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Aims and scope

The Kosin Medical Journal (Kosin Med J, KMJ, pISSN 2005-9531, eISSN 2586-7024, https://www.kosinmedj.org), the official publication of Kosin University College of Medicine, is a peer-reviewed and open access journal of research in all medical fields, including medicine, medical science, medical ethics, medical policy, and medical education. Its regional focus is Korea, but it welcomes submissions from researchers all over the world.

The KMJ aims to communicate new medical information to medical personnel, and to facilitate the development of medicine, medical science, medical ethics, medical policy, and medical education, as well as the propagation of medical knowledge by publishing high-quality, evidence-based articles.

The KMJ publishes editorials, review articles, original articles, and case reports. All manuscripts should be creative, informative, and helpful for the diagnosis and treatment of medical diseases and the communication of valuable information about all fields of medicine, medical science, medical ethics, medical policy, and medical education.

The first volume was published in Korean in February 1985. The KMJ has been published in English since 2016. A total of 1,165 papers were published by 2021. Since 2022, the KMJ has been published four times a year, on March 31, June 30, September 30, and December 31.

KMJ is indexed, tracked, and covered by National Research Foundation of Korea (NRF), KoreaMed, KoreaMed Synapse, Google Scholar, Index Copernicus International, Directory of Open Access Journals (DOAJ), Korean Medical Citation Index, and DOI/Crossref.

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Editorial

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Why should you not overlook the postoperative evaluation of steno-occlusive arterial disease for coronary artery bypass graft patients?

Jong Hyun Baek[1], Haeyoung Lee[2]

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See "Incidence of arterial steno-occlusive disease and related factors in patients undergoing coronary artery bypass graft surgery" by Seong Gyu Kim, Geun Woo Lee, Chul Ho Lee.

Atherosclerosis is a systemic disease. The pro-inflammatory stimulations such as high saturated fat diet, obesity, hyperglycemia, hypertension, smoking, etc., trigger the inflammatory process, which initiate macrophage and T-cell infiltration into vascular wall. This endothelial injury enhances the monocytes penetrating into intima and then the monocytes are transformed to scavenger cells which engulf cholesterol, building up a fatty streak. As other cells are accumulating around fatty streaks, they grow into plaque [1].

Coronary artery disease (CAD) is a well-known illness of atherosclerosis. Also, cerebrovascular disease (CVD) and peripheral artery disease (PAD) belong to the same spectrum of atherosclerosis [2]. CAD or CVD was accompanied with PAD more frequently than without PAD [3]. Therefore, PAD can be one of the sensitive markers or harbingers of CVD as well as CAD [4]. Above all, patients with steno-occlusive artery disease (SOD) were more likely to experience high cardiovascular mortality [5].

Despite all, clinical issues are clinician's attitude with ignored or missed diagnosis, inappropriate treatment, and growing prevalence of SOD in the present.

First, there remains the late diagnosis. Though there are pathologic lesions in some vasculatures, they can be sometimes underestimated due to asymptomatic or nonspecific symptoms. Bonacchi et al. [6] reported that the prevalence of PAD in patients after CAD treatment was 40%, whereas the prevalence of CAD undergone PAD surgery is 78%. In those cases, patients cannot undergo the best treatment due to failure of early diagnosis so that they can be the unavoidable victims of life-threatening complications derived from CAD or CVD. Therefore, more accurate and earlier diagnosis of SOD should be the challenging issues for clinical experts. In other words, clinicians should carefully evaluate patients for comorbidities both before and after attempting coronary artery bypass graft (CABG).

Second, there is a tendency in which SOD is treated more unsatisfactorily than CAD [4]. In other words, when physician's attention focused on CAD, there is a progressive SOD with the same pathology of CAD and then this can result in cardiovascular mortality in the end. Several studies report-

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ed that PAD is a well-known factor of poor short-term and long-term prognosis in patients undergoing CABG [6]. Even though manifestations are subclinical, PAD can increase the risks of cardiovascular events, stroke, acute renal failure, limbs ischemia, and mortality after CABG. The randomized on/off bypass (ROOBY) trial interested in the preoperative risk factors associated with post-CABG health-related quality of life (QoL). This ROOBY trial pointed out diabetes, smoking, chronic obstructive pulmonary disease, PAD, history of stroke, and depression as the worsening factors of health-related QoL following CABG [7]. As well as the minimization of related complications from CAD or CVD, desirable treatment results for CAD also will be guaranteed by application of the well-known treatment for noticed SOD; reduction of cholesterolemia, antiplatelet therapy, anticoagulation, peripheral vasodilators, high blood pressure management, physical exercise therapy, smoking cessation, alcohol reduction, lower-fat diet, etc.

Third, SOD has been one of relatively more common diseases in the future than now, which cause life quality decreased and requires more expenses for medical services globally [8]. So many researchers have been trying to reveal the clinical relationship and disparities between CAD and SOD [9]. On that point, we think that the subject covered in this target article is that authors emphasize the importance of diagnosis and treatment of SOD related with CAD and the warning to clinicians taking SOD more lightly than CAD.

In this issue of *Kosin Medical Journal*, Kim et al. [10] investigated that the incidence rate of SOD at another vasculatures in patients underwent CABG was 19.1%. Especially, the most commonly involved vessel was cerebral artery as 38% of them implying the importance of SOD due to vital issues. PAD was accompanied 2nd most commonly in 32% of them which suggested the inverse concept that the earlier you make accurate diagnosis of PAD, the earlier CAD or CVD can be diagnosed without missing.

Authors renewed the diagnosis and the progression of SOD which undoubtedly coexisted with CAD, not by the preoperative, but uniquely by the postoperative evaluation after CABG, resulting the importance of postoperative evaluation and management of SOD.

As mentioned in discussion, it is so pity that the evaluation for diagnosis of SOD could not be initiated until patients complained any symptoms. Therefore, we should pay attention to these patients postoperatively as well as preoperatively by the monitoring and the active treatment in correlated clinical departments under the conviction that SOD is inevitable.

They insisted that multidisciplinary approach to these groups is essential for the professionality and concentration on follow-up in regard to the risk factors of deteriorated renal function, hypercholesterolemia, and diabetes complications.

Dealing with study limitations, authors acknowledged poorly informative details about different medications affecting clinical results. We would like to suggest review of patient information about hemoglobin A1c level, dialysis and the preoperative ankle-brachial index at least. It might be more helpful to understand what the authors' saying. In addition, prognosis after postoperative medications and more accurate modality for early diagnosis after CABG should be addressed.

As the whole world goes by in the aging era, this study would be one of appropriate triggers leading a more various and attractive study idea, because the special issue focused on vascular deterioration might be required.

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Conflicts of interest
No potential conflict of interest relevant to this article was reported.

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Review article

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How to write an original article in medicine and medical science

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Excellent research in the fields of medicine and medical science can advance the field and contribute to human health improvement. In this aspect, research is important. However, if researchers do not publish their research, their efforts cannot benefit anyone. To make a difference, researchers must disseminate their results and communicate their opinions. One way to do this is by publishing their research. Therefore, academic writing is an essential skill for researchers. However, preparing a manuscript is not an easy task, and it is difficult to write well. Following a structure may be helpful for researchers. For example, the standard structure of medical and medical science articles includes the following sections: introduction, methods, results, and discussion (IMRAD). The purpose of this review is to present an introduction for researchers, especially novices, on how to write an original article in the field of medicine and medical science. Therefore, we discuss how to prepare and write a research manuscript for publication, using the IMRAD structure. We also included specific tips for writing manuscripts in medicine and medical science.

Keywords: Education; Journal article; Medicine; Methods

Introduction

Owing to the rapid development of medicine and the popularization of the internet, a vast amount of medical knowledge is available every day. To acquire new medical and scientific knowledge and data, researchers must read and assess published articles diligently and make continuous efforts to apply the findings to patient care or research. A researcher's ability to understand medical and scientific articles is very important.

Researchers can also obtain new knowledge by investigating unresolved queries, and informing other researchers of their studies. Researchers from all fields share their findings by reporting the results of studies in scientific journals. Researchers with expertise in a particular field are valued and their authority is evident by the impact of their published studies. It is not easy to conduct excellent research, and it is often difficult to effectively communicate our research outcomes. Many researchers have trouble with writing journal articles and find the process burdensome. Journals with high impact factors often allow English submissions for publication consideration only. For researchers whose native language is not English, writing manuscripts in English is a major obstacle.

Fortunately, there is a standard, preferred way to write original research papers, and the format follows the introduction, methods, results, and discussion (IMRAD) structure (Table 1). Scientific articles in medicine and medical

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Table 1. Composition of an original article in medicine and medical science

Section	Purpose
Introduction (I)	Why did you conduct the research?
Methods (M)	How did you conduct your research?
Results (R)	What have you discovered through your research?
And (A)	
Discussion (D)	What are the implications of your research findings?

science first began in the form of letters and descriptions in the 17th century [1]. The IMRAD structure was introduced in the twentieth century. In less than 40 years, the structure became widely used [2]. Following the IMRAD structure when preparing a research manuscript has many advantages. In this review, we described how to write an original article using the IMRAD structure.

The process of writing an original article

1. Preparation for writing

Before explaining the IMRAD structure, there are a few points that may assist with writing preparations. One aspect involves conducting literature searches and research. In general, an original article is written based on research results. A literature search is the first step in conducting research. It is essential to check whether there has been any prior research on the issue to be studied, and if so, understand the research findings, and identify the strengths and limitations of the research. In addition to landmark studies, recently published studies should be comprehensively investigated. This process allows researchers to view their research more objectively, and in turn, understand the strengths and weaknesses of their research. Furthermore, before starting the research, researchers should make the subject and target of the research as specific as possible, and consider whether the research to be conducted is feasible. Similarly, researchers need to ask themselves some questions before conducting their research and writing a manuscript: Did I perform something new and interesting? Is the task related to a current issue? Do I have solid evidence and clear answers to support my hypotheses and proposed goals? Are the conclusions of this study relevant? [3]. Only if the answer to each question is "yes," should the researchers begin to prepare their manuscript.

Journal selection is also an important consideration. Before writing a manuscript, the authors should take the time to carefully consider the most appropriate target journals. The most common way to determine if a journal is suitable is to look at the articles referenced by researchers who are conducting studies in the same area. To determine if a journal is interested in an article on a particular topic, the authors should refer to the aims and scope of each journal. It is also helpful to read the latest publications of the target journal. For example, the *Kosin Medical Journal* (KMJ) aims to "communicate new medical information to medical personnel and facilitate the development of medicine, medical science, medical ethics, medical policy, and medical education, as well as the propagation of medical knowledge by publishing high-quality, evidence-based articles."

Once the target journal has been decided, it is important to meticulously read the instructions to authors, which are usually available on the journal's website. Instructions to authors contain detailed information regarding the design and structure of the abstract and main context, preferred referencing style, the maximum number of words, format of figures and tables, etc. Authors must ensure that all the journal requirements are met. If authors soundly format the article according to the requirement of the target journal, journal editor will be happy to facilitate the review process.

2. Title page

The title page should consist of the full title, authors, institutional affiliations, the corresponding author's name and contact details, funding sources, and conflicts of interest. Please be mindful that while most journals require these in the title page. There are also journals that are very particular about what to include and not include. Some require two title pages for example a separate title page file with all the information and a simplified title page with the main text with only the full title. This is especially so, if the paper uses blinded peer review. Depending on the journal, a running head, word count, and the number of figures and tables may also be required.

1) Article title

A good title is cardinal because it arouses curiosity and can lead the reader to read the article. Simultaneously, the title should explain the content of the manuscript. A succinct title is easier to understand than a long and complex title. In addition, a succinct title that describe the study design. The

title should contain all sensitive and specific information so that the research can be detected through electronic retrieval [4].

2) Authors and institutional affiliations

Names are a sensitive issue. We recommend that you do not change your name throughout your career. Decide carefully what name you will publish under from the beginning. For authors, whose first language is not English, pay particular attention to the spacing between the letters of the English version of your name. Some journals also present the highest academic degree(s) for each author, while others do not. Always check the name of the department(s) and institution(s) to which the research is attributed.

Authorship and order of authors are also controversial. The International Committee of Medical Journal Editors (ICMJE) recommends the following criteria for authorship. Authorship credit should be based on (1) substantial contributions to the study concept and design, data collection, or data analysis and interpretation, (2) drafting or critically revising articles from important perspectives, and (3) final approval of the version to be published. The authors should satisfy all three conditions [4].

3) Corresponding author

The corresponding author receives all correspondences from the journal editors and is responsible for archiving the entire study. Therefore, the corresponding author must provide details of his/her full name, workplace, postal and e-mail addresses, telephone numbers, and fax numbers.

4) Funding and conflicts of interest

If the study was supported by funding or if there are any conflicts of interest, these must be declared.

5) Running head in the footer

Some journals demand a short or running title in the header or footer. A running title usually consists of no more than 50 characters (including letters and spacing). Running heads appear in most journals and are used in the editorial office to organize and locate manuscripts.

6) Word counts and the number of figures and tables

In general, word count refers to the number of words in the main text, excluding the abstract, acknowledgments, figure legends, and references. The number of figures and tables should be limited to the range permitted by each journal. For example, Table 2 shows the KMJ specifications for each publication type.

