700) ($p<0.001$). Using the Youden index for cut-off point, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were 39.78%, 85.28%, 58.3% and 73.3%, respectively. AUC of NLR, LMR, PLR and NMR values were 0.511, 0.544, 0.547 and 0.538 ($p=0.686$, $p=0.091$, $p=0.070$, $p=0.138$, respectively).

Discussion

PSA is widely used for screening PCa. High PSA level is the most common indication to perform prostate biopsy, which is the only method available to confirm the diagnosis of PCa.

Table 1. Patients' characteristics		
(n=546)		**Mean ± SD / Median (min-max)**
Age		63.7±7.1/64 (45-85)
Pathology, n (%)	BPH	300 (54.9)
	Prostatitis	60 (11)
	PCa	186 (34.1)
PCaGS, n (%)	6	138 (25.3)
	7	35 (6.4)
	≤8	13 (2.4)
	Non-PCa	360 (65.9)
NLR		2.46±1.46/2.06 (0.45-13.37)
LMR		3.94±2.07/3.57 (0.2-23.6)
PLR		120.69±60.73/106.8 (33.57-833)
NMR		8.52±7.97/7.5 (0.21-170)
tPSA		6.52±1.76/6.22 (4-10)
fPSA		1.32±0.67/1.17 (0.25-5.03)
f/t PSA		0.2±0.09/0.19 (0.04-0.56)

BPH: Benign prostatic hyperplasia, PCa: Prostate cancer, PCaGS: Prostate cancer-Gleason score, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte, PSA: Prostate-specific antigen, f/t: Free/total, min: Minimum, max: Maximum, fPSA: Free prostate-specific antigen, tPSA: Total prostate-specific antigen

Table 2. Comparison of study parameters of patients in non-PCa and PCa groups			
	Non-PCa (n=360)	**PCa (n=186)**	**P value**
Age	63.2±7.4	64.7±6.4	**0.018**[1]
NLR	2.06 (0.54-11.3)	2.08 (0.45-13.37)	0.681[2]
LMR	3.53 (0.2-16)	3.61 (0.5-23.6)	0.089[2]
PLR	110 (33.57-833)	102.75 (34.5-345.55)	0.073[2]
NMR	7.49 (0.21-58.8)	7.71 (3.25-170)	0.147[2]
tPSA	6.12 (4-10)	6.44 (4-10)	0.159[2]
fPSA	1.29 (0.25-5.03)	1.01 (0.29-3.27)	<**0.001**[2]
f/t PSA	0.2 (0.04-0.56)	0.16 (0.04-0.4)	<**0.001**[2]
f/t PSA groups			
≤**0.15**	93 (25.8)	93 (50)	<**0,001**[3]
>**0.15**	267 (74.2)	93 (50)	

[1]Student's t test [2]Mann-Whitney U test [3]chi-square test. Data are presented with mean ± SD, n (%) and median (min-max)
NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte, PSA: Prostate-specific antigen, f/t: Free/total, min: Minimum, max: Maximum

However, BPH and prostatitis may also increase PSA levels as PSA lacks sufficient sensitivity and specificity to diagnose PCa. Besides, one out of five men with PCa may be misdiagnosed in the first prostate biopsy (15). Attempts have been made to identify several molecular and biochemical markers that increase the diagnostic accuracy of the prostate biopsy. Nevertheless, no markers were universally accepted due to cost and availability. Cheap and widely used markers are needed to prevent unnecessary biopsies and reduce biopsy-related complications. Prostate health index and multiparametric prostate magnetic resonance imaging, which are frequently used recently, have been used to reduce unnecessary biopsies.

Table 3. Univariate and multivariate analysis to determine associated factors with PCa						
	Univariate			**Multivariate**		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age	1.029	1.004-1.056	**0.025**	1.061	1.031-1.092	<**0.001**
NLR	1.012	0.898-1.142	0.842	1.131	0.923-1.386	0.235
LMR	1.071	0.984-1.165	0.112	1.085	0.926-1.271	0.315
PLR	0.996	0.993-1.000	**0.042**	0.994	0.989-0.999	**0.018**
NMR	1.017	0.988-1.046	0.254	1.005	0.957-1.055	0.851
f/t PSA	0.001	0-0.009	<**0.001**	0.001	0-0.003	<**0.001**

PCa: Prostate cancer, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte, PSA: Prostate-specific antigen, OR: Odds ratio, CI: Confidence interval, f/t: Free/total

Increasing evidence has shown that systemic inflammatory factors are positively associated with various solid cancer types (16,17). The detection of immune response against tumor cells with certain markers is commonly used. The most studied marker, NLR, is related to immune function. NLR can be measured easily and inexpensively. Increased NLR is a poor prognostic factor in several types of cancer (18,19,20). Patients with high NLR have relatively low lymphocyte counts, which is associated with generalized state of immunosuppression. This insufficient immune effect seemed to be associated with the outcome of the patients. Some studies have shown that high NLR has a poor prognostic value in PCa after radical prostatectomy (21,22). Contrary to these studies, Maeda et al. (23) proposed that there was no relationship between NLR and biochemical recurrence after prostatectomy. Tang et al. (24) performed a meta-analysis including 18 studies and revealed that NLR could predict the prognosis for patients with locally advanced or castration-resistant PCa.

The predictive value of inflammation markers in the diagnosis of PCa was investigated (9,10,11,12,13,14). A meta-analysis showed that men with elevated leukocyte count were associated with higher PCa risk (25). However, Fujita et al. (26) concluded that elevated neutrophil count might be good indicator of a benign prostate biopsy. Beside these contradictory results, the predictive values of NLR and PCa detection rates were published with controversial results. Yuksel et al. (12) found that mean NLR values of patients with and without PCa were similar (p=0.944). PLR values of the cases in PCa group were significantly higher compared to the BPH group (p=0.018). Similarly, Gökce et al. (9) revealed that prostatitis prevents the use of NLR in differentiating PCa and benign conditions. Study by Huang et al. (13) demonstrated that NLR had a poor predictive value in entire cohort, but a promising superior predictive value among patients with PSA ranged from 4 to 10 ng/mL. Furthermore, two other studies showed that a higher NLR was significantly associated with PCa detection (10,11). In present study, there were no statistically differences in terms of NLR, LMR, PLR and NMR, (p=0.293, p=0.066, p=0.189 and p=0.334, respectively). Univariate and multivariate logistic regression analyses revealed

Table 4. Sensitivity, Specificity, PPV and NPV for study parameters							
	Cut-off value	AUC (95% CI)	P value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
NLR	≤1.48	0.511 (0.468-0.553)	0.686	23.12 (17.3-29.8)	82.22 (77.9-86.0)	40.2 (30.8-50.1)	67.4 (62.8-71.8)
LMR	>4.28	0.544 (0.501-0.587)	0.091	37.10 (30.1-44.5)	72.22 (67.3-76.8)	40.8 (33.3-48.6)	69.0 (64.0-73.6)
PLR	<104	0.547 (0.504-0.589)	0.070	52.69 (45.3-60.0)	55.56 (50.3-60.8)	38.0 (32.0-44.2)	69.4 (63.8-74.7)
NMR	>5.58	0.538 (0.495-0.580)	0.138	88.17 (82.6-92.4)	21.67 (17.5-26.3)	36.8 (32.3-41.4)	78.0 (68.6-85.7)
f/t PSA	≤0.13	0.660 (0.619-0.700)	**<0.001**	39.78 (32.7-47.2)	85.28 (81.2-88.8)	58.3 (49.2-67.0)	73.3 (68.8-77.4)

Cut-off values were calculated with Youden's index
PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte, PSA: Prostate-specific antigen, CI: Confidence interval, f/t: Free/total

that age, PLR and f/t PSA were associated with PCa detection. Although, PLR did not have a strong predictive value to detect PCa in ROC analysis, univariate and multivariate analyses have shown that PLR value will be promising for the future studies. The only study investigated that NLR and NMRs in the decision for prostate rebiopsy in patients with a previous benign pathology revealed that NLR and NMR values were significantly higher in patients with a diagnosis of PCa after the first negative biopsy (27).

Study Limitations

There were several limitations in our study. First, it was a retrospective cohort study. The second limitation was that the role of other various medical conditions such as smoking, metabolic syndrome, cardiovascular diseases and some other unknown factors that could affect the results was not evaluated in multivariate analyses.

Conclusion

Several studies have been published with controversial results trying to specify the predictive value of ratios of white blood cells in the diagnosis of PCa. Therefore, there might be a bias to select patients. PLR will be promising for the future studies. Large-scale prospective studies are needed to assess the presence of biomarkers to detect PCa.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: K.K., E.İ., S.T., Design: K.K., M.A., M.S., Data Collection or Processing: Y.A., H.A., Analysis or Interpretation: K.K., Y.A., S.T., Literature Search: K.K., Y.A., H.A., Writing: K.K., E.İ.