3. Abstract

The abstract should summarize the key concepts and results of the study to inform the subject and content of the research. It may be the only part that the readers read. Certainly, it is often the only part used in a bibliographic search system [3]. Therefore, it is important to communicate the most meaningful parts of the research in a concise and specific way. To identify which aspects of the article should be emphasized, writing the abstract after writing the manuscript is recommended. Authors should check the guidelines in each journal to determine if a structured abstract (e.g., using background, methods, results, and conclusion as headings) is required. In the medical and medical science fields, many journals recommend a word limit of 250 words for the abstract.

4. Keywords

These words are at the heart of the article. Articles can be searched through keywords to obtain more citations. Therefore, it is important to include the most relevant keywords

Table 2. *Kosin Medical Journal* specifications for different publication types

Type of article	Original article	Review article	Case report	Editorial
Abstract (word count)	250	250	250	NR
Text (word count)[a]	NL	6,000	1,500	1,000
References	40[b]	100	20	20
Tables and figures	NL	NL	6	3

Any article longer than these limits should be discussed with the editor.
NL, not limited; NR, not required.
[a]Maximum number of words excluding the abstract, references, and figure legends.
[b]Except for meta-analyses or systematic reviews.

so that others can find the article. Many journals require three to five keywords. Keywords should use terms retrieved from the Medical Subject Headings (MeSH) of the National Library of Medicine.

5. Introduction

The introduction presents the reasons why the researchers conducted this study. In general, it should be written in a format that introduces the research subject, the research purpose, the context, and the direction of the current research. The introduction should address three questions: (1) what is the problem? (2) what is the significance of the problem and what are the unsolved queries? and (3) what question does your research answer? [5]. In this last part, the hypotheses and goals of the research study should be articulated. An introduction with three paragraphs is usually sufficient. The content should be clear and concise. Only essential and relevant references should be cited. If the introduction is too long, the reader will not want to read the remainder of the article. We have not included the results or conclusions in the introduction. Any details, conjectures, or comparisons with other studies should also be left for discussion.

6. Methods

The methods section specifically presents how the research was conducted. In other words, the details included should be sufficient to allow other researchers to reproduce the research study in whole or in part. Findings from the research is included in the results section, and not in the methods section [3]. The following information should be included in the methods section.

1) Study design

A detailed description of the study design should be provided in a separate paragraph and the type of study should be specified (e.g., cohort, clinical trial, randomized, cross-section).

2) Participants

When describing the methodology, where the research was conducted, how the participants were selected, the inclusion and exclusion criteria, how the randomization process was created, the number of participants who were withdrawn from the research, the number of participants who finally participated, and so on, should be provided.

3) Variables

The variables must be clearly defined for readers to understand. In general, variables may be classified into numerical and categorical variables. If a variable is divided or categorized into two or more groups, the procedure for setting the cutoff point should also be distinctly described [3].

4) Measurement and monitoring criteria

Describe in detail what the variables were measured, how they were measured, how many times they were measured, and so on [3]. If a commercial kit was used for the research, the accurate name of the kit and the location of headquarters have to be provided.

5) Estimation of the sample size

In order to obtain accurate results, optimal sample size calculation and power analysis are one of the most important issues of research. Generally, the sample size calculation and power analysis are arranged by effect size, power (1-β), significance level (α), and type of statistical analysis [6,7].

6) Statistical methods

The statistical tools used in the research should be detailed, including the *p*-value cutoff for statistical significance, and the measures of effect sizes used. In general, numerical data is compared using the Student *t*-test (a parametric test) and Mann-Whitney *U* test (a nonparametric test) [8]. And, categorical data is compared by using the chi-square test (a parametric test) and Fisher exact test (a nonparametric test) [9]. In terms of the risk factors, logistic regression analysis is used to assess the risk factors of the diseases, and Cox proportional hazards regression analysis is used to investigate risk factors affecting survival rate. If an uncommon statistical analysis is used, it is recommended that references be provided.

7) Research ethics

The approval from a relevant Ethics Committee or Animal Experimentation Committee to conduct the research must be articulated. For prospective human research studies, informed consent must be explained.

7. Results

This section should include only research findings. Your interpretation of the results should be provided in the discussion section [5]. Results may be presented visually as tables and figures so that readers can understand easily and clearly. The information presented in tables and figures should not be repeated in the text. Results are usually written in the past tense. The number of tables, figures, and graphs allowed by the journal may be limited. Each table, figure, or graph requires a descriptive title. The names of the variables, units, legends, etc. should be included so that the results can be interpreted without reference to the text.

8. Discussion

This section describes the research subject that the researcher presented in the introduction, with particular emphasis on the meaning and relationship of the research findings to the subject [3]. Information already detailed in the introduction or results sections do not need to be repeated in this section. It is often useful to briefly begin with a lead paragraph accentuating the most important findings in the area to date. Then, provide possible explanations for the research findings. Plausible or possible underlying mechanisms can also be suggested. Discuss the similarities or differences of the study's findings with evidence from other associated studies in the literature [4]. If the results differ from previous studies, highlight the reasons for these differences.

Any limitations of the research must be mentioned. Also, explain how the limitations may influence the interpretation and substantiation the research results. The relevance of the results should not be overestimated. In addition, the strengths of the research should be described based on the findings. The author's responsibility is to mention all aspects and suggest future research directions and clinical practice applications [5].

The discussion section closes with a conclusion. Conclusions should be drawn based on the research findings. A key message to the reader should be articulated. Importantly, researchers must not arbitrarily interpret research findings and draw conclusions that have not been clearly demonstrated in the study.

9. References

The Harvard and Vancouver systems are the most common referencing styles in medicine and medical science. Of these, the Vancouver system is commonly used in medical journals [10]. To ensure the correct referencing style is used, refer to the "instructions to authors" of the target journal. For Vancouver style, the references are numbered sequentially in the order in which they appear in the manuscript. If possible, it is better to use recent references. A citation management software (e.g., Endnote or Mendeley) can be useful for managing the bibliography [11].

10. Acknowledgments

In acknowledgments, include the names of individuals or organizations who have contributed to the research but who are not listed as the authors. Any assistance provided during research exploration to manuscript submission should be acknowledged [10].

Additional information related to KMJ

KMJ is international, open access, peer-reviewed quarterly journal of medicine published online only in English (31st March, 30th June, 30th September, 31st December). The aims of KMJ are to publish high-quality evidence-based, scientific research articles from various disciplines of the medical sciences including basic and clinical studies. The categories of KMJ are consisted of editorials, review articles, original articles, and case reports. As mentioned above, the instructions to authors and more specific details are listed on the website of KMJ (https://www.kosinmedj.org). If researchers need further information from KMJ, feel free to contact KMJ office (office@kosinmedj.org).

Writing tips

- Before writing the manuscript, we suggest creating the tables and figures. Then, write the results, methods, introduction, and discussion sections in this order. Finally, write the abstract and insert the title [3].
- Make the manuscript publication valuable by finding simple, concise ways to tell your story. Check the logical arrangement of the argument and the flow of the experiment, and remember to use the summary statements [12].
- Write a good cover letter and pay attention to references. Make sure you fully understand the material you refer to, and that it defends the work in the way you think it does [12].

· Ask your colleagues to review your manuscript. Reading your manuscript aloud to your colleagues may also help with checking the logical and structural flow with checking the logical and structural flow. When writing the manuscript, write carefully and with enough detail so that even non-professionals in your field can understand your manuscript.
· Make no mistakes, especially grammatical errors. If you plan to submit your manuscript to an English journal, you may need assistance with proofreading and editing to review your manuscript, especially for syntax and grammatical errors. For researchers whose first language is not English, professional English proofreading services are available.
· Read the comments of the reviewer carefully and apply the suggestions to actively improve the quality of your manuscript.

Conclusions

Writing an original article in medicine and medical science is challenging, especially initially. Similar to establishing a research method, manuscript writing can be improved with constant practice. Reading research articles written by others is paramount to writing a good original article. Taking notes of relevant sentences, including structures and expressions, can also help with your writing. The IMRAD method is a traditional method. It is likely to continue to be used in the future. Thus, researchers need to be familiar with this structure.

Article information

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How to write case reports in medicine

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Medical research has become an important part of providing care to patients. Case reports published in medical journals can communicate information to the medical community about rare or unreported features, conditions, complications, or interventions. Case reports are generally short, focusing on key components such as a summary and introduction, case presentation, and discussion. Authors now have access to free, continuously updated case reports of different types from multiple journals. This review introduces the process and mechanisms for how and when to prepare a case report. We briefly review the editorial process of each of these complementary journals, along with author anecdotes, hoping to inspire authors to write and continue writing case reports; and discusses the essentials of a case report, aiming to provide guidelines for improving medical writing skills.

Keywords: Case reports; Guideline; Medical writing; Publications

Introduction

From the Hippocratic era to date, physicians from all specialties have described interesting cases [1,2]. For most researchers, case reports can be the first effort to publish in medical journals, and it is considered a useful practice in scientific writing due to the similarities in its basic format [3]. Notwithstanding the variety of case reports, all reports represent the reader's clinical symptoms, diagnostic approaches, or therapeutic alternatives for a disease [4]. Thus, case reports should be educational as well as contain a useful practical message [5]. While some experts ignore case reports in the structure of evidence-based literature of medicine, these publications allow you to share anecdotes of individual clinical practice, providing a substantial source of information for optimal patient treatment. Randomized controlled trials are at the top, followed by systematic reviews and meta-analyses, prospective experimental trials, observational studies, case-control studies, and case series in general evidence-based medicine. However, in the treatment guideline and updated experts' consensus, systematic reviews and meta-analyses are considered at the top of the hierarchy of evidence-based medicine [6]. On the other hand, it has been pointed out by others that carefully written and interpreted case reports play an important role in the advancement of medical knowledge and education [7]. A previous report [8] listed five potential contributory roles of case reports: (1) description and recognition of a new disease; (2) rare manifestations recognition of a known disease; (3) mechanism elucidations of a disease; (4) adverse or beneficial effects detection of medications; and (5) medical education and deep gratitude.

The number of case reports published annually has increased over the past 20 years [9]. However, the quality of published case reports has been inconsistent. A systematic assessment of the quality of case reports revealed frequent

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technical and editorial errors. Common problems in case reports include a lack of description of symptoms, failure to identify the main outcomes, and failure to draw conclusions [10]. Thus, case reports need to be improved in several ways.

To address this issue, Gagnier et al. [11] developed the CARE (CAse REport) Statement and Checklist, which includes reporting guidelines for case reports. The CARE guidelines consist of a 36-item checklist that provides a comprehensive framework for enhancing the transparency and accuracy of reporting [12]. The CARE guidelines are available online on the CARE Case Report Guidelines homepage (open access via https://www.care-statement.org/) and have been published in several journals.

The aims of this review are to give guidelines for beginner authors to write new case reports. Although these guidelines to write case reports are not sufficient to become a great writer, they do help inexperienced authors exercise and develop the basic skills needed in medical publishing.

General principles of case reports

Case reports are generally short, focusing on key components such as summary & introduction, case presentation, and discussion about detailed review and conclusions [13]. Figures, tables, illustrations, and graphs can be supplementary and enhance the flow and clarity of the case report. Differently from original papers, the authors do not need to follow the usual format of manuscript organization (i.e., introduction, methods, results, and discussion) [5]. Since the format of case reports varies widely from journal to journal, authors should recognize the guidelines of journal where they want to publish. A case report is a design to study for unexpected or completely new cases, each with an individualized report of the patient's results, clinical progression, and outcomes. Such a report might also include a review of existing studies.

Some scientists have classified case reports as designing a study qualitatively, while other scientists have considered them designing a study quantitatively or with combined method design [14]. In addition, considering most approaches for research in medical science, case reports can be classified as a new discovery or corroboration. There are several types of case reports and they are organized into the following categories: a single case, >2 combined cases, or a series of multiple cases. Case reports are usually presented retrospectively. However, they can be prepared prospectively by using a new diagnostic approach or guidelines to discover new cases in a certain health situation [15]. Table 1 shows examples of case types considered suitable for report [1]. Notwithstanding these limits to publish, All case reports have the potential to be published if they are genuine, lead to a gradual advance in medicine, or have educational value despite not containing new discoveries.

Format for writing a case report

The word count for case reports varies among journals. However, Authors should be aware that they should not exceed 1,500 words in general. And then, case reports should be concise, concrete, and organized, embracing sufficient data to inform relevantly [1]. If the journal required a shorter version, the discussion section of the article can be shortened. *Kosin Medical Journal* (KMJ) requires the manuscript to include in a total of six tables or figures. For human cases to report, the case description should include a statement regarding informed consent to publish about clinical data. The submission guidelines of KMJ are available online on the KMJ homepage (open access via https://www.kosinmedj.org/authors/authors.php).

1. Title

The readers see the title page as first step to a case report. Therefore, the title should be attractive, clear, organized to grab the reader's attention to the topic. An ideal title should not sound too broad or artificial, and should be understandable to the reader [16]. A title can be placed on the first page of the manuscript or as a separate file labeled "title" depending on journals. Unnecessary words, double

Table 1. Criteria for the publication of a case report

Unexpected or rare adverse events
Drug-related side effects
Unexpected or abnormal manifestations of a disease
New link in the disease progression
New development, diagnosis, and/or treatment of emerging diseases
New associations between various diseases and symptoms/signs
Unexpected side effects during treatment
Consequences that explain the possible etiology of a disease or side effect

meanings, and abbreviations should be avoided in the title, and the phrase "case report" should be included. Beneath the title and on the same page, all the authors, their affiliations, and email addresses should be listed. Most reports for case are not written by a single author. However, if the number of authors exceeds six, some publishers may return case reports for publications back. For KMJ, there is no limitation of number of authors. Finally, one author should be assigned the role of "corresponding author," communicating with editorial board members of the journal, and this author should provide contact information to the journal. Corresponding authors must submit a case report to receive comments on the case report from journal editors, and the corresponding author's name must be included in the list.