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Original Article

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A Retrospective Study of Patients with Recurrent or Refractory Testicular Germ Cell Tumors Treated with High-dose Chemotherapy and Autologous Peripheral-blood Stem-cell Transplantation Single-center Experience

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Abstract

Objective: Patients with recurrent metastatic germ cell tumor (GCT) can be treated with second-line or even third-line regimens; 20-30% of testicular GCT (TGCT) relapse or become refractory after first-line therapy and optimal treatment for this group is not very well defined.

Materials and Methods: We presented the analysis of the efficacy of high-dose chemotherapy and peripheral-blood stem-cell transplantation in patients treated between 2016 and 2019. Five patients with five autologous stem-cell transplantations (ASCT) were analyzed retrospectively. All patients were treated with bleomycin, etoposide, cisplatin as first-line therapy and paclitaxel, ifosfamide, cisplatin was given as salvage chemotherapy. Stem–cell collection was performed with granulocyte stimulating factor. ASCT was performed with carboplatin (700 mg/m^2) and etoposide (750 mg/m^2). The results were provided as median (min-max).

Results: After ASCT, all patients were in complete remission (CR). The follow-up after ASCT was 12 months. At the 12-month follow-up, four patients were still alive and in CR, and only one patient died at 6th month after ASCT due to recurrence. Grade 2/4 toxicities were observed in five patients. Only one patient died due to complications of transplantation.

Conclusion: Although the number of the patients in this study was limited, ASCT seemed to be a safe and effective treatment modality in recurrent refractory non-seminomatous TGCT, and treatment-related mortality was very low in this heavily pretreated group.

Keywords: High-dose chemotherapy, autologous stem–cell transplantation, efficacy, germ cell tumors

Introduction

Testicular cancer is one of the most common solid tumors affecting men between the ages of 15 and 40. Testicular germ cell tumors (TGCT) consist of 95% of all testicular cancers (1). The International Germ Cell Cancer Collaborative Group (IGCCCG) classifies patients with metastatic GCT into good, intermediate, and poor risk disease on the basis of specified prognostic criteria (2). According to IGCCCG, the good risk category represents 60%, intermediate risk represents 26%, and poor risk represents 14% of patients with metastatic GCT. The cure rates in treatment with cisplatin-based front-line combination chemotherapy are found to be 90%, 84%, and 51% in good, intermediate, and poor risk disease, respectively (3). After treatment with first-line chemotherapy, more than 80% of patients with good risk and 50%-60% of patients with poor risk have long-term remissions. Patients with relapse after initial chemotherapy can still be treated with salvage chemotherapy, and the most effective regimen for these patients is not clear. The 5-year overall and disease-free survival rates for patients with poor prognosis are 41% and 48%, respectively, after standard-dose chemotherapy. Salvage therapy alternatives have gained importance in this 20-30% of patients who are refractory or have recurrence after the initial chemotherapy. High-dose

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chemotherapy with autologous stem–cell transplantation (ASCT), mostly performed as tandem transplantation, is an alternative in recurrent or refractory cases (4).

The second-line standard-dose chemotherapy options include etoposide plus ifosfamide plus cisplatin (VIP), vinblastine plus ifosfamide plus cisplatin, or paclitaxel plus ifosfamide plus cisplatin (5,6,7). High-dose chemotherapy (HDCT) followed by bone marrow transplantation was first investigated at the University of Indiana in 1986 (8). In previous series of 184 consecutive patients with recurrent metastatic GCT treated with HDCT and peripheral-blood stem-cell transplantation (PBSCT) between 1996 and 2004, long-term disease-free survival was achieved in 70% of patients in the second-line setting, and in 45% of patients who received a third-line or subsequent regimen (9). Memorial Sloan Kettering Cancer Center pioneered another widely used HDCT regimen, which incorporates paclitaxel and ifosfamide as induction chemotherapy and stem-cell mobilization followed by high-dose carboplatin and etoposide with PBSCT for three cycles (10). The high-dose chemotherapy was mainly based on carboplatin and etoposide as high as 1500 mg/m^2 and 1500 mg/m^2 (11). Other conventional-dose combination chemotherapies may also be used as salvage therapies. They are mostly based on ifosfamide and cisplatin with the addition of a third agent. The third agent may be vinblastine, etoposide, or paclitaxel. Combination chemotherapy with gemcitabine, etoposide and ifosfamide is another salvage therapy (11). There are contradictory statements in the literature about the superiority of ASCT over other salvage chemotherapies. While Pico et al. (12) could not demonstrate a survival benefit of addition of ASCT to VIP/VeIP (vinblastine, ifosfamide, cisplatin) chemotherapies, Lorch et al. (13) showed an improvement in overall survival (OS) when ASCT was performed.

In this study, we aimed to evaluate ASCT data in patients with recurrent or refractory non-seminomatous testicular stem cell.

Materials and Methods

Patients who had metastatic GCT that progressed after one or more standard cisplatin-etoposide-based combination chemotherapy regimens were scanned retrospectively. After the approval of the institutional review board 2018/354, we conducted a retrospective analysis of five patients with recurrent/refractory GCT who received HDCT and PBSCT between 2016 and 2019 in our hospital.

Patients ≥18 years of age at the time of ASCT were enrolled in the study. Patient characteristics and laboratory findings including beta-human chorionic gonadotropin (β-HCG), lactate dehydrogenase (LDH) and alpha-fetoprotein (AFP) were documented. Clinical data were retrieved from clinical records of the patients. All patients had metastatic disease and they were classified as poor or intermediate risk group according to the IGCCC (2).

Treatment Protocol

All patients were treated with BEP (bleomycin 30 mg/day at D1, 8, 15, etoposide 100 mg/m^2/day D1-D5, cisplatin 20 mg/m^2/day D1-D5, every 21 days) as first-line therapy for two or more cycles. TIP (paclitaxel 175 mg/m^2/day D1, ifosfamide 1000 mg/m^2/day D1,2,3, mesna 1000 D1,2,3, cisplatin 60 mg/m^2/day D1, every 12 days) regimen was given as salvage second-line chemotherapy for at least two or more cycles in all patients. Peripheral-blood stem–cells were harvested after stimulating the bone marrow using granulocyte colony-stimulating factor (G-CSF). Stem–cell harvesting was performed by subcutaneous injection of G-CSF at 10 micrograms/kg/day started for 5 days and stem cell collection was performed on the 5th day. Only one patient received TIP before harvesting CD 34+ stem–cell, G-CSF at 10 micrograms/kg/day started on the 5th day of the therapy. ASCT consisted of 700 mg/m^2 of carboplatin in combination with 750 mg/m^2 etoposide on days 1-3 (9). Patients were treated with G-CSF after 5th day of transplantation. Patients received bacterial, viral and fungal prophylaxis according to the following regimen: levofloxacin 500 mg orally once a day, acyclovir 400 mg orally twice a day, fluconazole 400 mg orally once a day. Prophylactic antiemetic drugs were also added to the standard therapy in all patients. Platelet and red blood cells were transfused to maintain 10×10^9/L and 6 g/dL levels, respectively. Patients were treated according to neutropenic fever guidelines. Thrombocyte engraftment was defined as thrombocytes more than 20×10^9/L for three consecutive days and neutrophil engraftment was defined as neutrophil number ≥500×10^9/L.

Response Evaluation

The radiologic response to treatment was evaluated with positron emission tomography-computed tomography (PET/CT) before ASCT and two months after ASCT.

Biochemical evaluation was performed with tumor markers, LDH, β-HCG and AFP that were measured after each course of chemotherapy and approximately two months after ASCT.

Responses were classified as complete response (CR) and partial response (PR), and CR was evaluated with PET/CT of the disease together with tumor markers within normal range. PR was defined as PET/CT of the disease with an evidence of response. PR was divided into PR with negative tumor markers (tumor markers within normal range) and PR with positive tumor markers (high tumor marker levels) (14). Progressive disease (PD) was accepted as more than 25% increase in PET/CT measurable mass or more than 10% increase of elevated tumor markers. Stable disease was classified as a response that did not fit the criteria of PR or PD (15).

Intoxications were evaluated according to World Health Organization criteria.