2. Abstract

This is the most important section of the report, since it will be freely accessible for others to read when retrieved from any medical database during a relevant search. Similar to other types of articles, case reports should include a short summary of the contents of the case report. Initialisms, acronyms, and informal abbreviations should be avoided wherever possible or kept to an absolute minimum and accompanied by proper definitions. The abstract should not contain references and preferably should be written within 250 words, although the word limit differs among journals, indicating the importance of submission guidelines. The abstract usually has three parts: background, presenting case, and conclusion. The background should clearly explain why this unique case should be reported. The conclusion should be brief, and explain the lessons learned from this case report and its impact. The abstract should provide information that is easily searchable in electronic databases and should help researchers draw attention to the case report [17].

3. Keywords

Keywords are extremely important to index the case report and should contain minimum of three and a maximum of 10 words or phrases. Keywords should be selected very carefully to allow optimal retrieval during research. In KMJ, there should be three to five keywords listed under the abstract on the same page. For selection of keywords, it is advisable to reference the Medical Subject Headings (MeSH) in PubMed or http://www.nlm.nih.gov/mesh/MBrowser.html.

4. Introduction

In this section, a definition and brief description of the pathology, including common presentations and disease progression, are discussed, explaining the background of the selected topic. The introduction should be concise and get the reader's interest by providing background information about its importance and an explanation of key contents of the case report. This is followed by describing about the case briefly to be reported and its significance of this case compared to previous studies. Although a comprehensive review of literature is covered in the discussion section, a brief description of the literature is available here [5].

5. Case presentation

A case summary or description focuses on any case presentation. The case should be presented over time with sufficient data to enable the reader to understand the author's own conclusions about the validity of the case. A clear figure and data for the patient's cases should be provided with sufficient details and explanation [14]. The demographic information about cases should be described, in details that could confirm the patient's identity. Case presentation should begin with major complaints and detailed medical conditions and, including past medical history, social and occupational history, medications. The results from the physical examination should be presented in brief, along with laboratory results, images, and analyses. The differential diagnosis approach and rationale for treatment should be described, with the follow-up data and conclusions. Over-interpreted approach should be avoided. This section can be divided into smaller subsections if necessary, and relevant images and tables should be added to make the case easier for the reader to understand. A clinically significant negative outcome should be mentioned. Authors' interpretation or reasoning should be avoided in the body of the case report. Tables and figures should be used to reveal chronological findings or to compare observations using different methods. All figures require a concise and definite explanation. For cases of using surgical or pathological data, The authors are encouraged to provide about the surgical procedure and a concrete pathology results [17].

Crucially, patient confidentiality must be preserved. In the first sentence, mentioning patient demographics, in-

cluding race, age, gender, and occupation, is recommended but not required. Patient initials, and other records to identify must be avoided to reduce the possibility of identification.

6. Discussion

The discussion explains the author's rationale and approach in detail with information about the uniqueness of the case. It also helps to make summary of the main points, compare the uniqueness of case with previously reported cases, draw new information and practical applicability, and clinically useful conclusions [13]. When comparing the new case with existing knowledge, the author should concisely summarize what was previously well known. And elucidate the novelty of these reported cases from previously reported ones and why it is worth reading and publishing. Case report discussions are not intended to provide a comprehensive literature data or all references citations. Therefore, the authors should reconfirm all citations of references concretely. Authors must be aware of limitations of the case and explain each of limitation and importance of those. The values added by case reports to update existing literature and data should be emphasized and mentioned together with the lessons learned from the case, especially if there is a potential for new recommendations for patient diagnosis or management [13,18].

7. Conclusion

The conclusion section should include a concise statement to explain the importance and relevance of the case, which should relate to the purpose of the paper. The author should conclude the key findings from case reports with verified references in the discussion. A brief explanation of lessons learned from case reports may be included with concrete evidence and recommendations. The authors should conclude briefly within one paragraph [19]. Case reports have far less potential impacts on clinical practice than well-organized prospective original research articles. However, case reports are of value with newness and uniqueness. Researchers can share individual clinical practices, increase awareness of unique manifestations, and provide instructive messages through case reports. Case reports with well-written and proper structure will contribute to the medical science and enhance the current evidence-based medicine.

8. References

The references listed at the end of the case report should be carefully selected based on relevance. Readers who wish to obtain more information than from the case reports are able to get more information from references and the references should support key findings in the case reports [20]. There are some restrictions in some journals about the number of references to 15. However, KMJ recommends no more than 20 references for case reports.

Conclusions

Case reports are of value as source of information with newness and uniqueness and enable researchers to provide their clinical practices on individual cases, increase knowledge of unique manifestations, and convey important messages for education. Case reports will continue to provide new research ideas through hypothesis generation, contribute and enhance knowledge of the medical science in the present evidence-based medicine. Although these guidelines to write case reports are not sufficient, this review will help beginner authors to practice and achieve their writing skills improvement for writing or case reports in medicine.

Article information

Conflicts of interest

Sung Il Im is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Alcohol-related liver disease and liver transplantation

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Alcohol-related liver disease (ALD) has become the major cause of liver transplantation (LT) in Korea, and is currently the most common cause of LT in Europe and the United States. Although, ALD is one of the most common indications for LT, it is traditionally not considered as an option for patients with ALD due to organ shortages and concerns about relapse. To select patients with terminal liver disease due to ALD for transplants, most LT centers in the United States and European countries require a 6-month sober period before transplantation. However, Korea has a different social and cultural background than Western countries, and most organ transplants are made from living donors, who account for approximately twice as many procedures as deceased donors. Most LT centers in Korea do not require a specific period of sobriety before transplantation in patients with ALD. As per the literature, 8%–20% of patients resume alcohol consumption 1 year after LT, and this proportion increases to 30%–40% at 5 years post-LT, among which 10%–15% of patients resume heavy drinking. According to previous studies, the risk factors for alcohol relapse after LT are as follows: young age, poor familial and social support, family history of alcohol use disorder, previous history of alcohol-related treatment, shorter abstinence before LT, smoking, psychiatric disorders, irregular follow-up, and unemployment. Recognition of the risk factors, early detection of alcohol consumption after LT, and regular follow-up by a multidisciplinary team are important for improving the short- and long-term outcomes of LT patients with ALD.

Keywords: Alcohol use disorder; Liver disease; Liver transplantation

Introduction

Liver transplantation (LT) is the only available treatment option for survival in the cases of liver failure in patients with terminal disease. Repeated and continuous alcohol consumption has been identified as a substantial risk factor for chronic liver disease, and the net effect of alcohol consumption on health is estimated to account for approximately 3.8% of deaths worldwide [1,2].

Alcohol-related liver disease (ALD) has become the major cause of LT in Korea, and is currently the most common cause of LT in Europe and the United States (US). One of the biggest reasons for the increase in LT in ALD is the decrease in chronic hepatitis B patients in Korea, which is related to the decrease in LT patients with hepatitis C owing to the use of direct-acting agents in Western countries. However, the decrease in hepatitis B and C, which have been the main indications for LT, cannot fully explain the increase in LT in patients with ALD. LT for ALD has several ethical dilemmas. Alcoholism has recently been considered as a chronic and relapsing-remitting neurological disease with a definite biological background, and the change in attitude towards LT in ALD is also one of the reasons why LT has become the main treatment for ALD [3-5].

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ALD is one of the major causes of chronic liver disease, accounting for approximately 48% of cirrhosis-related deaths in the US. Persistent alcohol consumption is the main cause of alcoholic steatohepatitis (ASH), cirrhosis, and liver cancer [6].

Despite the environmental and genetic factors associated with alcohol use disorder (AUD), ALD is still regarded as self-harm by some transplant doctors. Transplantation access for LT candidates with ALD is still marginal. Of the potential candidates with ALD, only approximately 5% to 10% of the patients have been selected for LT [7,8].

These results could may at least partially explain why transplant waitlist registrants with ALD present have high Model for End-stage Liver Disease scores, and why a higher proportion of waitlist registrants have severe decompensated complications compared with other patients on the waiting list.

Current status of LT for ALD

According to the United Network for Organ Sharing report, up to 2015, the LT waitlist and LT surgeries for patients with hepatitis C virus (HCV)-related chronic liver disease, the first indication for LT, accounted for 33% and 28%, respectively. Since 2016, ALD (30%) and nonalcoholic steatohepatitis (21%) have accounted for more than half of the total waiting list, and LT surgeries due to ALD and nonalcoholic steatohepatitis have surpassed HCV. In Europe, LT for alcoholic cirrhosis (AC) increased abruptly from approximately 35% in 1988–1995 to 45% in 1996–2005. Since 2019, AC has become the major cause of LT. Therefore, ALD has become the leading cause of LT in the US and Europe [9,10].

In Korea, the number of patients waiting for an LT increased from 4,279 in 2010 to 5,804 in 2019, and the number of deaths due to the unavailability of a liver for transplantation steadily increased from 631 in 2010 to 972 in 2019 (Fig. 1). Korea is an endemic region for hepatitis B virus (HBV), with approximately 5% of the general population being HBV carriers; however, it has been controlled by a national vaccination program for all neonates in Korea since 1995 and a national screening program for antiviral treatment. Thus, the patients who received liver grafts from deceased donors for ALD has increased sharply, from 34% in 2015 to 51% in 2019, while the number of patients undergoing living donor LT has increased gradually, from 20% in 2015 to 25% in 2019. Similar to Western countries, ALD has pushed HBV as the second causative disease and has become the primary etiology of LT in Korea (Fig. 2) [3,11].

Alcohol-related liver disease

ALD represents a spectrum of liver damage due to repeated and continuous alcohol misuse, from alcoholic fatty liver to more advanced forms, including ASH, liver fibrosis, AC, and

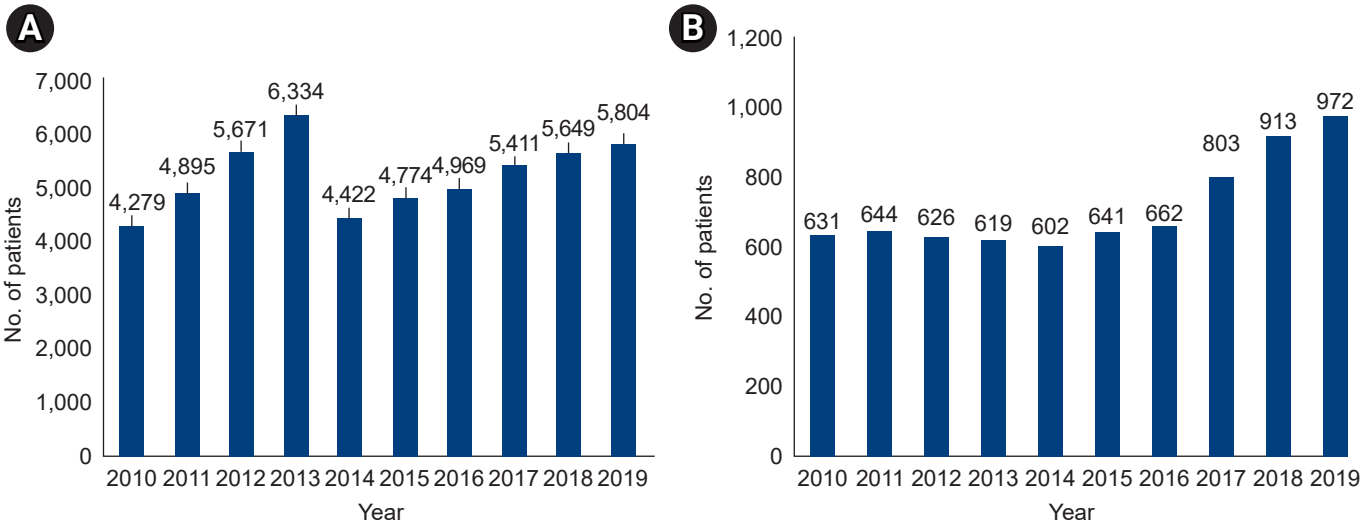

Fig. 1. Liver transplant waiting list (A) and waiting list mortality (B) in Korea from 2010 to 2019. Data are from Korean Network for Organ Sharing [3].