Results

Patient and Disease Characteristics

We retrospectively analyzed five ASCT in five patients with refractory or recurrent non-seminomatous TGCT. The median age at diagnosis was 36 years (range, 29-58 years). In one patient, tandem transplantation was performed. In four patients, one cycle of ASCT was performed. According to the IGCCC, five patients were classified as intermediate and poor risk group. LDH levels at diagnosis were above the normal limits. Also, AFP and beta-HCG were normal or high at diagnosis. All patients

were at advanced stage with lymph node metastasis and organ metastasis including lung and liver. Five patients had remission, PR, or CR after three or more cycles of BEP. The characteristics are shown in Table 1. TIP regimen was administered as first-line salvage to all patients before ASCT. The median line of chemotherapy before ASCT was seven (range, 5-11). Four patients were transplanted as first salvage therapy and one patient was treated with ASCT as tandem transplantation.

Three patients were treated with radiotherapy before ASCT. Radiotherapy was performed due to pulmonary and/or lymph node metastasis in these patients. The median time to ASCT was 8 months (range, 7-12 months). In four patients, remission (PR or CR) was achieved before ASCT. Only one patient was refractory before ASCT. In the PR group, all patients had normal levels of AFP and beta-HCG.

Table 1. The Characteristics of the patients	
	n %
Age (median, range)	36 (29-58)
IGCCC	
Intermediate	2 (40)
Poor	3 (60)
At The diagnosis (level-median range)	
ß-HCG	4 (80)
AFP	3 (60)
LDH	3 (60)
Lymph node metastasis	5
Organ metastasis	
Lung	2 (40)
Brain	None
Liver	1(20)
Bone	None
Multiple organ	3 (60)
Remission after first line	
PR	4 (80)
CR	1 (20)
Refractory	
Number of chemotherapy linesbefore ASCT (median, range)	7 (5-11)
Response before ASCT	
PR	2 (40)
CR	2 (40)
Refractory	1 (20)
Before ASCT (level-median range)	
Beta HCG	1 (20)
LDH	All patients level were in normal range
AFP	1 (20)
IGCCC: International Germ Cell Consenus Classification, ß-HCG: Beta-human-chorionic gonadotropin, AFP: Alpha Fetoprotein, LDH: Lactate Dehydrogenase, PR: Partial response, CR: Complete response, ASCT: Autologous stem cell transplantation	

The median number of stem cells collected per patient was 5.5×10^6/kg (range, 4.2-8.11×10^6/kg).

Safety and Efficacy

Neutropenic fever episodes were observed in all patients during transplantation procedures, and they were treated according to neutropenic fever guidelines. Only one patient experienced Grade 4 mucositis that required total parenteral nutrition. Table 2 summarizes the toxicities. Transplantation-related mortality was observed in one patient. He died due to uncontrolled infection, sepsis and multiorgan failure during the neutropenic period.

The median number of transfused thrombocyte apheresis and red blood cell was 1 (range, 1-5) and 1 (range, 1-5), respectively. Thrombocyte and neutrophil engraftments were observed at a median of 11 day (range, 10-20) and 10 day (range, 9-20), respectively. Engraftment failure was not documented. During the median 12-month follow-up period, we did not observe any secondary malignancy. After ASCT, all patients were in CR.

Discussion

In the last decade, there have been several reports published on the use of HDCT with ASCT in recurrent/refractory GCTs. These reports are consistent in providing information about the

Table 2. Toxicities associated with autologous stem cell transplantation (n=5)	
Toxicity	**n**
Neutropenia fever (yes/no)	5
Apheresis thrombocyte transfusion (median, range)	1 (1-5)
Erythrocyte transfusion (median, range)	1 (1-5)
Mucositis	
Grade 1/2	4
Grade 3/4	1
Diarrhea	
Grade 1/2	5
Grade 3/4	None
Neuropathy Grade 1	None
Hearing loss Grade 1	5
Requirement for TPN 1	
Requirement for oral nutrition solutions	4
Requirement for intensıve care unit	1
Toxic hepatitis	
Grade 1/2	None
Grade 3/4	None
Renal toxicity	
Grade 1/2	None
Grade 3/4	None
TPN: Total parenteral nutrition	

superiority of HDCT compared to conventional chemotherapy. However, reports have great variability in patient selection, prior treatments, selection of conditioning regimen and variability of the doses within the same regimen. In addition, some reports in the literature describe the effectiveness of a single HDCT cycle, while others use a tandem transplant strategy (9,16,17,18).

In the literature, most of the studies consisted of heterogeneous patient groups with poor or good prognostic factors or including both seminomatous and non-seminomatous (4,15,19,20). In this regard, our study group was relatively homogenous, consisting of only recurrent or refractory primary non-seminomatous TGCTs. We aimed to analyze the efficacy and safety of ASCT in this patient group. Nowadays, cisplatin-based combination chemotherapy will cure 83% of patients with metastatic GCT (21). Ninety percent of patients with IGCCCG good risk disease will achieve cure with primary treatment chemotherapy. Patients with intermediate and poor risk disease have less favorable outcomes and a significant proportion will relapse and require recovery therapy. ASCT can be considered as a relatively safe procedure with only one death related to transplantation (20%).

The most common non-hematologic side effects were mucositis and diarrhea. While prophylactic antiemetic therapy was given in all patients, Grade 3-4 nausea or vomiting was not documented. Among hematologic adverse events, the most common one was neutropenia. In a retrospective study consisting of 364 recurrent metastatic GCT patients treated with ASCT, the treatment-related mortality was found to be 2.4% (nine patients). Infection was the most common cause of treatment-related mortality (19). In another prospective study, the treatment-related mortality was 5.5% in primarily treated patients and 8.3% in recurrent group (4). The vast majority of patients had oropharyngeal mucositis, diarrhea and febrile neutropenia as non-hematologic toxicities, as in our results (4).

In the long-term follow-up, one of the most important adverse side effects were secondary malignancies. There are conflicting data in the literature about secondary malignancies, including acute leukemia. Adra et al. (19) reported that five patients developed secondary leukemia within a range of 17–120 months after transplantation, but no acute leukemia was reported in another study (4). Also, solid tumors were reported in the literature after ASCT (19). In our study, , no secondary malignancy or leukemia was observed during a median follow-up of 12 months.

In a retrospective study of patients with poor or intermediate prognostic factors according to the IGCCC including seminomatous and non-seminomatous subtypes, CR, PR and RD rates were 50%, 36%, and 14%, respectively (19). In another study in 2003, lower response rates and higher mortality rates were reported (21). Yilmaz et al. (22) reported that CR and PR rates were 47.3% and 31.5%, respectively, after ASCT. The median OS and progression-free survival (PFS) were 18 (range, 0-37.4 months) and 7 (range, 0-15months) months, respectively. The estimated 2-year OS was 47.4% and PFS was 35.3%. Although the number of patients (19 patients) in this study was limited, they accepted that ASCT was a safe and effective treatment modality in recurrent and refractory non-seminomatous TGCT with an acceptable OS, PFS and mortality rates.

A wide range of OS (30-66%) and PFS (25-50%) rates were reported in the literature (23,24,25). In a study by Rick et al. (23), TIP chemotherapy followed by one cycle of high-dose carboplatin and etoposide with stem cell transplantation was evaluated. The 3-year survival rates were 30% for OS and 25% for event-free survival. Our estimated rates at 1-year seemed to be similar to the estimated rates at 3-years in this analysis. In a retrospective analysis of 364 patients, 2-year PFS and OS were 60% and 66%, respectively. We think that such changes in these rates may be related to several factors. The first reason is that most of the patients (n=303) in this study were transplanted as a first salvage therapy. Only six of 364 (1.6%) patients were heavily treated. However, in our study, all patients were heavily treated with equal or more than three or more lines of chemotherapy. The second reason is that the cohort of 364 patients consisted of both seminoma and non-seminoma patients, but we only enrolled nonseminoma patients. Finally, the third reason is that 151 of 364 patients were classified as good prognosis according to the IGCCCG. In our study, we only included patients with intermediate and poor prognostic factors.

Several causes have been investigated as possible risk factors affecting PFS and OS. We think that high beta HCG, AFP levels, response of the patient before and after ASCT, LDH levels before ASCT negatively affected the response of the patients. However, in the multivariate analysis, only high LDH levels were associated with poor OS rates. High serum beta HCG levels and AFP levels, initial IGCCCG risk and the time of ASCT (second vs third or later) were possible variables, but it could not be confirmed with other study data (26). We could not demonstrate the statistical difference of variables in multivariate analysis possibly due to small sample size.

There is also conflicting data in the literature about the exact time of the ASCT in GCT. It can be performed as a first-line, second-line therapy or it can be an alternative therapy in heavily treated refractory patients. Although high-survival rates (more than 70%) were documented in the literature when ASCT was performed as first-line therapy in patients with poor prognostic factors (14), the role of ASCT as a first-line treatment in patients with poor prognostic markers is not clear. The International Prognostic Factors Study Group offered data of 1594 patients with GCT, who have progressed after at least three cycles of cisplatin-based chemotherapy. Patients were treated with standard dose or ASCT as first salvage therapy. 2-year PFS (49.6% vs 27.8%; $p<0.001$) and 5-year OS (53.2% vs 40.8%; $p<0.001$) were significantly longer in the ASCT group than in the standard-dose group (26). However, the exact time and the number of ASCT should be determined with Phase III large cohort prospective studies.