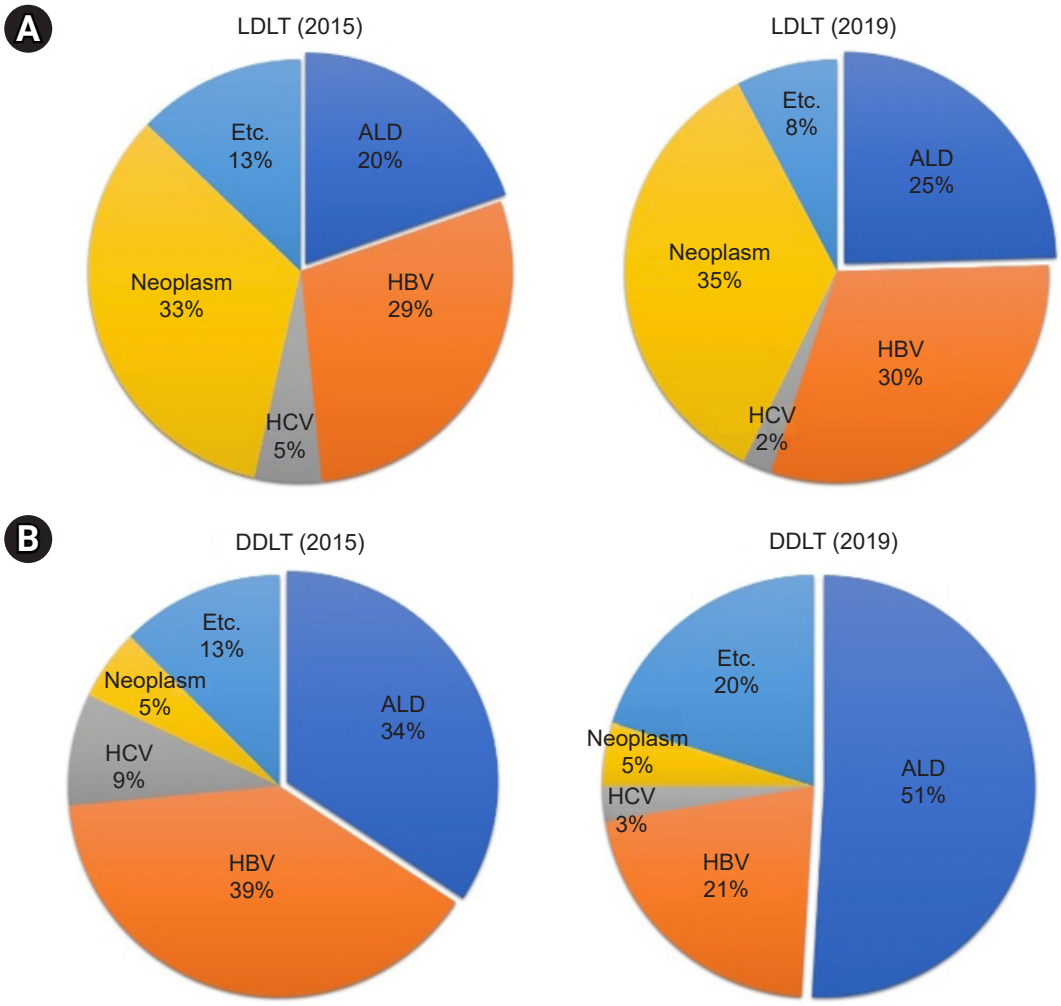

Fig. 2. Changes of etiology for liver disease in liver transplantation in Korea. (A) Living donor liver transplantation (LDLT). (B) Deceased donor liver transplantation (DDLT). HCV, hepatitis C virus; HBV, hepatitis B virus; ALD, alcohol-related liver disease. Data are from Korean Network for Organ Sharing [3].

severe alcoholic hepatitis presenting with acute-on-chronic liver failure. ALD is one of the major causes of chronic liver disease worldwide, both by itself and as a contributor to the aggravation of chronic viral hepatitis, non-alcoholic fatty liver disease, and other liver diseases [6].

In general, the risk of ALD increases based on the amount of alcohol intake (≥30 g/day). The minimum amount required to cause cirrhosis is approximate ≥30 g/day in men and ≥20 g/day in women. In most studies, alcohol intake of 40–80 g/day increases the risk of liver damage. People who consume more than 60 g of alcohol per day develop steatosis, and some people with steatosis develop ASH, of which 10% to 20% eventually develop cirrhosis. In addition, both genetic and non-genetic factors can influence both individual susceptibility and the clinical course of ALD [12-14].

Disease spectrum

1. Fatty liver

Fatty liver is the first response to repeated alcohol misuse and is incarnated by the deposition of adipose tissue in the hepatocytes. Macrovesicular steatosis, with its characteristic foamy cytoplasmic appearance, is the early and most common observed pattern of alcohol-induced liver damage from alcohol. There are four major pathogenic factors as follows: (1) increased nicotinamide adenine dinucleotide

synthesis caused by alcohol oxidation; (2) increased hepatic inundation of chylomicrons and free fatty acids; (3) ethanol-mediated blocking of adenosine monophosphate-activated kinase effect, which inhibits peroxisome proliferating-activated receptor α and stimulates sterol regulatory element binding protein-1c to increase adipogenesis and reduce lipolysis; (4) acetaldehyde-induced mitochondrial and microtubules damage, reduced nicotinamide adenine dinucleotide oxidation, and very low-density lipoprotein accumulation, respectively [15-17].

2. Steatohepatitis

ASH is an advanced form of hepatic injury in patients with repeated alcohol misuse and can present as an acute-on-chronic hepatic failure, resulting in rapid deterioration of liver function, and increased mortality. Despite the aggressive therapy, approximately 30% to 50% of patients with severe ASH eventually die [18]. ASH is pathologically determined as the existence of steatosis, hepatocyte swelling, and the inflammatory infiltration of polymorphonuclear neutrophils. ASH is a clinical syndrome that refers to the recent onset of symptoms, such as ascites and/or jaundice in patients with persistent alcohol intake. Although clinical symptoms may appear abruptly, the term “acute” is not recommended, as it is an aggravation of the underlying ALD and usually follows a chronic long course. However, the exact prevalence of ASH remains unknown. According to a study that used the National Inpatient Database in the US, ASH contributed to 0.8% of all hospitalizations in the US, with approximately 325,000 hospitalizations in 2010. The population burden of AC is underestimated and is not clearly known, and there is a higher probability of AC in patients hospitalized for alcohol-related problems. The clinical features of ASH are characterized by severe jaundice and are associated with the risk other related other complications. ASH can occur at any stage of liver disease and up to 80% of patients with severe ASH presenting with underlying AC. Patients with severe ASH are hospitalized for treatment, which can also lead to complications such as hepatic failure and sepsis. Alcoholic fatty livers can cause repeated parenchymal inflammation and cellular damage, which increase the risk of progression to fibrosis and cirrhosis. Various factors can contribute to ASH development, which are as follows: (1) toxic effects of acetaldehyde; (2) lipid peroxidation due to reactive oxygen species production and Deoxyribonucleic acid adduct formation; (3) pro-inflammatory cytokines levels. Repeated alcohol misuse can also result in changes in the colonic microbiota and increased intestinal permeability, resulting in increased serum lipopolysaccharides levels, which induce inflammatory actions in hepatic Kupffer cells [19-21].

3. Cirrhosis

AC is an advanced, chronic form of ALD. Progressive alcoholic steatosis can result in septal fibrosis and cirrhosis, which are characterized by the development of extensive scarring (fibrosis) and regenerative nodules. The development of fibrosis is a major change in ALD as it is an essential prerequisite for the exacerbation of cirrhosis. The progression of fibrosis varies according to the histological lesions of ALD. As with other etiologies, patients with AC are susceptible to decompensation-related complications due to high portal pressure and hepatic failure and are at risk of developing hepatocellular carcinoma [12,22]. A study of patients diagnosed with AC showed that the 1- and 5-year mortality rates were approximately 30% and 60%, respectively. Hepatic encephalopathy is considered the most dangerous sign and symptoms among the complications that define hepatic decompensation [23].

Alcohol use disorder

AUD is the leading cause of cirrhosis in European countries and the US. AUD is a chronic medical condition defined as an unhealthy pattern of alcohol consumption. When alcohol is not consumed, it can cause clinically significant impairment that can include compulsive alcohol quest, positive reinforcement, and negative emotional states. These include alcohol abuse, alcohol dependence, alcohol addiction, and a condition that some people colloquially call alcoholism [2,24].

AUD has become a significant public health concern over the past decade, and its prevalence has increased at an alarming rate. According to an epidemiological survey conducted in the US between April 2012 and June 2013, 12-month and lifetime prevalence rates of AUD were approximately 13% and 29%, respectively. The prevalence rate increased from 8.5% to 12.7% between 2001–2002 and 2012–2013, constituting an increase of approximately 50%. This increase was particularly prominent among minority-race

groups, city dwellers, women, and low-income individuals. AUD affects 10% of the European population [2,25].

AUD diagnosis using the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) requires at least two of the 11 criteria. According to the following DSM-5 criteria, the AUD is classified as follows according to the severity: mild, moderate, or severe (2–3, 4–5, or ≥6, respectively) (Table 1) [26-28]. Addiction specialists consider AUD as a chronic disorder of relapse and remission. For addiction experts, relapses in drinking should be defined by the amount and frequency of alcohol intake. However, most LT centers have an absolute view of alcoholic consumption and define any drinking as relapse [29-31].

Pre-LT management

Treating patients with AUD is extremely challenging both before and after LT. The most important and major treatment plan for patients who undergo LT after surgery is complete abstinence; if alcohol consumption continues even after medical or surgical treatment, the treatment effect is inevitably limited. If there is no improvement in liver function even after complete abstinence, and symptoms of decompensation persist, LT is the best treatment option.

All patients preparing for LT should be screened for AUD before surgery, even if the reason for LT is not ALD. AS the role of the pre-LT assessment is to identify the best fit for transplantation, an initial recommendation may be that an individual needs treatment for addiction before a final decision is made. Establishing abstinence before LT is the most important starting point. The AUD identification test (AUDIT) comprises 10 questions with a scoring system. An AUDIT score of >8 is considered a positive screening test result indicative of the presence of AUD. A score of >20 indicates alcohol dependence, and the patient should be referred to an addiction specialist (Table 2) [28]. Patients with AUD often undergo LT evaluation after hepatic decompensation (i.e., ascites, variceal bleeding, or hepatic encephalopathy) or are managed palliatively without considering a transplant.

1. Behavioral therapy for pre-LT AUD

Behavioral therapy is the mainstay of AUD treatment in LT candidates and recipients. LT programs in many Western transplant centers require regular attendance at alcoholic anonymous meetings after completing behavioral therapy before being put on a waiting list.

2. Pharmacotherapy for pre-LT AUD

The American Psychiatric Association (APA) has suggested the AUD treatment guidelines. This guideline describes several pharmacological agents, including naltrexone, acamprosate, disulfiram, topiramate, and gabapentin. The APA recommends the administration of naltrexone or acamprosate to patients with moderate-to-severe AUD as the first-line treatment. Naltrexone blocks the effects of opioid receptors and suppresses alcohol intake and desire. Drug levels of naltrexone after administration are different in

Table 1. DSM-V diagnostic criteria for alcohol use disorder

1. Do you end up drinking more, or longer, than you intended?
2. Have you more than once wanted to cut down or stop drinking, or tried to, but could not?
3. Do you spend a lot of time drinking alcohol or being sick or getting over other aftereffects?
4. Have you ever wanted a drink so badly that you could not think of anything else?
5. Have drinking or being sick from drinking often interfered with taking care of your home or family? Or caused job troubles? Or school problems?
6. Have you continued drinking even though it was causing trouble with your family or friends?
7. Do you give up or cut back on activities that are important or interesting to you, or give you pleasure, in order to drink?
8. Have you gotten into situations more than once while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?
9. Have you continued drinking even though it makes you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?
10. Do you have to drink much more than you once did to get the effect you want? Or do your usual number of drinks have much less effect than before?
11. When the effects of alcohol are worn off, do you experience withdrawal symptoms, such as trouble sleeping, shakiness, restlessness, nausea, sweating, a racing heart, or a seizure? Or do you sense things that are not there?

DSM-V, Diagnostic and Statistical Manual of Mental Disorders.

Table 2. The Alcohol Use Disorder Identification Test questionnaire

1. How often do you have a drink containing alcohol?
(0) Never (1) Monthly or less (2) 2–4 times per month (3) 2–3 times per week (4) 4+ times per week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?
(0) 1 or 2 (1) 3 or 4 (2) 5 or 6 (3) 7 or 9 (4) 10 or more
3. How often do you have six or more drinks on one occasion?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
4. How often during the last year have you found that you were not able to stop drinking once you had started?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
5. How often during the last year have you failed to do what was normally expected of you because of drinking?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
7. How often during the last year have you had a feeling of guilt or remorse after drinking?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
(0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily
9. Have you or someone else been injured as a result of your drinking?
(0) No (2) Yes, but not in the last year (4) Yes, during the last year
10. Has a relative, friend, doctor, or other health worker been concerned about your drinking or suggested that you should cut down?
(0) No (2) Yes, but not in the last year (4) Yes, during the last year

patients with compensated cirrhosis and those with decompensated cirrhosis; therefore, it is not recommended in the decompensated cirrhosis group with severe decompensated complications. Acamprosate has shown efficacy in treating AUD, especially for preventing alcohol relapse in previously sober patients. A study in patients with Child-Turcotte-Pugh (CTP) class A or B liver cirrhosis showed safety outcomes and, although results in the severe cirrhosis group; however, studies on patients with CTP class C liver cirrhosis are limited. Disulfiram is an alcohol-insensitive drug that alters the patient's response to alcohol, resulting in a dispersant and loathe experience. Disulfiram has hepatotoxic effect; therefore, it is not recommended for use in patients with advanced liver disease. The APA also suggests that topiramate or gabapentin should be administered be as the second-line treatment for patients with moderate-to-severe AUD. Topiramate is a Food and Drug Administration (FDA)-approved anticonvulsant that works as a glutamate blocking agent in addition to gamma-aminobutyric acid agonistic effects. The APA recommended its use in cases of intolerance or suboptimal responses to first-line drugs (i.e., naltrexone and acamprosate). Although topiramate causes no direct hepatotoxicity, it exhibits indirect hepatotoxicity because it is metabolized by cytochrome P3A4. Therefore, it increases the levels of valproic acid and other anticonvulsants, which may cause hepatic injury. Gabapentin is in a class of anticonvulsant medication approved by the FDA for treating epilepsy and nerve pain. The APA recommends gabapentin for patients who show first-line treatment failure or cannot tolerate first-line treatment.

In the future, there may be more drugs related to the treatment of AUD. For example, a randomized controlled trial reported that varenicline, a drug for smoking cessation, reduced binge drinking and usual social alcohol drinking days, and increased smoking cessation compared to with a placebo.

LT for ALD

Although ALD is one of the most common causes of LT in the US and Europe, LT has traditionally been not considered as an option for patients with ALD due to organ shortage and concerns for relapse [10,25,32].

The survival rates of patients at 1, 3, 5, and 10 years after LT for AC are reported to be 84%–89%, 78%–83%, 73%–79%, and 58%–73%, respectively. The overall survival rates were similar to those of LT in patients with HCV cirrhosis or hepatocellular carcinoma. However, the 10-year survival

rate for patients with harmful drinking after LT is 45%–71%, compared with 75%–93% for patients who refrain from drinking or occasionally drink [10,33-37].

Grat et al. [37] reported that there was no difference in the overall survival rate at postoperative 5 years in patients with ALD who underwent LT; however, the survival rate deteriorated beyond the fifth year after transplantation in patients with ALD, and ALD was a risk factor.

Hong et al. [11] reported that the 1-year and 3-year overall survival rates of deceased donor LT (DDLT) for the patients with ALD and HBV were 90.7% and 82.1% in the HBV patients group and 92.1% and 82.3% in the ALD patients group, in Korea, respectively. They reported that there were no significant differences in the 1-year and 3-year overall survival rates between the two groups [11].

The improvement in survival rate after LT in patients with ALD patients differs according to the severity of liver disease, especially in severe AC with CTP grade C decompensated cirrhosis. CTP grade C patients who underwent LT showed significantly higher 1-year and 5-year survival rates compared with the control group; however, there was no significant increase in survival benefits for AC patients with CTP grade A or B compared with the control group [38,39].

Prevalence of alcohol relapse after LT

LT can cure liver disease, but not AUD. Therefore, the LT teams should be aware that patients who have undergone LT for ALD may experience alcohol relapse whenever after surgery. However, the precise proportion of patients with alcohol relapse after LT remains unknown. The prevalence of alcohol relapse varies from 10% to 90% in several studies due to differences in the definition of relapse and follow-up time after LT. As per literature, 8%–20% of patients resume alcohol consumption 1 year after LT, and it gradually increases to 30%–40% at 5 years post-LT. Studies estimate that up to 20%–50% of patients who undergo LT will be readmitted for alcohol-related problems within 5 years after LT, and approximately 10%–15% of the patients resume heavy alcohol consumption [14,29,40-43].

A meta-analysis showed an alcohol relapse and heavy alcohol relapse rate of 22% and 14%, respectively, at a mean follow-up of 24 months after LT. Studies have shown poor outcomes in patients with alcohol relapse after performing LT for ALD compared with patients with no or intermittent alcohol intake. A meta-analysis by Rustad et al. [44] showed that patients with alcohol relapse had a higher risk of steatohepatitis, rejection, or liver failure, and mortality. The results of this study highlight the importance of examining alcohol use after LT and identifying steps to avoid negative consequences [5,44,45].

Definition of alcohol relapse after LT

The reported definitions of alcohol relapse vary widely, from sobriety to alcohol-related consequences, such as hospital readmissions, or physical, social, and legal consequences. Failure of sobriety is the commonly used definition because it emphasizes the recommendation to abstain from alcohol completely; however, there is no evidence that mild relapses (occasional "slips," less than once per month) have effects on graft or patient survival. The most effective methods for identifying alcohol relapse post-LT are the clinical interviews and the Alcohol Timeline Followback questionnaire; however, more research on the utility of combining several methods needs to be conducted [32,46].

Some experts stipulate relapse as four or more alcoholic drinks per day or 14 or more total drinks per week for at least 4 weeks [47,48]. Arab et al. [49] suggested a three-stage definition of alcohol relapse according to the relapse severity: (1) mild; (2) moderate (continuous and repeated drinking, at daily and weekly doses within the recommended standards of the National Institute on Alcohol Abuse and Alcoholism); and (3) severe (associated morbidity or mortality, which includes alcohol-induced hepatitis, pancreatitis, hepatic graft loss or other medical problems associated with alcohol recurrence). DiMartini et al. [50] suggested four patterns of alcohol consumption stage depending on the time of relapse, quantity, and duration as follows: (1) minimum drinking over a long period; (2) early relapse that progresses rapidly to moderate consumption; (3) early relapse that progresses to a repeated and continuous harmful drinking; (4) moderate drinking with a late start.

Many LT centers consider even a single drop of alcohol consumption after LT to be a relapse, and addiction experts do not support this definition. Data accumulated over the past decade show that reducing alcohol consumption, whether intoxicated or not, lowers overall morbidity, mortality, and health costs, and improves the psychosocial status. Alcoholism experts define AUD as a chronic disease

that recurs with exacerbation and improvement, and the treatment focuses on changing it from severe to mild [51-53].

Unfortunately, there are few well-designed studies on interventions to improve outcomes in patients with ALD after LT, as the treatment goals of post-LT studies for ALD are still unclear. Mathurin and Lucey [54] proposed that, in order to achieve this goal, future studies should recalibrate the outcome goals of recipients with ALD as either abstinence (the ideal outcome) or low-risk drinking (acceptable outcome).

Six-month abstinence rule

A 6-month abstinence rule has been proposed because of organ shortages and concerns regarding sharing limited resources with patients with self-inflicted conditions who are at risk of alcohol relapse after LT. In this context, pre-LT alcohol abstinence is one of the hottest and most controversial issues. This rule has been a general requirement since 1997. During a national meeting for LT in ALD in 1997, and all transplant professionals agreed that abstinence from alcohol prior to LT is an important prerequisite for selecting patients for LT and most European and US programs require a definite period of abstinence lasting 6 months (the so-called 6-month abstinence rule) [40,55]. To identify patients with ALD who are eligible for LT, many transplant centers in Western countries require the 6-month rule to followed before transplantation can be considered. However, unlike the US and Europe, Korea has a different cultural and social background than Western countries, and most organ transplants are made from living donors, which is about twice that of deceased donors. Most transplant centers in Korea do not require a specific period of sobriety before LT either living donor LT or deceased donor LT for patients with ALD. In recent years, more patients with ALD have received transplants from deceased donors in Korea. Of the deceased liver donors in 2012, 18.7% were allocated to patients with ALD, and the proportion was 38.0% in 2017, without the requirement of any abstinence period [3,43].

Although abstinence before transplantation is the most widely studied predictor, it has not been shown to be a significant predictor of recurrence after LT in many studies. Additionally, different cutoffs for pre-transplant abstinence (3 months and 1.5 years) have been found in various studies [56,57].

Table 3. Predictors of alcohol relapse after liver transplantation

First author	Year	No.	Relapse rate (%)	Abstinence predict post-LT relapse	Predictors of alcohol relapse
Jauhar [58]	2004	111	15	No	Family history of alcoholism
Kelly [59]	2004	100	20, harmful	No	Amount of pre-LT consumption, smoking, family support
Perney [60]	2005	61	52	Yes	Younger age, alcohol abuse in first relatives
DiMartini [61]	2006	167	42	Yes	NS
De Gottardi [62]	2007	387	11.9, harmful	Yes	Age >50 yr
Pfitzmann [63]	2007	300	11.9	Yes	Absence of companion, presence of young children
Gedaly [64]	2008	142	19	Yes	Drug abuse
Karim [65]	2010	80	10, harmful	Yes	Female sex
Hartl [56]	2011	120	16	Yes	NS
Egawa [66]	2013	140	22.9	No	Psychological disease history, smoking, Noncompliance with clinic visits after LT
Rice [67]	2013	300	16	Yes	Young age, biliary complication
Deruytter [68]	2013	108	29	No	Family history of alcohol abuse
Rodrigue [69]	2013	118	33.8	No	No hepatocellular carcinoma, smoking, social activity
Grat [37]	2014	97	33.5	No	Younger age
Satapathy [70]	2015	148	10.8, harmful	No	Older age, family support
Saigal [71]	2015	408	9.5	No	Younger age
Skladany [72]	2019	89	26	No	Smoking
Kitajima [57]	2019	190	13.7	No	Complication after LT, alcohol relapse before LT
Chung [43]	2021	129	13.9 (LDLT) 31.7 (DDLT)	No	Smoking

LT, liver transplantation; LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; NS, not studied.

The 6-month abstinence rule serves two purposes. First, to give the patient time to demonstrate abstinence (which is thought to be predictive of abstinence after LT) and second, to give the patient an opportunity to recover through medical treatment after abstinence [73].

Risk factors for alcohol relapse after LT

Various predictors of recurrence after LT have been investigated. Many studies are retrospective and used different definitions of alcohol relapse after LT, making comparisons challenging (Table 3) [37,43,56-73]. Several studies have identified the associations between demographic and clinical factors and alcohol relapse after LT. According to the literature, the commonly reported risk factors in the several studies are poor social and familial support, young age, smoking, psychiatric disorder, family history of AUD, previous treatment history for AUD, short abstinence period before transplantation, irregular follow-up, divorce, separation by death, and unemployment [5,43,49,50,61,72,74].

Conclusions

It is a known fact that abstinence is a pivotal factor for improving the long-term prognosis in patients who have undergone LT for ALD, and alcohol relapse is the most serious problem for patients. Few prospective studies have been conducted to reduce recurrence after LT for ALD. It has been considered that continuous intervention before and after LT is extremely important, and this can be achieved by the accurate identification of risk factors for recurrence after LT. In this sense, the key to the main treatment of these patients is the role of the multidisciplinary ALD team before and after surgery. A multidisciplinary team approach in combination with biochemical screening, can identify early recurrence and improves post-LT survival in patients with ALD. Recognition of risk factors, early detection of relapse after LT, and regular follow-up by a multidisciplinary team are essential to improve the prognosis of LT patients with ALD.

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Conflicts of interest

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Postoperative effects of bariatric surgery on heart rate recovery and heart rate variability

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Background: Several studies have reported associations between obesity and autonomic dysfunction. However, little research has investigated the effect of bariatric surgery on heart rate recovery (HRR) in the treadmill test and heart rate variability (HRV) in 24-hour Holter monitoring. We investigated the effects of bariatric surgery on HRR and HRV, which are parameters related to autonomic dysfunction.

Methods: We retrospectively investigated patients who underwent bariatric surgery in 2019. The treadmill test, 24-hour Holter monitoring, and echocardiography were performed before and 6 months after surgery. We compared the changes in HRR in the treadmill test and HRV parameters such as the time domain and spectral domain in 24-hour Holter monitoring before and after surgery.

Results: Of the 40 patients who underwent bariatric surgery, 25 patients had the treadmill test or 24-hour Holter monitoring both before and after surgery. Body weight and body mass index significantly decreased after surgery (112.86±24.37 kg vs. 89.10±20.26 kg, p<0.001; 39.22±5.69 kg/m^2 vs. 31.00±5.09 kg/m^2, p<0.001, respectively). HRR significantly increased (n=23; 43.00±20.97 beats/min vs. 64.29±18.49 beats/min, p=0.001). The time domain of HRV parameters increased (n=21; standard deviation of the N-N interval 123.57±28.05 ms vs. 152.57±39.49 ms, p=0.002 and mean N-N interval 791.57±88.84 ms vs. 869.05±126.31 ms, p=0.002).

Conclusions: Our data showed that HRR after exercise and HRV during 24-hour Holter monitoring improved after weight reduction with bariatric surgery through improved cardiac autonomic function.

Keywords: Autonomic nervous system disorders; Bariatric surgery; Heart rate recovery; Heart rate variability

Introduction

Obesity is a state in which body weight is increased due to excessive accumulation of fat, defined by a body mass index (BMI) of over 30 kg/m^2. The World Health Organization estimated that 13% of the population worldwide was obese in 2016 [1]. An epidemiological study on obesity conducted in the United States reported that obesity prevalence has been gradually increasing over time [2]. Obesity is an important factor of metabolic syndrome and independent risk for cardiovascular diseases and mortality [1,3]. Of the physiologic changes occurring in obesity, autonomic dysfunction is no-

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table. Generally, the autonomic function in obese individuals is disturbed. The main mechanisms influencing various factors, such as insulin resistance, are reduced vagal tone and overactivated sympathetic nervous function [1,4,5]. Impaired autonomic function is associated with all-cause and cardiovascular mortality [6]. One study reported that cardiac autonomic dysfunction could be responsible for the 40 times higher rate of sudden cardiac death in obese people as compared to those with normal body weight [4]. Previous studies reported improvement of the autonomic function after weight loss in obese people [7-10].

Bariatric surgery is very effective intervention for weight loss that improves metabolic complications. Long-term follow-up data showed effective remission and prevention of type 2 diabetes mellitus (DM), dyslipidemia and hypertension after Roux-en-Y gastric bypass surgery [11]. After gastric bypass surgery, both disease-related mortality and risk of cardiovascular events are reduced significantly [12,13]. However, the effect on autonomic function after bariatric surgery is inconclusive.