Study Limitations

There are some limitations in our study such as small number of patients and retrospective nature of the study. There was also no control group as the study was a retrospective study.

Conclusion

GCTs have an excellent prognosis with platinum-based therapy in recurrent or refractory patients. There are many reports in the literature on the use of HDCT and ASCT in improving the outcome in patients with relapsed GCTS or platinum-refractory disease and patients with poor prognostic features. However, reports have great variability in patient selection, prior treatments, selection of the conditioning regimen and variability of the doses within the same regimen.

In conclusion, HDCT followed by PBSCT is a safe and effective treatment modality in recurrent/refractory non-seminomatous TGCT. Patients with platinum-refractory or recurrent disease and patients with poor prognostic features are primary candidates for HDCT. Treatment with high-dose carboplatin and etoposide was associated with low treatment-related mortality. Nevertheless, final results of ongoing phase III randomized trials are needed to define the role of HDCT as a part of initial treatment of extragonadal GCT with poor prognosis.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Dr. Sadi Konuk Training and Research Hospital Research Committee (no: 2018/354).

Informed Consent: All patients were informed verbally and in writing, and gave written informed consent before the procedure.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ş.İ.G., E.G., Design: Ş.İ.G., E.G., Data Collection or Processing: Ş.İ.G., E.G., Analysis or Interpretation: Ş.İ.G., E.G., Literature Search: Ş.İ.G., E.G., Writing: Ş.İ.G., E.G.

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Original Article

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Prognostic Value of the mRNA Expression of Members of the Toll-like Receptor Family in Clear Cell Renal Cell Carcinoma

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Abstract

Objective: The aim of this study was to examine *TLRs* expression in tumoral and non-tumoral kidney tissue in patients with clear cell renal cell carcinoma (ccRCC) and to evaluate the prognostic significance of *TLRs* expression profile in ccRCC.

Materials and Methods: *TLR* 1-10 mRNA expressions were measured by real-time polymerase chain reaction (RT-PCR) in formalin-fixed paraffin-embedded (FFPE) 23 ccRCC tumoral tissue samples and 23 non-tumoral kidney tissue samples.

Results: A total of 46 individuals were included in the study. None of the patients had rhabdoid or sarcomatoid features. Lymphovascular invasion was observed in only three patients. RT-PCR analyses revealed *TLRs* mRNA expressions in 23 ccRCC samples and 23 non-tumoral FFPE kidney tissue samples. TLR (*TLR1*-10) mRNA expression was significantly increased in FFPE ccRCC tissues according to RT-PCR results ($p<0.05$).

Conclusion: The results demonstrated that *TLRs* might have function in ccRCC pathogenesis. This present study will shed light on research to understand the role of the *TLR* gene family expression in tumor progression of ccRCC.

Keywords: RCC, *TLR*, mRNA expression

Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer worldwide with an increasing incidence. During the diagnosis process, one third of the patients have metastasized and half of the remaining patients will experience a recurrence after treatment (1). Clear cell RCC (ccRCC) is the most frequent pathological subtype, representing approximately 70% of RCC cases (2). ccRCC has a poor prognosis with low response rates to conventional therapies such as chemotherapy (3).

Studies have shown that the most important genetic alteration in ccRCC is the loss of function of *von Hippel-Lindau tumor suppressor* (*VHL)* gene. In 90% of sporadic ccRCC, one copy of *VHL* is mutated, while another copy is lost through 3p deletions (4). According to The Cancer Genome Atlas, ccRCC is characterized by recurrent mutations in *PI3K/AKT/MTOR* (5). Tumor microenvironment has an important role in many processes observed in tumor progression, such as immune-escaping, chemotherapy resistance and metastasis. Recently, studies related to genetic changes in Toll-like receptors (*TLRs*) that recognize danger-associated molecular patterns derived from cancer cells in tumor microenvironment are increasing rapidly.

TLRs are a conserved family of receptors capable of recognizing pathogenic structures known as pathogen-associated molecular patterns (6). Until today, 13 *TLR* analogues have been identified in mammals, *TLR*11, 12 and 13 are not expressed in humans but are functional only in mice (7). They are located on the cell surface or on endosomes within the cell. Although endosomal *TLRs* primarily detect viral and bacterial nucleic acids, surface *TLRs* such as *TLR*2 and *TLR*4 primarily recognize bacterial proteins (8). *TLRs* are mainly expressed in immune system cells such as macrophages and DCs, and are key sensors of pathogen invasion (9). Recent data suggest that functional *TLRs* are

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expressed not only in immune system cells but also in cancer cells (10). Damage-associated molecular patterns derived from damaged normal epithelial cells and necrotic cancer cells are found in the tumor microenvironment, and these patterns are thought to stimulate chronic inflammation by inducing specific *TLR*s (11,12). However, the expression patterns of *TLR*s in human cancer tissues are largely unknown. To our knowledge, there is no previous study of *TLR*s (*TLR*1-10) mRNA expression in ccRCC.

Thus, the aim of this study was to investigate *TLR*1-10 expression in non-tumoral kidney tissue and tumoral tissue in patients with RCCs and to evaluate the prognostic significance of *TLR*s expression profile in ccRCCs.

Materıals and Methods

Study Samples

Twenty-three tumoral ccRCC and 23 non-tumoral kidney tissue nephrectomy specimens were provided by İstanbul Gaziosmanpaşa Hospital. Patients were histopathologically diagnosed as having ccRCC at our hospital between 2007 and 2017. Cases with cystic RCC were excluded from our study since this subtype of RCC is composed of hypocellular tumor areas.

All patients were staged based on the Union for International Cancer Control Tumor-Node-Metastasis classification. The retrospective study design was approved by the Institutional Review Board (2017-KAEK-189_2018.10.10_02).

Tumor Selection

The histopathological slides stained with Hematoxylin-Eosin (H&E) were microscopically examined to select paraffin embedded blocks with preserved, viable tumor tissue comprising over 90% of the block. The tumor area was marked and cut. Areas containing necrosis and hemorrhage were excluded from the study. Two pieces of 10-µm-thick sections were cut from each selected paraffin block.

RNA Extraction and cDNA Synthesis

Total RNA from 10 µm Formalin-Fixed Paraffin-embedded (FFPE) sections was isolated using High Pure FFPET RNA isolation kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Total RNA concentrations were measured and 1 µg RNA was used as a template for the synthesis of complementary DNA (cDNA) using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). The cDNAs were stored at -80°C until used as a template in real-time quantitative PCR.

Real-time Quantitative PCR

Real-time PCR analyses were performed using quantitation of *TLR*1-10 genes and an internal reference gene (*β-actin*) at mRNA level using the LightCycler 480 platform (Roche Diagnostics). PCR primers and Universal Probe Library probes for *TLR*1-10 and internal reference gene (*β-actin*) are presented in Table 1. The final reaction volume for the analysis of *TLR*s expression was 20 µ; 1 µL from each primer and probe set, 4 µ of ×5 LightCycler TaqMan Master Mix, 2 µ cDNA sample, and 13 µ PCRgrade water. The cycle conditions were 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for one second. All runs included one negative cDNA control consisting of DNase- and RNase-free water. The housekeeping β-actin gene was used as a control to normalize expression of each gene and the final results were obtained with LightCycler 480 software.

Statistical Analysis

SPSS 18 package program was used for statistical analysis. Non-parametric statistical methods were used to determine the differences between the groups. Mann-Whitney U test was used for variables with two groups and Kruskal-Wallis H test was used when the number of groups was more than two. Values were expressed as mean ± standard deviation. $p< 0.05$ was considered statistically significant.