The cardiac autonomic function can be assessed based on the heart rate recovery (HRR) in the exercise test and the heart rate variability (HRV) in 24-hour Holter monitoring. HRR is commonly defined heart rate decrease after cessation of exercise, as a predictor of coronary arterial disease and cardiovascular mortality [14]. HRV is regulated by the interaction between sympathetic and parasympathetic activities, and decreased HRV is associated with cardiovascular events [15,16].

We investigated the effects of bariatric surgery on the autonomic function and the factors influencing the improvement in autonomic function.

Methods

Ethical statements: This study was approved by the Ethics Committee of Kosin University Gospel Hospital (No. 2021-05-018). The need for written informed consent from the participants was waived because of the retrospective nature of this study.

1. Study design and population

This was a retrospective, observational, single-cohort study. We reviewed the medical records of patients who underwent bariatric surgery in 2019 at Kosin University Gospel Hospital. We excluded patients who did not undergo baseline or follow-up treadmill test and 24-hour Holter monitoring. We compared the HRR 1 minute after the treadmill test and the HRR parameters such as time domain and spectral domain on 24-hour Holter monitoring at baseline and 6 months after surgery. We also compared the body weight, BMI, glycated hemoglobin (HbA1c), fasting insulin, C-peptide, lipid profile, and echocardiographic parameters. The patients were divided according to the type of surgical procedure which is one of the factors for autonomic function.

Hypertension was defined as systolic blood pressure (BP) >140 mmHg or diastolic BP >90 mmHg or previous diagnosis. DM was defined when the patient had fasting plasma glucose level >126 mg/dL, HbA1c level >6.5%, or was treated for DM. Dyslipidemia was considered if the patient was being treated with lipid-lowering drugs. Coronary arterial disease was defined as luminal stenosis of coronary artery over 50% on coronary angiography or computed tomographic coronary angiography or a history of previous coronary artery bypass graft or percutaneous coronary angioplasty. Chronic kidney disease was defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2 in the baseline laboratory test. Obstructive sleep apnea was confirmed using nocturnal polysomnography.

2. Exercise test

The exercise test was a treadmill test that was performed according to the Bruce protocol using GE CASE version 6.51 (GE Healthcare, Boston, MA, USA) before and after surgery. Participants were encouraged to exercise up to 80% of their age-predicted maximal heart rate (HR) and at stage III of the Bruce protocol. The BP and HR were recorded during the last 30 seconds of each stage immediately after the exercise and at 1, 2, and 5 minutes of recovery. HRR was defined as the difference between the maximal HR during exercise and the HR at 1 minute of recovery. S-T segment abnormality is defined as an upsloping depression ≥1.5 mm or horizontal or down-sloping depression ≥1 mm [14].

3. 24-Hour Holter monitoring

The patients were subjected to 24-hour Holter monitoring before and after surgery. The three-channel 24-hour register was obtained from seven electrodes on the thoracic surface. The electrodes were connected to a Seer Light recorder (General Electric, Milwaukee, WI, USA). While the

parasites were cleaned semi-manually during the analysis, the time domain parameters, such as standard deviation of the N-N interval (SDNN), standard deviation of sequential 5-minute N-N interval means (SDANN), mean number of normal sinus R (mean NN), percentage of successive normal sinus RR interval >50 ms (pNN50), and frequency domain parameters, such as very low frequency, low frequency, high frequency, and low to high frequency ratio, were recorded automatically.

4. Echocardiography

Standard two-dimensional echocardiography was performed for all subjects using a 3.5-MHz transducer. The examiners were blinded to the information of the subjects. The echocardiographic parameters measured included the left ventricle (LV) cavity diameter, LV end-diastolic volume, and LV end-systolic volume, according to the criteria of the American Society of Echocardiography. The LV ejection fraction was measured using the Simpson's method. The pulse-wave Doppler imaging of the trans-mitral LV inflow was performed in the apical four-chamber view, with the sample volume placed at the level of the mitral valve tips. Doppler variables were analyzed during three consecutive beats. The following measurements of the global LV diastolic function were also recorded: peak early (E) and late (A) diastolic mitral flow velocity, E/A ratio, and early (E') diastolic mitral annular velocity.

5. Statistical analysis

The data were analyzed using the Statistical Product and Service Solutions version 25.0 for Windows (IBM Corp., Armonk, NY, USA). All continuous values are reported as means±standard deviations. Normally distributed variables were compared using Student *t*-test. If the data were non-normally distributed, a Wilcoxon signed-rank test was used.

Results

Of the 40 subjects, 25 were enrolled in the final analysis. The mean age of the subjects was 37.4 years. The male was 14 (56%). Moreover, 14 subjects had DM (56%) and mean duration was 5.1 years (Table 1). Laparoscopic sleeve gastrectomy was performed in 14 patients (56%), laparoscopic Roux-en-Y gastric bypass surgery in 10 (40%), and gastric band ligation in one (4%) (Table 1).

Compared to the baseline, the postoperative body weight values reduced significantly (112.86±24.37 kg vs. 89.10±20.26 kg, $p<0.001$), and the BMI decreased according to the weight loss (39.22±5.69 kg/m^2 vs. 31.00±5.09 kg/m^2, $p<0.001$). The HbA1c, fasting blood sugar, fasting insulin, and C-peptide levels reduced significantly after surgery, as well as the Homeostatic Model Assessment for Insulin Resistance score that assesses insulin resistance. In the lipid profile, the triglyceride level and high-density lipoprotein cholesterol level were improved significantly (Table 2).

The echocardiographic variables are presented in Table 3. There were no significant differences in the LV end-diastolic diameter, LV end-systolic diameter, LV end-diastolic volume, LV end-systolic volume, end-diastolic interventricular septal thickness, and end-diastolic posterior LV wall thickness values after surgery. Moreover, no changes were noted in the Doppler parameters of E velocity, A velocity, E/A ratio, E', and E/E'.

1. Exercise test

Of the 25 patients, 23 underwent the exercise test before and after the surgery. No significant ST-T segment changes were observed. The total exercise time and metabolic equivalents improved significantly after surgery, whereas the baseline HR decreased and peak HR increased significantly. The HRR after 1 minute was significantly increased from the baseline (43.00±20.97 beats/min vs. 64.29±18.49 beats/min,

Table 1. Baseline characteristics

Characteristics	Value (n=25)
Age (yr)	37.40±10.49
Male sex	14 (56.0)
Hypertension	15 (60.0)
Diabetes mellitus[a)]	14 (56.0)
Dyslipidemia	11 (44.0)
Chronic kidney disease	1 (4.0)
Congestive heart failure	0
Coronary arterial disease	3 (12.5)
Fatty liver	13 (52.0)
Stroke	1 (4.0)
Sleep apnea	12 (48.0)
Laparoscopic sleeve gastrectomy	15 (60.0)
Laparoscopic Roux-en-Y gastric bypass	10 (40.0)

Values are represented as mean±standard deviation or number (%).
[a)]Mean duration, 5.1 years.

Table 2. Changes in metabolic parameters

Variable	Mean±SD		*p*-value
	Before surgery	After surgery (6 mo)	
Weight (kg)	112.86±24.37	89.10±20.26	<0.001
Body mass index (kg/m^2)	39.22±5.69	31.00±5.09	<0.001
HbA1c (%)	7.14±1.93	5.64±0.71	<0.001
Insulin, fasting (mIU/L)	32.13±33.57	7.41±4.06	<0.001
C-peptide, fasting (ng/mL)	2.64±1.32	1.90±1.86	<0.001
Fasting blood sugar (mg/dL)	127.50±56.83	89.16±14.16	<0.001
HOMA-IR	9.26±8.61	1.63±0.96	<0.001
Triglycerides (mg/dL)	190.41±134.68	110.76±63.09	0.002
Total cholesterol (mg/dL)	174.75±51.04	168.80±42.55	0.475
High-density lipoprotein (mg/dL)	45.31±12.74	49.52±11.99	0.025
Low-density lipoprotein (mg/dL)	111.08±48.01	104.96±36.87	0.357

SD, standard deviation; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

Table 3. Changes in echocardiographic parameters

Variable	Mean±SD		*p*-value
	Before surgery	After surgery (6 mo)	
LVEF (%)	64.97±4.17	61.19±4.08	0.681
LVEDD (mm)	50.60±4.39	50.22±3.59	0.823
LVESD (mm)	32.33±3.02	31.20±5.50	0.601
LVEDV (mL)	106.70±37.24	99.12±32.48	0.455
LVESV (mL)	37.69±13.60	36.02±11.54	0.823
IVSTd (mm)	10.82±1.46	10.45±1.47	0.140
PWTd (mm)	10.40±1.40	10.80±5.33	0.179
E velocity (cm/sec)	0.73±0.19	0.72±0.14	0.779
A velocity (cm/sec)	0.63±0.18	0.62±0.15	0.841
E/A ratio	1.27±0.55	1.18±0.43	0.888
E' (cm/sec)	0.10±0.11	0.08±0.01	0.794
E/E'	9.47±2.50	8.42±1.70	0.159

SD, standard deviation; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; IVSTd, interventricular septal end diastole thickness; PWTd, posterior left ventricular wall thickness; E velocity, peak early diastolic mitral flow velocity; A velocity, late diastolic mitral flow velocity; E', early diastolic mitral annular velocity.

p=0.001) (Table 4).

2. 24-Hour Holter monitoring

Of the 25 patients, 21 patients were subjected to 24-hour Holter monitoring before and after surgery. Although maximal HR was not significantly different before and after surgery, the average HR and minimal HR showed a significant reduction after surgery. Of the time domain parameters, the mean NN, SDNN, and SDANN significantly increased after surgery. However, the frequency domain parameters like very low frequency, low frequency, high frequency, and low to high frequency ratio, did not differ statistically (Table 4).

3. Types of bariatric surgery

In laparoscopic sleeve gastrectomy cases, the HRR in the exercise test and all parameters of the time domain and frequency domain in the 24-hour Holter monitoring showed significant differences. Although SDNN, SDANN, and mean NN almost showed a statistical difference (*p*=0.008, *p*=0.006, and *p*=0.015, respectively), the HRR and other HRV parameters had no significant difference in laparoscopic Roux-en-Y gastric bypass surgery cases (Table 5).

Table 4. Changes of exercises test parameters and 24-hour Holter monitoring parameters

Variable		Mean±SD		*p*-value
		Before surgery	After surgery (6 mo)	
Exercise ECG test				
Time (sec)		397.52±91.14	495.48±104.18	0.001
Metabolic equivalents		8.14±1.53	9.60±1.84	0.002
Baseline	SBP (mmHg)	124.95±16.95	121.19±17.63	0.104
	DBP (mmHg)	71.90±15.80	69.62±12.55	0.475
	HR (beats/min)	78.90±18.31	68.81±15.90	0.039
Peak	SBP (mmHg)	186.19±24.81	175.48±30.75	0.080
	DBP (mmHg)	77.90±24.05	81.38±20.38	0.455
	HR (beats/min)	143.00±21.47	156.57±19.13	0.007
Recovery	SBP (mmHg)	171.19±36.67	159.67±25.66	0.104
	DBP (mmHg)	68.81±21.05	69.67±15.16	0.398
	HR (beats/min)	100.00±11.84	82.29±21.09	0.010
Change in	SBP (mmHg)	61.24±31.08	54.29±25.11	0.139
	DBP (mmHg)	6.00±19.27	11.76±20.29	0.204
Heart rate recovery (beats/min)		43.00±20.97	64.29±18.49	0.001
24-Hour Holter monitoring				
QRS interval (beats)		97,967±17,304	77,020±20,365	<0.001
HR	Minimum	51.86±6.82	47.23±7.75	0.003
	Average	76.86±8.89	71.09±9.88	0.005
	Maximum	126.38±15.88	127.59±20.07	0.835
Mean NN (ms)		791.57±88.84	869.05±126.31	0.002
SDNN (ms)		123.57±28.05	152.57±39.49	0.002
SDANN (ms)		106.19±26.44	134.76±36.82	0.001
pNN50 (%)		11.66±8.01	15.29±11.42	0.181
Frequency (ms)	Very low	31.41±11.56	33.64±12.39	0.058
	Low	19.98±11.96	20.81±8.40	0.076
	High	13.34±5.04	14.89±5.62	0.181
L/H ratio		1.48±0.40	1.72±1.69	0.263

SD, standard deviation; EGC, electrocardiogram; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; mean NN, mean number of normal sinus R; SDNN, standard deviation of the N-N interval; SDNN, standard deviation of the N-N interval; SDANN, standard deviation of sequential 5-minute N-N interval means; pNN50, percentage of successive normal sinus RR intervals >50 ms; L/H, low frequency/high frequency.

DISCUSSION

The major results of this report were increased HRR and exercise capacity; increased HRV parameters of time domain including SDNN, SDANN, and mean NN; and reduced baseline HR on exercise test and mean HR on 24-hour Holter monitoring in patients after bariatric surgery. The metabolic parameters of DM and dyslipidemia also improved.

Previous studies have reported an association between autonomic function and weight reduction [7-9,17]. Brinkworth et al. [10] showed that the HRR improved after weight loss. Similarly, Wasmund et al. [18] reported improvement in the HRR 2 years after gastric bypass surgery, and Nault et al. [19] reported an increase in HRV on 24-hour Holter monitoring following weight loss after biliopancreatic diversion and duodenal switch surgery. Maser et al. [20] reported favorable effects on the cardiovascular autonomic function 12 months after surgery. However, some studies reported unfavorable results after bariatric surgery [21-23]. Zhang et al. [22] reported that approximately 4.2% of patients experienced orthostatic intolerance within 5 years of bariatric surgery, and 16.5% of them had severe symptoms that needed treatment with vasopressor agents. Our results showed improvement of the autonomic function, and no orthostatic intolerance was reported in the participants after bariatric surgery.

The mechanisms underlying the improvement of auto-

Table 5. Changes in parameters related to sleeve gastrectomy and bypass surgery

	Laparoscopic sleeve gastrectomy cases (n=15)			Laparoscopic Roux-en-Y gastric bypass surgery cases (n=10)		
	Before surgery	After surgery (6 mo)	*p*-value	Before surgery	After surgery (6 mo)	*p*-value
Weight (kg)	113.97±16.80	87.56±14.45	0.001	113.41±33.65	91.45±27.51	0.005
Body mass index (kg/m^2)	39.47±2.93	30.63±3.07	0.001	38.83±8.53	31.56±7.34	0.005
HRR (beats/min)	44.53±20.04	67.00±20.20	0.003	39.22±28.56	59.00±13.20	0.123
Mean NN (ms)	796.08±87.30	895.83±146.54	0.012	785.56±95.84	833.33±88.59	0.066
SDNN (ms)	117.08±30.58	152.17±38.88	0.008	132.22±23.12	153.11±42.65	0.086
SDANN (ms)	98.67±29.21	130.42±32.62	0.006	116.22±19.41	140.56±43.13	0.051
pNN50 (%)	9.58±6.10	18.42±12.90	0.015	14.43±9.71	11.12±7.92	0.374
Low frequency (ms)	18.48±4.98	22.22±10.10	0.060	21.98±17.78	18.94±5.38	0.594
High frequency (ms)	12.05±3.41	16.24±6.07	0.023	15.06±6.47	13.11±4.71	0.314
L/H ratio	1.57±0.38	1.33±0.39	0.008	1.35±0.42	2.23±2.53	0.173
HbA1c (%)	6.58±1.83	5.45±0.74	0.001	7.94±1.89	5.92±0.59	0.005
Insulin (mIU/L)	28.21±26.29	8.37±4.45	0.005	38.02±43.19	5.98±3.07	0.005
C-peptide (ng/mL)	2.60±1.49	1.66±0.65	0.004	2.70±1.11	2.28±2.88	0.139
HOMA-IR	8.19±9.80	1.78±1.03	0.004	10.87±6.62	1.41±0.84	0.005

Values are represented as mean±standard deviation.
HRR, heart rate recovery; mean NN, mean number of normal sinus R; SDNN, standard deviation of the N-N interval; SDANN, standard deviation of sequential 5-minute N-N interval means; pNN50, percentage of successive normal sinus RR intervals >50 ms; L/H, low frequency/high frequency; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

nomic function are complex and undefined. A prospective study with type 1 DM patients showed that BMI is an independent predictor of autonomic dysfunction as well as poor glycemic control [15]. Another recent study reported that the metabolic benefits after bariatric surgery are related to weight loss itself in type 2 DM patients [24]. Some studies suggest that the improvement of autonomic function is associated with weight reduction after bariatric surgery [18,20,25]. A previous study reported that the HRV improved after bariatric surgery, and this was associated with adipose tissue and adipocyte secretors that are affected by weight loss [26]. On the other hand, Perugini et al. [27] showed that the HRV improved after laparoscopic Roux-en-Y gastric bypass surgery, which correlates with insulin resistance but not with the degree of obesity. In this study, we could not determine a correlation between the autonomic function parameters and the independent factors such as weight reduction or insulin resistance.

Autonomic function is also influenced by the surgical technique [25,28,29]. A recent meta-analysis showed gastric sleeve gastrectomy is more effective on the parasympathetic nervous function due to preserving the vagal trunk of the stomach's lesser curvature [30]. other prospective study reported that both sleeve gastrectomy and gastric bypass surgery are effective in weight reduction, remission of obesity-associated comorbidities, and improvement of quality of life but less nutritional deficiency in sleeve gastrectomy [28]. In our data, only sleeve gastrectomy group showed significant increase of HRV and HRR parameters. however, previous multiple studies reported improvement of autonomic function with gastric bypass surgery. this study is inconclusive because of small sample size and uncontrolled other factors such as diabetes (35.71% in sleeve gastrectomy vs. 90% in gastric bypass).

HRR and HRV are one of the noninvasive methods of autonomic function and reflect balance of sympathetic activities and parasympathetic activities. Previous studies reported that the risk of coronary arterial disease, death from myocardial infarction, cardiovascular mortality was increased as reduced HRR and increased resting HR [31,32]. The ARIC study showed decreased HRV parameters were important risk factor of coronary heart disease [33]. The reduction of HRV on 24-hour Holter monitoring indicates the risk of increased arrhythmia after myocardial infarction and long-term cardiac events [34,35]. So, reduction of HRR and HRV parameters are associated with cardiac autonomic dysfunction and risk of cardiovascular events. Postoperative increase of the HRR and HRV suggests improvement of autonomic function and a reduction in the cardiovascular mortality risk. It supports the findings of a prior long-term study on the mortality benefits

after bariatric surgery, especially disease-related death including cardiovascular death [12].

The limitations of this study include the single-center design, small sample size, and relatively short follow-up duration. Other confounding factors, such as obstructive sleep apnea, were not well controlled. Adipocyte secretors and other neurohormones such as adiponectin and leptin were not considered in this study. Additional case-control studies with further long-term period data involving mortality and cardiovascular events are warranted.

In conclusion, our results showed that the HRR on exercise test and HRV on 24-hour Holter monitoring improved after bariatric surgery. This suggests that the improvement in the autonomic function with weight reduction after bariatric surgery could be one of the major factors in reduction of long-term cardiovascular mortality.

Article information

Conflicts of interest

Sung Il Im is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Analysis of ultradian rest-activity rhythms using locomotor activity in mice

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Background: Locomotor activity in mice may have an ultradian rest-activity rhythm. However, to date, no study has shown how locomotor activity can be explained statistically using fitted cosine curves. Therefore, this study explored whether the ultradian rhythm of locomotor activity in mice could be analyzed using cosine fitting analysis.
Methods: The locomotor activity of 20 male mice under a 12/12-hour dark/light cycle for 2 days was fitted to a cosine function to obtain the best fit. The mean absolute error (MAE) values were used to determine the explanatory power of the calculated cosine model for locomotor activity. The cosine fitting analysis was performed using R statistical software (version 4.1.1).
Results: The mean MAE was 0.2944, whereas the mean MAE for integrating the individual analyses in the two experimental groups was 0.3284. The periods of the estimated ultradian rest-activity rhythm ranged from 1.602 to 4.168 hours.
Conclusions: These results suggest that locomotor activity data reflect an ultradian rhythm better than a circadian rhythm. Locomotor activity can be statistically fitted to a cosine curve under well-controlled conditions. In the future, it will be necessary to explore whether this cosine-fitting analysis can be used to analyze ultradian rhythms under different experimental conditions.

Keywords: Activity; Locomotion; Mice; Ultradian rhythm

Introduction

The gross locomotor activities in mice show circadian patterns according to a 12/12-hour dark/light cycle [1]. Nocturnal mice are likely to be less active during the light phase and are more active during the dark phase [1]. Locomotor activities have been widely used to predict sleep-wake states [1-4]. Previous studies have reported that the total sleep duration of mice ranges from 6.2 to 14.9 hours and the awake state occupies 37.93%–74.2% of a 24-hour period [1,2,4].

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However, when the rodents were observed more closely, the locomotor activities showed a polyphasic pattern during the active phase beyond their circadian rhythms under the 12/12-hour dark/light entrained condition as well as the free running state [5,6]. The locomotor activity of mice can be expressed depending on their adaptation to the external environment [7,8]. Some studies suggest that the ultradian rhythm of locomotor activities can be related to feeding rhythms [9,10]. Other studies have reported that biological signals, such as body temperature, insulin, and glucocorticoid might be related to the ultradian rhythm of locomotor activities [11,12]. Considering the previous findings, locomotor activities might reflect the adaptation response to the environment or feeding conditions. Furthermore, these ultradian rest-activity rhythms can be reflected by dopaminergic neurotransmission [13,14]. Dopaminergic oscillation is closely related to a hedonic drive and novel seeking [15,16]. Movements to satisfy internal drive or navigate to changing environments might be expressed into gross locomotor activities.

In previous studies, the ultradian rhythm of locomotor activity was analyzed by the observation of peaks and troughs using graphical representations [12,17]. Recently, the ultradian rhythm was analyzed by autocorrelations, spectral analysis, periodograms, or wavelets [5,13,18]. However, there is a lack of studies on the extent to which locomotor activities can be explained statistically by fitted cosine curves based on ultradian rest-activity rhythms. Therefore, this study explored whether the real locomotor activity data in mice could be statistically fitted to the cosine curve within the range of ultradian rhythms, and investigate the amount of locomotor activity that can be explained by the ultradian rest-activity rhythms. In addition, based on the cosine fitting analysis, this study attempted to find and obtain the ultradian rest-activity rhythm of each mouse and estimate their ranges using cosine curves.

Methods

Ethical statements: This study was approved by the Institutional Animal Care and Use Committee of the Pusan National University Hospital (No. PNUH-2017-118).

1. Subjects

Male mice from the Institute of Cancer Research (n=20, weight 34–38 g, age 5–6 weeks) were used in this study. The subjects were acclimated to the new environment for 7 days at a constant room temperature (20°C±1°C) and humidity (40%–60%). The subjects were housed individually in cages. Food and water were provided ad libitum. The dark/light cycle was entrained by a 12/12-hour cycle using a light control system (Philips, Burlington, MA, USA).

2. Experimental procedure and measurement

After the acclimation period, free locomotor activities of each mouse in the cage were observed for 2 days under the 12/12-hour dark/light cycle to compare each ultradian rest-activity rhythm by light entrainment. The locomotor activity of each mouse was measured using infrared motion detectors and the Mlog system (Biobserve Inc., Bonn, Germany). Each detector was placed at the top of the cage. The raw data measured by the Mlog system reflect the intensity of locomotor activity, and a larger value indicates that the animal is more active. This study comprised two experiments that were performed at different times, and the mice were divided into two groups of 10 mice (groups 1 and 2) for each experiment. The data from each experiment were analyzed using the cosine curve fitting. To discover the ultradian rest-activity rhythms, we compared the values analyzed using the data of each mouse and the integrated data of all mice.

3. Individual data analysis

Individual locomotor activity data collected by the Mlog system for 2 days were preprocessed for cosine curve fitting. To reduce data variability with the neighboring data of locomotor activities, data were converted to minutes using an average. Outliers of the data beyond the range of 1.5 × interquartile range (IQR, Q3–Q1) were removed. The data were normalized within the range to obtain values between 0 and 1 (0–1 transformation). Considering that the locomotor activity data were displayed as 0 when there was no movement, the value of the cosine function was also transformed to a value between 0 and 1 (0–1 transformation). The cosine function with the highest fit was calculated repeatedly by changing the period and phase of the cosine function to obtain the best fit, as shown in Fig. 1. The following formula was used for the cosine function in this model:

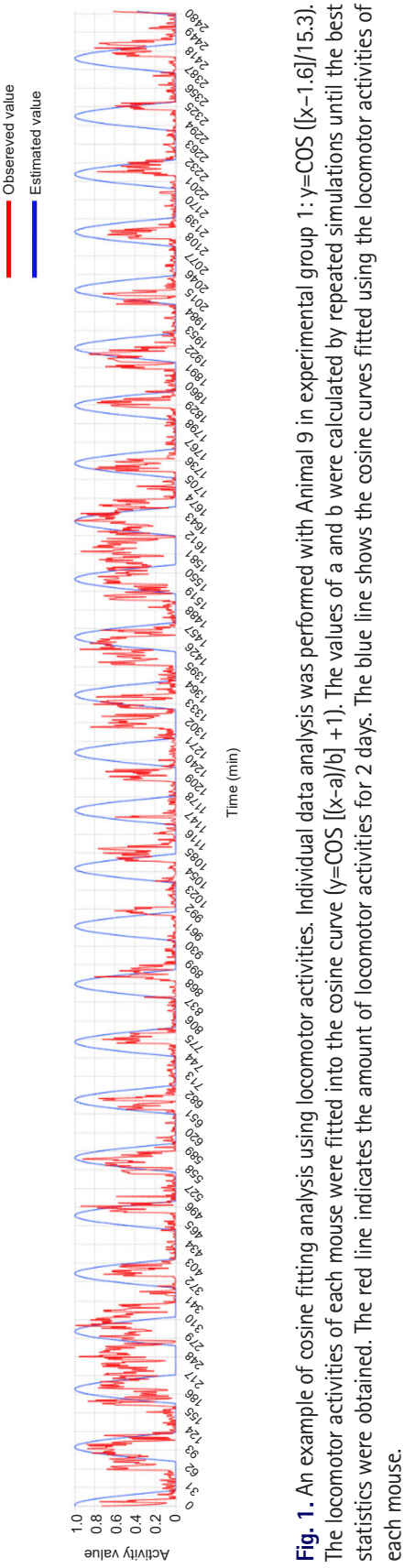

Fig. 1. An example of cosine fitting analysis using locomotor activities. Individual data analysis was performed with Animal 9 in experimental group 1 : y=COS ([x–1.6]/15.3). The locomotor activities of each mouse were fitted into the cosine curve (y=COS [(x–a)/b] +1). The values of a and b were calculated by repeated simulations until the best statistics were obtained. The red line indicates the amount of locomotor activities for 2 days. The blue line shows the cosine curves fitted using the locomotor activities of each mouse.

$$y=COS([x-a]/b)+1$$

where "a" and "b" are arbitrary real numbers for statistical simulation; a and b reflect the phase and amplitude of the cosine curve, respectively. The values of a and b were calculated using repeated simulations, until the best statistics were obtained. For intuitive judgment, it was judged based on the mean absolute error (MAE), which has a relatively small influence on outliers, and MAE values were used to determine the explanatory power of the calculated cosine model in explaining the measured locomotor activities.