Table 1. Primers and UPL probes used for real-time gene expression analysis (5′®3′)

Primer sequences	UPL number
TLR1	
CCTAGCAGTTATCACAAGCTCAAA (Forward) CCTTGGGCCATTCCAAATA (Reverse)	#79 (04689020001)
TLR2	
GGCCAGCAAATTACCTGTGTG (Forward) AGGATCAGCAGGAACAGAGC (Reverse)	#56 (04688538001)
TLR3	
GTGGCCCTTAAAAATGTGGA (Forward) GTGTTTCCAGAGCCGTGCTAA (Reverse)	#151 (04694376001)
TLR4	
TCATTGTCCTGCAGAAGGTG (Forward) TCC CAC TCC AGG TAA GTG TT (Reverse)	#62 (04688619001)
TLR5	
TGAGGGACTTTCTCATCTTCAAGT (Forward) CCTTAATGCAGTCAGATGGCTA (Reverse)	#31 (04687647001)
TLR6	
TTTGGATTTATCTCATAATCAGTTGC (Forward) GATCTAAATGCCTGAAACTCACAA (Reverse)	#121 (04693558001)
TLR7	
GTCTAAAGAACCTGGAAACTTTGG (Forward) TCTCAGGGACAGTGGTCAGTT (Reverse)	#102 (04692209001)
TLR8	
CAGAATAGCAGGCGTAACACATCA (Forward) TGTTGTCATCATCATTCCACAA (Reverse)	#59 (04688562001)
TLR9	
CTGGGACCTCTGGTACTGCT (Forward) CTGCGTTTTGTCGAAGACCA (Reverse)	#98 (04692152001)
TLR10	
TGTCACCATTGTGGTTATTATGC (Forward) GCAGATCAAAGTGGAGACAGC (Reverse)	#76 (04688996001)
β-actin	
ATTGGCAATGAGCGGTTC (Forward) CGTGGATGCCACAGGACT (Reverse)	#11 (04685105001)
UPL: Universal ProbeLibrary	

Results

A total of 46 individuals were included in the study. The mean age of the ccRCC group (six female and 17 male) and the control group (nine female and 14 male) was 58.4±7.5 years (range, 48-72 years) and 56.3±6.9 years (range, 45-70 years), respectively. None of the patients had rhabdoid/sarcomatoid features. Lymphovascular invasion was observed in only three patients. The tumor characteristics are summarized in Table 2.

Table 2. Tumor characteristics of ccRCC patients

Characteristics	Number of patients (%)
Gender	
Female	6 (26)
Male	17 (74)
Affected side	
Right	12 (52)
Left	11 (48)
Pathological grade	
Grade I Grade II Grade III Grade IV	2 (8) 7 (30) 13 (56) 1 (6)
pT stage	
pT1a	7 (30.4)
pT1b	8 (34.7)
pT2a	1 (4.5)
pT3a	7 (30.4)
Tumor size	
<5	8 (34.7)
≥5	15 (65.3)
LN involvement	
Nx	5 (21.7)
N0 N1	17 (74) 1 (4.3)
Capsular infiltration	
Negative	15 (65.3)
Positive	8 (34.7)
Lymphovascular infiltration	
Negative	20 (87)
Positive	3 (14)
Perirenal infiltration	
Negative	19 (82.6)
Positive	4 (17.4)
Renal sinus infiltration	
Negative	19 (82.6)
Positive	4 (17.4)
Necrosis	
Negative	16 (69.5)
Positive	7 (30.5)

ccRCC: clear cell renal cell carcinoma, LN: Lymph node

TLR (*TLR*1-10) mRNA expression was significantly increased in FFPE ccRCC tissues according to real time PCR results ($p<0.05$) (Figure 1). There was no significant relationship between *TLR* mRNA expression and tumor localization (right vs left kidney), tumor size, pT-class, capsular invasion, renal sinus invasion and necrosis ($p>0.05$). *TLR*5 overexpression in ccRCC tissue samples showed a significant association with tumor grade III ($p=0.028$). In addition, a negative correlation was found between *TLR*1-4-7-9 expression and perirenal invasion, respectively ($p=0.023$, $p=0.041$, $p=0.041$, $p=0.031$). *TLR2* overexpression in ccRCC tissue samples showed a significant association with Nx category ($p=0.044$).

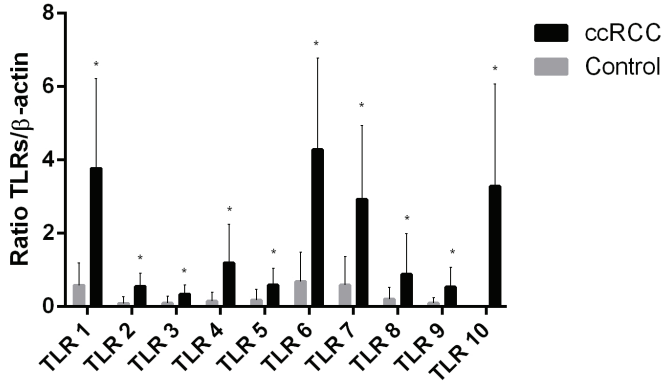

Figure 1. mRNA expression level of TLRs in FFPE kidney samples. All TLR mRNA expression was followed by real-time PCR and the results were normalized to β-actin mRNA. Values were expressed as mean ± standard deviation. Asterisks (*) denote significant differences ($p<0.05$)

ccRCC: Clear cell renal cell carcinoma

Discussion

It is believed that *TLR*s play important roles in innate immunity; and chronic inflammation is one of the vital events in carcinogenesis. *TLR*s are expressed in macrophages, natural killer cells (NK), dendritic cells (DCs) and T cells. Today, it is known that *TLR*s are also expressed in cancer cells (13). For this reason, it is considered that *TLR* gene expression profiles may be important markers in cancer development and progression.

Here we demonstrated for the first time that *TLR*1-10 mRNA is frequently expressed in FFPE kidney tissues in patients with ccRCCs. The expression of *TLR* 1-10 mRNA was significantly increased in RCC patients compared to the control group. There were differences in *TLR* expression in many cancers (14). *TLR* expression studies in ccRCC are quite limited in the literature. In the current study, *TLR*5 overexpression in ccRCC tissue samples showed a significant association with tumor grade III. Similarly, it was suggested that *TLR*5 expression had become more diffuse during the progression to dysplasia (15). Further studies are needed to clarify the true role of *TLR*5 expression in ccRCC.

Immunogenic processes are effective in the pathogenesis of RCC and RCC may affect different effector cells of the natural and adaptive immune system including NK cells, DCs and various T cells (16). Failure in desired full response to

target-specific therapies has revived the interest in immune modulation in RCC treatment. The importance of the role of the immune response to RCC was understood when it was shown that metastatic lesions might regress spontaneously (17). Additionally, it was confirmed that there was a complex interaction between the tumor and host immune response with demonstration of increase in cytokines (18) and chemokines (19) as well as tumor-infiltrating lymphocytes (20) in circulation in patients with RCC.

Low levels of oxygen in the cellular environment occur in many pathophysiological conditions such as infection, inflammation, and solid tumor development (21). The relation of tumor microenvironment with RCC development can be explained especially with the production of proangiogenic factors, which end up with the hyperactivation of Hypoxia-Inducible Factor 1 (*HIF-1*) in lesions with VHL mutations (22). In one study, it was reported that *TLR*2 and *TLR*6 expressions were increased in hypoxia (23). Morikawa et al. showed that *TLR*3 expression was increased in ccRCC patients compared to the control group (24). For this reason, probably, some cytokines produced by cancer cells or by infiltrating immune cells may induce the *TLR*3 expression in ccRCCs.

TLR expression profiles have been investigated in many types of cancer. Some studies have shown that TLRs inhibit tumor growth, while others have indicated that they enhance tumor progression. In a study, Bednarczyk found that three proteins namely Dual specificity protein phosphatase 2, Interferon gamma and Eukaryotic initiation factor 4A-I (DUSP2, IFNγ, EIF4A1) were associated with TLR system, which differentiate early stages of colorectal cancer from healthy tissue (25). TLRs also play a critical role in the induction of colitis, which in consequence can lead to cancer. One study reported that chronic stress could increase the expression of TLR in the colonic mucosa (26). Furthermore, ovarian cancer cells showed overexpression of TLR2, TLR3, TLR4, and TLR5 (27,28), while there was a high expression of TLR5 and TLR9 in human cervical cancer (29). The results underline the role of pathways associated with TLR activation in the pathogenesis of several cancers.

In line with these studies, *TLR*3 mRNA expression was found to be higher in ccRCC patients compared to the control group in our study. In a study that was conducted with the immunohistochemical technique by Wang et al.(30), it was reported that *TLR*4 expression was increased in RCC tissues compared to neighboring normal tissues. In another study that was conducted on RCC FFPE tissues, it was shown that *TLR*9 was associated with good prognosis and that low *TLR*9 expression was associated with short-term survival (1). In the present study, *TLR*1-4-7-9 expressions were increased in patients with no perirenal invasion, which is an aggressive clinicopathological parameter for ccRCC.

Study Limitations

The main limitation of our study was the small number of patients. In addition to the expression of mRNA, it would be appropriate to show protein expression in these tissues.

Conclusion

As a result, *TLR* mRNA expressions were significantly increased in ccRCC FFPE tissues compared to non-tumoral tissue samples. It is important to elucidate the potential mechanisms underlying the formation and progression of ccRCC to facilitate the identification of new prognostic markers and development of promising targeted strategies. For this reason, determining *TLR* protein expressions as well as mRNA expression and comparing these data with clinicopathological data in more patients will reveal the role of the changes in expression of *TLR* genes in ccRCC pathogenesis.

Ethics

Ethics Committee Approval: The retrospective study design was approved by the Institutional Review Board (no: 2017-KAEK-189_2018.10.10_02).

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: S.S.Ö., P.H., S.Y., Design: S.S.Ö., Data Collection or Processing: S.S.Ö., P.H., Analysis or Interpretation: S.S.Ö., P.H., S.Y., Literature Search: S.S.Ö., Writing: S.S.Ö.

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Original Article

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Comparison of Robotic and Laparoscopic Partial Nephrectomy in Robotic Surgery Era

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Abstract

Objective: To compare the results of robotic partial nephrectomy (RPN) and laparoscopic partial nephrectomy (LPN) operations and to determine whether they have any superiority to each other in terms of oncological and functional outcomes.

Materials and Methods: The data of patients who underwent partial nephrectomy due to renal tumor in our clinic were evaluated retrospectively. The data included demographic information, operative technique, tumor size, operative time, duration of warm ischemia, amount of intraoperative bleeding, length of hospital stay and complications.

Results: A total of 60 patients were included in the study. There was no significant difference between age (52.5±13.3 years vs 50.1±12.4 years, p=0.48), body mass index (26.9±3.7 vs 27.3±3.3 kg/m^2, p=0.69) and tumor size (3.2±1.4 cm vs 3.4±1.2 cm, p=0.79) of patients who underwent LPN and RPN. The mean operative time (194.5±44.6 min vs 203.3±22.2 min, p<0.001) and length of hospitalization (5±1.4 days vs 6.2±2.1 days, p=0.009) were significantly shorter in the RPN group. Although intraoperative bleeding was less in RPN patients, it was not statistically significant (p=0.065). Similarly, the duration of warm ischemia was lower in RPN patients than in LPN patients, but it was not statistically significant (14.3±7.8 min vs 16.3±7.3 min, p=0.298).

Conclusion: RPN and LPN, which are minimally invasive surgical treatment options, can be used safely in the treatment of kidney tumors. RPN can be recommended to patients for early return to daily life.

Keywords: Partial nephrectomy, renal tumor, robotic surgery, laparoscopic surgery

Introduction

Nowadays, the development of radiological imaging methods and increasing accessibility have increased the incidence of incidentally detected renal masses. The majority of the detected masses are T1 tumors smaller than 7 cm and if technically feasible, it is recommended to remove only the mass by partial nephrectomy (1). Partial nephrectomy is traditionally performed with open technique, and can also be performed successfully with minimally invasive laparoscopic and robotic techniques. Minimally invasive techniques have been reported to offer shorter hospital stay, less blood loss and lower complication rates (2). Some studies have shown that robotic partial nephrectomy (RPN) reduces ischemia and suturing time even for an experienced laparoscopic surgeon.

In this study, we aimed to compare the results of RPN and laparoscopic partial nephrectomy (LPN) operations in our clinic and to determine whether they have superiority in terms of oncologic and functional aspects.

Materials and Methods

After obtaining the approval of the Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (no: 2019/217), the data of patients who underwent partial nephrectomy due to renal tumor in the urology clinic of Bakırköy Dr. Sadi Konuk Training and Research Hospital between January 2015 and February 2019 were evaluated retrospectively. In patients undergoing LPN and RPN, renal hilar vascular control was performed by clamping the renal artery and vein separately. The operative time in RPN did not include the robot docking time.

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The data analyzed included demographic information, pre- and post-operative glomerular filtration rate (GFR) values, operative technique, tumor size, R.E.N.A.L. nephrometry score, operative time, duration of warm ischemia, amount of intraoperative bleeding, length of hospital stay and complications. Patients who were operated for benign pathologies and patients under 18 years of age were excluded from the study. The patients were divided into two groups as LPN group and RPN group. GFR was calculated using MDRD (Modification of diet in renal diseases study) formula=186x(Creatinine)$^{-1.154}$x(Age)$^{-0.203}$x(0.742 if female) x (1.210 if black).

Statistical Analysis

IBM SPSS v.21 (Chicago, IL, USA) was used for statistical analysis of the data. Continuous variables were given as mean ± standard deviation, while categorical variables were given as numbers and percentages. Kolmogorov-Smirnov test was used to test the normality of data. For pairwise comparison, Student's t-test and Mann-Whitney U test were used for numerical data and chi-square test was used for categorical variables. P<0.05 was considered statistically significant.

Results

A total of 60 patients (38 male, 22 female) were included in the study. The mean age of the patients was 51.3±12.8 years. The mean tumor size was 3.3±1.3 cm. There were 30 consecutive patients whose data were available for LPN and RPN groups (Table 1). There was no significant difference between mean age (52.5±13.3 years vs 50.1±12.4 years, p=0.48), body mass index (26.9±3.7 vs 27.3±3.3 kg/m^2, p=0.69) and tumor size (3.2±1.4 cm vs 3.4±1.2 cm, p=0.79) of patients who underwent LPN and RPN. The mean R.E.N.A.L. score of both groups were similar (p=0.642). ASA scores were similar for both groups (p=0.254). The mean operative time (194.5±44.6 min vs 230.3±22.2 min, p<0.001) and length of hospital stay (5±1.4 days vs 6.2±2.1 days, p=0.009) were significantly shorter in the RPN group. Although the amount of intraoperative bleeding was less in RPN patients, it was not statistically significant (206±94.4 mL vs 246.8±72.2 mL, p=0.065). Similarly, the duration of warm ischemia was lower in RPN patients than in LPN patients but it did not reach statistical significance (14.3±7.8 min vs 16.3±7.3 min, p=0.298). Post-operative complication rates and characteristics were similar in LPN and RPN groups according

Table 1. Comparison of the robotic and laparoscopic partial nephrectomy groups

Variance		Laparoscopic PN (n=30)	Robotic PN (n=30)	P value
Age (year)		52.5±13.3	50.1±12.4	0.48
Gender	Female	13	9	0.42
	Male	17	21	
Side	Right	17	18	1.00
	Left	13	12	
ASA score	1	8	7	0.25
	2	16	21	
	3	6	2	
Body mass index (kg/m^2)	-	26.9±3.7	27.3±3.3	0.69
Tumor size (cm)	-	3.2±1.4	3.4±1.2	0.57
RENAL score	-	5.5±1.6	5.7±1.6	0.64
Pre-operative GFR	-	110.1±26.4	111.4±22.7	0.84
Duration of the operation (min)	-	230.3±22.2	194.5±44.6	<0.001
Intraoperative bleeding (mL)	-	246.8±72.2	206±94.4	0.06
Warm ischemia time (min)	-	16.3±7.3	14.3±7.8	0.29
Post-operative GFR	-	75.3±21.4	94.5±35.7	0.01
Surgical margin	Positive	1	1	0.75
	Negative	29	29	
Absolute GFR exchange	-	-34.8±33.5	-16.9±29.6	0.29
Percentage of GFR exchange (%)	-	-25.3±34.8	-15±24	0.31
Post-operative complication	Clavien-Dindo=1	20	24	0.382
	Clavien-Dindo=2	10	6	
Duration of stay in the hospital (day)		6.2±2.1	5±1.4	0.009

PN: Partial nephrectomy, GFR: Glomerular filtration rate, ASA: American Society of Anaesthesiologists

to Clavien-Dindo classification (p=0.382). Surgical margin positivity was detected in one patient (3.3%) in both patient groups. Patients with positive surgical margins were followed up conservatively and no recurrence was observed at a mean follow-up of 18 months. The decrease in GFR was higher in the LPN group in both units and percent (p=0.029 and p=0.031, respectively).

Discussion

Partial nephrectomy is the gold standard in the treatment of renal tumors, especially those smaller than 4 cm (3). However, it is recommended to remove larger renal masses by partial nephrectomy when technically possible. Over the years, partial nephrectomy has evolved from open to minimally invasive laparoscopic and finally to robotic techniques. In many different studies, RPN has been shown to be superior to LPN in various aspects (4). In this study, we demonstrated that RPN is superior to LPN in some aspects, but they are similar in terms of oncologic outcomes and preservation of renal function.

In a multicenter prospective study by Alimi et al. (5), short-term oncologic and functional results of RPN and LPN were found to be similar. According to this study, LPN was associated with longer warm ischemia (23 min vs 15.7 min) and longer hospital stay (4.6 days vs 3.6 days), whereas intraoperative blood loss was higher in RPN (381 mL vs 215 mL). Perioperative complications and positive surgical margin rates were reported to be similar in RPN (2%) and LPN (6%) groups (5). In another study by matching the patients according to nephrometry scores, RPN operative time was found to be shorter than LPN in all nephrometry scores, whereas ischemia time and hospital stay were lower in nephrometry scores greater than 7 (2). In another study conducted by Faria et al. (6), RPN was found to be superior to LPN in terms of renal function, warm ischemia time, suture time, renorrhaphy time ($p<0.05$). In another study by Kim et al. (7), similarly, operative and warm ischemia times were found to be shorter in RPN patients than in LPN. In addition, recovery of renal function after partial nephrectomy has been reported to be better in RPN patients. In our study, RPN was superior to LPN in terms of operative time and hospital stay. Although the amount of bleeding and warm ischemia time was lower in RPN patients, no statistical significance was observed. However, absolute and percent GFR reduction was significantly higher in LPN patients than in RPN patients. This difference may be related to the amount of bleeding or ischemia time.

In a multicenter study by Zargar et al. (8), RPN and LPN were compared in terms of trifecta (negative surgical margin, zero perioperative complications, and duration of warm ischemia less than 25 min). In the study, 1185 RPN and 646 LPN patients were included, and the rate of achieving trifecta was 70% in RPN and 33% in LPN. In a meta-analysis by Choi et al. (3) comparing RPN to LPN, no difference was found between the two types of operations in terms of complication rates, serum creatinine change, operative time, estimated blood loss and surgical margin positivity according to Clavien-Dindo classification. However, less switching to open surgery or radical surgery has been reported in RPN. In addition, the duration of warm ischemia and hospitalization were lower in RPN patients. The surgical margin positivity and blood loss data in our study were also in line with the mentioned studies. There was no significant difference between the complication rates according to Clavien-Dindo classification.

When comparing LPN and RPN operations and recommending them to patients, it would be appropriate to perform cost-effectiveness analysis. Even in a study in the United States, the cost of RPN was $1066 more than LPN per case (9). The cost of maintenance of the robotic system as well as the purchase costs should be well analyzed for developing countries like us.

Study Limitations

Our study has, of course, limitations. First of all, this study was performed retrospectively in a single center. Failure to randomize patients may have led to bias during patient selection. Although all of the surgeons were experienced in the field, different surgeons may also have an impact on the results.

Conclusion

RPN and LPN, which are minimally invasive surgical treatment options, can be used safely in the treatment of renal tumors. RPN can be recommended to patients for better preservation of renal reserve and early return to daily life.

Ethics

Ethics Committee Approval: This study is approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (no: 2019/217).

Informed Consent: Due to this study is retrospective, informed consent had not obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.G., S.Ş, Design: E.G., S.Ş, Data Collection or Processing: E.G., S.Ş, Analysis or Interpretation: E.G., S.Ş, Literature Search: E.G., S.Ş, Writing: E.G., S.Ş.

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Contribution: There is not any other contributors who may not be listed as authors.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report
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Challenges in Differential Diagnosis: A Case Series of Four Adult Patients with Renal Leiomyoma

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Abstract

Leiomyomas are rare, benign and solid tumors of the kidney. Although the developments in radiological imaging methods provide early detection of kidney tumors, it is difficult to differentiate leiomyomas radiologically from other malignant renal tumors. Moreover, the definitive diagnosis of leiomyomas can only be achieved by histopathological and also immunohistochemical evaluation after surgical intervention. Immunohistochemically, positive staining with smooth muscle actin and vimentin, whereas negative staining with cytokeratin, S100, Mart1 and HMB45 are the methods used in the differential diagnosis of leiomyomas.

In this case series, after preoperative radiological evaluation, two female and two male patients between 45 and 89 years of age underwent laparoscopic treatment with the diagnosis of a malignant mass in the kidney. We aimed to illustrate the clinical, radiological and histopathological relationship of four adult patients who were managed by laparoscopic approach and diagnosed with renal leiomyoma. Renal leiomyomas should be kept in mind in patients with a renal mass before definitive treatment.

Keywords: Benign renal tumor, laparoscopy, radical nephrectomy, partial nephrectomy, renal leiomyoma

Introduction

Leiomyomas are infrequent solid, benign lesions of the kidney which arises from smooth muscle cells of various structures of kidney such as renal capsule, pelvis or vascular smooth muscle (1). In spite of their rarity within the genitourinary system, leiomyomas are frequently detected in the kidneys and are less often non-epithelial benign tumors of the bladder (2). Kidney leiomyomas are often identified between the second and fifth decade of life, with a median age of 42 years and a female predominance. Although the majority of kidney leiomyomas in the literature are adults, they can also be detected in children. Symptoms of leiomyomas vary according to localization in the urinary system, whereas those originating from the kidney often do not cause symptoms and may radiologically mimic other malignant masses of the kidney (3,4).

The differential diagnosis can be difficult with clinical evaluation. Therefore, a variety of immunohistochemical staining methods such as cytokeratin, S100, Mart1 and HMB45 are needed for the definitive diagnosis of leiomyoma (1).

In this article, we aimed to discuss the rarely seen cases of renal leiomyomas from clinical evaluation to the definitive diagnosis in terms of clinical, radiological evaluation, surgical management, and histopathological and immunohistological assessment.

Case Reports

This study was conducted in accordance with the declaration of Helsinki. All patients read the patient information form and written informed consents were obtained.

Case 1: A 45-year-old woman with a history of open uterine myomectomy five years ago presented with pain in the left lower quadrant for one week. Physical examination, urine and blood analyses were normal. Abdominal ultrasonography (US) revealed a 5 cm mass in the lower pole of the right pelvic ptotic kidney. Further imaging with computerized tomography (CT) was performed and revealed a completely exophytic, well-circumscribed, homogeneously contrast enhancing, 6x5 cm renal mass (Figure 1A and Figure 2). Since renal cell carcinoma could not be ruled out, laparoscopic partial nephrectomy was carried out for the management of the renal mass. Postoperative course was uneventful and the patient was discharged on the 3rd post-operative day. Pathological gross examination of the surgical specimen revealed a 7x5x4 cm fibrillary mass in the form of a benign smooth muscle tumor with a hard-rubbery

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appearance surrounded by a thin fibrous capsule. A positive immunohistochemical staining with SMA (smooth muscle actin) also confirmed the pathologic diagnosis leiomyoma. Preoperative serum creatinine and eGFR were 0.5 mg/dL and 141.80 mL/min/1.73 m^2, respectively. The patient was followed up for 11 months with a serum creatinine and eGFR of 0.6 mg/dL and 114.90 mL/min/1.73 m^2, respectively, at the last follow-up visit.

Case 2: On US examination, a 58-year-old man with microscopic hematuria was found to have a large mass located in the mid-lower pole of the right kidney. The patient had no additional systemic disease. Contrast-enhanced CT scan revealed an encapsulated right renal mass measuring 80x80x70 mm that medially pushed the renal pelvis and extended downward in the medial part of the right kidney. After contrast agent administration, homogeneous enhancement was observed in the central part of the mass with neovascularization in the inferior aspect of the mass, as well as peripheral heterogeneous enhancement. The remaining abdominal structures and left kidney had normal appearance, and no pathological lymph nodes were visualized. In the light of these radiological findings, the patient underwent a laparoscopic right radical nephrectomy. Histopathological examination of the mass revealed a leiomyoma that was uniformly separated from renal parenchyma, encapsulated, without mitosis, and immunohistochemically positive for SMA. The postoperative course was uneventful and the patient was discharged on the 3rd postoperative day. Preoperative serum creatinine and eGFR were 0.9 mg/dL and 86 mL/min/1.73 m^2, respectively. The patient was followed up for 67 months. Serum creatinine and eGFR levels were 1.4 mg/dL and 58 mL/min/1.73 m^2, respectively, five years after surgery.

Case 3: A 4-cm middle pole mass was found on US in the right kidney of a 62-year-old man who was evaluated for lower urinary tract symptoms. The patient had no additional disease except for Type II diabetes mellitus that was regulated by oral antidiabetic. Two separate masses were detected on CT scan, one at the level of renal hilum, 38x30 mm in size, located medially and the small one was 10x7 mm in size, located anterolaterally in the right kidney (Figure 1B). Both masses had contrast-enhancement and a solid appearance. Laparoscopic right radical nephrectomy was carried out and histopathological evaluation was reported as leiomyoma for both masses. Immunohistochemical examination revealed negative staining with cytokeratin, CD10, S100 and positive staining with SMA and vimentin. Post-operative course was uneventful and the patient was discharged on the 2nd post-operative day. Preoperative serum creatinine and eGFR were 0.7 mg/dL and 101 mL/min/1.73 m^2, respectively. The patient was followed-up for 47 months with a serum creatinine and eGFR levels of 1.6 mg/dL and 45 mL/min/1.73 m^2, respectively.

Case 4: An 89-year-old woman who was evaluated for dyspeptic complaints was found to have two solid masses in her right kidney on abdominal US. Therefore, abdominal magnetic resonance imaging (MRI) was performed to obtain additional information about radiological characteristics of the masses. One 4 cm exophytic mass in the upper pole of the right kidney and one 3 cm solid mass in the medial cortex of the middle pole were detected. Moreover, the lesions were isointense on T1W images and hypo-intense on T2W images. The lesions were found to restrict diffusion on diffusion-weighted series. On dynamic multiphasic series, masses had more hypo-intense appearance and less contrast enhancement than the renal cortex. In the light of these findings, laparoscopic right radical nephrectomy was performed. Histopathological evaluation of the surgical specimen was reported as leiomyoma for both masses and immunohistochemical examination revealed negative staining with Mart1, HMB45 and positive staining with SMA. The postoperative course was uneventful and the patient was discharged on the 3rd postoperative. The patient was followed-up for 14 months and, serum creatinine and eGFR levels were 1.12 mg/dL and 44 mL/min/1.73 m^2, respectively.

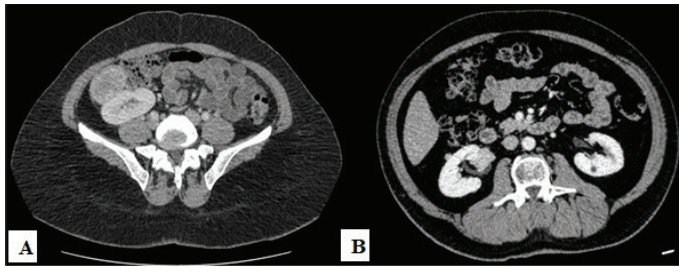

Figure 1. Contrast-enhanced CT images of Case 1 **(A)** and Case 3 **(B)**
CT: Computerized tomography

Discussion

Leiomyomas may originate from smooth muscle cells of the renal capsule, renal pelvis and renal vascular structures of the upper urinary tract. Besides, it may also arise from other

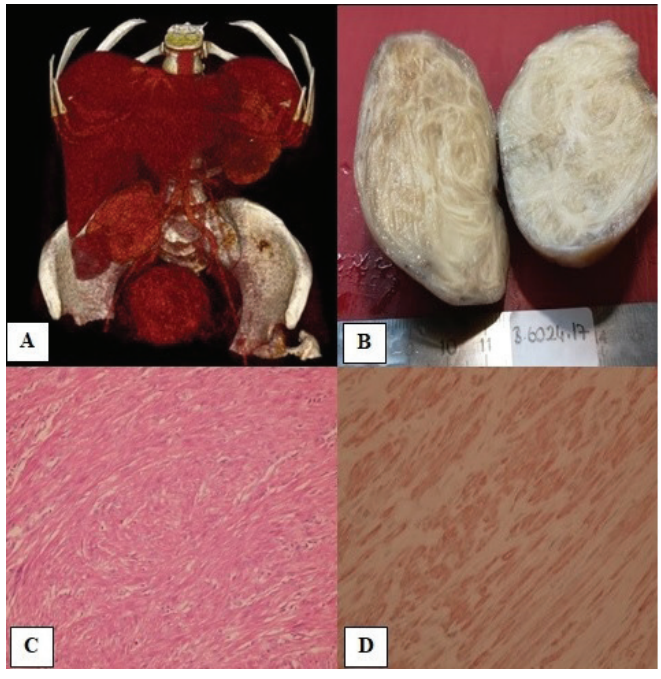

Figure 2. 3D-CT images of right pelvic kidney with mass **(A)** Macroscopic appearance of the lesion (**B**) Hematoxylin & eosin staining x400 (**C**) SMA staining x400 (**D**)
CT: Computerized tomography, SMA: Smooth muscle actin

genitourinary tract organs including smooth muscle cells, such as ureter, bladder, urethra, prostate, seminal vesicles, testicles, penis and scrotum (5). Renal leiomyomas are extremely rare benign tumors of the kidney with a prevalence of up to 5%, based on incidental findings of autopsy series. Renal leiomyomas constitute 1.5% of benign and 0.3% of overall renal tumors (6).

Although the vast majority of kidney leiomyoma cases reported in the literature are adults, they may also be detected in childhood. Leiomyomas are two times more common in women than men, and the incidence increases after the fourth decade of life (3,5). In our series, leiomyoma was detected equally in both men and women. The cases were older than 40 years old and ranged between 45-89 years.

Renal leiomyomas are divided into three groups in terms of location: a) subcapsular (53%): small, multifocal, asymptomatic, incidentally detected tumors during autopsy or imaging, b) capsular (47%): large, solitary and symptomatic masses (37%), and c) rare renal pelvis leiomyomas (10%) (4). Renal leiomyomas are detected incidentally on imaging as non-symptomatic masses. However, when they reach large sizes and create mass effect, the common presenting symptoms are palpable flank mass, flank pain and hematuria. It was pointed out that symptomatic masses were seen more frequently in young patients and asymptomatic ones more frequently in the elderly (5). In our series, none of the four patients had palpable mass and flank pain, but one patient had microscopic hematuria and a large renal mass was detected incidentally in the right kidney.

In recent years, renal tumors are being detected at increasingly smaller sizes with the developments in radiological imaging. US is the first-line diagnostic tool if there is any solid or cystic renal masses. Contrast-enhanced CT and MRI provide detailed information about unidentified renal masses. However, radiologically, it is difficult to differentiate leiomyomas from other malign renal masses. In abdominal CT, small, peripherally located leiomyomas are seen as well-defined, capsulated, homogeneously enhancing solid lesions. But, it is stated that lesions show a lower enhancement than renal cortex at the corticomedullary phase, and also a cleavage plane between the renal cortex and lesion can be defined. As tumor size increases, the contrast pattern also shows heterogeneity due to hemorrhage, cystic or myxoid degeneration (3,7). Besides, detailed preoperative anatomical information can be obtained by 3D-CT angiography for a renal mass with planned partial nephrectomy (8). In addition to the enhancement properties of leiomyomas, restricted diffusion may be detected in MRI due to different cellular and fibrous component contents (9). The radiological findings of our cases were consistent with the literature. Since the right kidney is within the pelvic localization in Case 1, 3D-CT was performed for detailed anatomical information before laparoscopic partial nephrectomy.

In the light of current literature, the precise diagnosis of renal leiomyomas can be made after pathological examination. The cases described here could not be diagnosed preoperatively. Moreover, special immunohistochemical staining may be required for pathological diagnosis. Histopathologic differential diagnosis is determined according to staining characteristics with several stains such as SMA, vimentin, CD10, S100 and HMB45 (4).

Due to the difficulties of differential diagnosis with other malignant renal lesions, surgical removal is usually performed for benign kidney lesions such as leiomyomas. Open surgical methods have been preferred for patients with large renal masses in previous case reports. In recent years, minimally invasive methods such as laparoscopic or robot-assisted laparoscopic partial or radical nephrectomy have been preferred as surgical methods in appropriate patients (10,11). To the best of our knowledge, this case series is the largest series in the literature in which renal leiomyomas were managed by laparoscopic methods. The characteristics of the cases are summarized in Table 1.

Conclusion

Today, despite the advancement in radiological diagnostic methods, it is difficult to differentiate renal leiomyomas from other malignant lesions in the preoperative period. Detailed pathological and immunohistochemical methods are needed for definitive diagnosis after surgical treatment. But, it is noticed that renal masses can be detected earlier in recent years. Therefore, the choice of minimally invasive treatment modalities, including partial nephrectomy, reduces patient morbidity and even mortality in long-term follow-up in appropriate patients.

Ethics

Informed Consent: All patients read the patient information form and written informed consents were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.N.M., E.Ö., Design: M.N.M., E.Ö., Data Collection or Processing: M.N.M., E.Ö., Y.K.Y., Analysis or Interpretation: M.N.M., E.Ö., Y.K.Y., Literature Search: M.N.M., E.Ö., Writing: M.N.M., E.Ö., Y.K.Y.

Table 1. Summarization of the characteristics of the cases

	Age (year)	Sex	Number of lesions	Size (mm)	Approach	Follow-up (months)	Change in e-GFR in follow-ups (%)
Case 1	45	F	1	60x50	LPN	11	-19.1
Case 2	58	M	1	80x70	LRN	67	-32.5
Case 3	62	M	2	38x30 and 10x7	LRN	47	-55.4
Case 4	89	F	2	40 and 30	LRN	14	-24.1

LPN: Laparoscopic partial nephrectomy, LRN: Laparoscopic radical nephrectomy, mm: Millimeter, F: Female, M: Male

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