4. Statistical analysis of measured data

If it is assumed that individual data can have similar patterns compared with each other, the data measured at the same time are integrated according to each period and converted into one data point for analysis of similarity on the fitted cosine curves. For data integration, mean, median, and max were used as group functions. In the process of integrating the data from the experimental group, the first quartile values sufficiently increased, and the process of transforming values using 0–1 transformation to change the negative value of the cosine function to 0 was unnecessary. Thus, the following formula was used for cosine function:

$$y=COS([x-a]/b).$$

Furthermore, as the data were merged, the y range limit of the cosine function increased from 40 to 100. The rest of the process was the same as that for individual data analysis. Mathematical simulations for cosine curve fitting were performed using R (version 4.1.1.; Foundation for Statistical Computing, Vienna, Austria).

Results

When the coefficients were fitted using individual data, as shown in Table 1, the mean MAE was 0.2944, variance was 0.000176, and standard deviation was 0.0132. Although the cosine graph of the fitted function showed a variance from the actual locomotor activities, the cosine functions were well fitted for each data based on the results that the variances of MAE were quite low. The mean MAE value (0.2944) in the data showed that approximately 70% of the fluctuations in locomotor activity were explained by a single cosine

Table 1. Individual statistical data analysis of measured locomotor activities using cosine curve fitting

Subject	Mean	Max	Min	Q1	Q3	SD	VAR	MAE[a]	Estimated a	Estimated b	Estimated period (hr)
Experimental group 1											
Animal 1	1.1879	10	0	0	2	2.3628	5.5831	0.3005	9.3	21.9	2.293
Animal 2	2.2189	17	0	0	3	4.0803	16.6494	0.2741	39.9	26.8	2.806
Animal 3	0.8924	10	0	0	0	2.2141	4.9024	0.2916	0	39.8	4.168
Animal 4	1.0802	10	0	0	0	2.3513	5.5289	0.2810	0	23.7	2.482
Animal 5	2.7746	20	0	0	4	4.9152	24.1596	0.2764	29.0	19.7	2.063
Animal 6	0.4770	5	0	0	0	1.2229	1.4955	0.2655	24.4	29.2	3.058
Animal 7	1.5964	12	0	0	2	2.9273	8.5694	0.3031	24.5	15.4	1.613
Animal 8	1.2225	10	0	0	2	2.3943	5.7326	0.3025	0	23.4	2.450
Animal 9	1.2444	10	0	0	2	2.4576	6.0398	0.2913	1.6	15.3	1.602
Animal 10	1.2014	10	0	0	1	2.4175	5.8445	0.2834	0	30.3	3.173
Experimental group 2											
Animal 11	1.1983	10	0	0	2	2.3813	5.6707	0.2934	39.9	33.6	3.519
Animal 12	2.1716	15	0	0	4	3.6793	13.5377	0.3017	39.9	16.6	1.738
Animal 13	1.5758	15	0	0	1	3.3122	10.9710	0.3026	0	39.4	4.126
Animal 14	1.0208	10	0	0	0	2.3011	5.2954	0.2924	39.9	25.9	2.712
Animal 15	0.8971	10	0	0	0	2.1892	4.7928	0.2871	39.9	38.7	4.053
Animal 16	2.3471	15	0	0	4	3.8717	14.9901	0.3148	0	19.9	2.084
Animal 17	1.2127	10	0	0	0	2.4627	6.0653	0.3023	0	22.9	2.398
Animal 18	1.2015	10	0	0	1	2.4119	5.8177	0.3131	0	16.8	1.759
Animal 19	1.1246	10	0	0	1	2.3053	5.3147	0.3151	7.2	16.7	1.749
Animal 20	1.3212	10	0	0	2	2.5055	6.2778	0.2973	8.3	39.9	4.178

The function for cosine curve fitting was used as y=COS ([x–a]/b)+1. The estimated period was calculated into data per hour based on the b value (period=b×2π/60).
Q1, first quartile; Q3, third quartile; SD, standard deviation; VAR, variance; MAE, mean absolute error.
[a]Mean MAE, 0.2944; VAR of MAE, 0.000176; SD of MAE, 0.0132.

Table 2. Statistics of cosine curve fitting analysis using statistics of measured data

Integrated data	Mean	Max	Min	Q1	Q3	SD	VAR	MAE[a]	Estimated a	Estimated b	Estimated period (hr)
Experimental group 1											
Mean	4.7175	17	0	1	7	3.9671	15.7382	0.3298	17	49	5.131
Median	2.1515	13	0	0	4	3.1019	9.6222	0.3581	48	32	3.351
Max	24.6241	83	0	8	37	19.0927	364.5324	0.3140	24	49	5.131
Experimental group 2											
Mean	4.7747	18	0	1	8	4.3435	18.8661	0.3049	5	56	5.864
Median	2.7803	15	0	0	5	3.8083	14.5036	0.3614	91	42	4.398
Max	23.9667	84	0	7	37	19.8799	395.2106	0.3027	10	56	5.864

The function for cosine curve fitting was used as y=COS ([x–a]/b). The estimated period was calculated into data per hour based on the b value (period=b×2π/60).
Q1, first quartile; Q3, third quartile; SD, standard deviation; VAR, variance; MAE, mean absolute error.
[a]Mean MAE, 0.3284; VAR of MAE, 0.000566; SD of MAE, 0.0237.

function.

Meanwhile, as shown in Table 2, the mean MAE for integrating each individual analysis in the two experimental groups was 0.3284, variance was 0.000566, and standard deviation was 0.0237. The results of the integrated data were inferior to those of individual data analysis. The similarity between the behavior patterns fitted by the cosine curves in the subjects was not high.

The estimated ultradian rest-activity rhythm ranged from 1.602 to 4.168 hours and 1.738 to 4.126 hours in the exper-

imental groups 1 and 2, respectively, as shown in Table 1. The periods of ultradian rest-activity rhythm estimated using integrated data ranged from 3.351 to 5.131 hours and 4.398 to 5.864 hours in the experimental groups 1 and 2, respectively, according to the data integration method in Table 2.

Discussion

The results of this analysis suggested the possibility of using a single cosine curve-fitting analysis to explore the ultradian rest-activity rhythm using locomotor activity in mice. In addition, the fitted cosine curve explained approximately 70% of the fluctuating locomotor activities. To compare the estimated ultradian rest-activity rhythm, these analyses were performed for the locomotor activities of the mice that were acclimated under well-controlled light conditions, including an illumination system with the 12/12-hour dark/light cycle. Therefore, it should be cautiously considered that these influences may be different under irregular or disrupted conditions undergoing a major change. Nevertheless, it is expected that locomotor activity in mice can be predicted to a large extent under the influence of the internal ultradian rest-activity rhythms of the individual in well-controlled stable environments.

However, the graph of the fitted function differed from the actual locomotor activity. Based on the MAE, the errors ranged from 26.7% to 31.5%. These differences may be caused by irregular locomotor activities due to adaptive responses to external environments [8,17]. Based on these findings, approximately 70% of the mouse's movement information may be affected by the internal ultradian oscillating rest-activity rhythms and 30% may be affected by external events or environment. In addition, some studies have reported that ultradian rest-activity rhythms may be reflected by the state of dopaminergic neurotransmission [13,14]. Meanwhile, dopaminergic neurotransmission has been related to addiction and depression [19,20]. Research on the relationship between addiction and depression with ultradian rhythms using cosine fitting analysis might be facilitated.

Meanwhile, the dopaminergic state can be influenced by various factors, such as genetic components, dopaminergic medications, and substances [13,14,21]. Considering the changeability of the dopaminergic state, these intrinsic ultradian oscillators may also change during long observation periods. Because fitting was attempted under the assumption that the ultradian rhythm will have a constant cycle, there is the possibility that these results may not be accurate in longitudinal observations. In case of the analysis of ultradian rhythms, wavelet analysis that can evaluate the changeable period of ultradian rhythms according to time would be useful [22].

The fitted cosine curves for the locomotor activities of each mouse were different with a period range from 1.602 to 4.168 hours, yet some mice showed similar movement patterns. The inter-mice differences were also supported by the finding that the MAE values after the integration of data in the experimental groups increased compared to the values before integration. These findings suggest the possibility that each individual has its own rest-activity rhythm depending on their own biological states or genetic characteristics. In this study, the period for cosine analysis of locomotor activity was not determined, and any period was allowed to be fitted within 2 days. As such, if the data strongly suggested a circadian rhythm (24-hour period), the circadian rhythm would be estimated as the best rhythm. However, in this study, cosine curves with a period ranging from approximately 1.6 to 4.2 hours were optimally fitted. These results suggest that the locomotor activity data reflect the ultradian rhythm better than the circadian rhythm; that is, even though the locomotor activities are influenced by circadian rhythms, they reflect the ultradian rhythm better. Previous studies have implied that the patterns of locomotor activities show an ultradian rhythm of several hours reflecting dopaminergic oscillation [13,14]. In studies using spectral analysis, the ultradian rhythms ranged from 2 to 6 hours, similar to the results of this study [14]. Thus, considering the findings of this study, cosine fitting analysis using locomotor activities can be useful in evaluating ultradian rest-activity rhythms.

This study had some limitations. First, because this study analyzed the locomotor activities of mice under relatively stable conditions, the results of this study cannot be generalized to the analysis of locomotor activities under every condition, including major stressful events or disrupting conditions. Second, cosine fitting analysis cannot be used with data that were observed for long-term periods, which cannot be assumed to have constant ultradian rhythms. Third, this study did not examine other supporting bio-

logical conditions that may affect movement. Fourth, the results of this study cannot be extended to female mice, because only male mice were included. Considering the sex difference between male and female mice, it is necessary to investigate whether these results are reproduced in female mice in the future study. However, this study suggests an appropriate analytical method for exploring ultradian rest-activity rhythms using locomotor activity in mice.

In conclusion, this study suggests that locomotor activities can be statistically fitted into a single cosine curve under well-controlled conditions with light entrainment of the 12/12-hour dark/light cycle. Each mouse might have its own ultradian rest-activity rhythm related to the biological conditions, such as the state of dopaminergic ultradian oscillation, as reported in previous studies [13,14]. In addition, this statistical analysis model using single cosine fitting analysis can be effectively utilized in research on ultradian rest-activity rhythms. As such, this ultradian rest-activity model using locomotor activity may be used to investigate the effects of any pharmacological agent on ultradian rhythms. In the future, it is necessary to explore whether this cosine-fitting analysis can be used to analyze ultradian rhythms under different experimental conditions.

Article information

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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STAT3 inhibition decreases ATP-induced *MUC8* gene expression in human airway epithelial cells

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Background: Contact between the human pulmonary system and bacteria, viruses, or other pathogens can induce airway diseases. Although pathogen-induced mucus oversecretion and hyperproduction are frequently observed in the human respiratory tract, the molecular mechanisms of pathogen-induced mucus hypersecretion and overproduction remain unclear. The objective of this study was to investigate the physiological signaling mechanism of adenosine triphosphate (ATP)-induced *MUC8* gene expression in human airway epithelial cells.

Methods: Real-time reverse transcription polymerase chain reaction, a cytokine array, and a Ca^{2+} concentration assay were performed to investigate the ATP/P2Y2-induced *MUC8* gene expression levels in human airway epithelial cells.

Results: The ATP/P2Y2 complex robustly secreted interleukin (IL)-6 in a time-dependent manner, whereas siRNA-P2Y2 did not. Moreover, ATP/P2Y2 induced *MUC8* gene expression. IL-6 secreted by ATP strongly elevated ATP/P2Y2-induced *MUC8* gene expression compared to ATP/P2Y2. Interestingly, a specific signal transducer and activator of transcription 3 (STAT3) inhibitor, 5,15-DPP, dramatically inhibited ATP/P2Y2/IL-6-induced STAT3 phosphorylation and resulted in an approximately 5-fold decrease in *MUC8* gene expression.

Conclusions: We showed that IL-6-activated STAT6 is essential for ATP/P2Y2-induced *MUC8* gene expression as part of inflammatory signaling by cytokines during airway inflammation. Our results provide a new molecular understanding of the signaling mechanism of *MUC8* gene expression during airway inflammation.

Keywords: Airway; Inflammation; Adenosine triphosphate; Mucin 8; STAT3 transcription factor

Introduction

Mucus has an essential function in the innate immune system against pathogens such as bacteria, virus, air pollutants, or fungus in the human respiratory track. Mucus hyperproduction and hypersecretion in airway track are frequently observed in a number of respiratory diseases, including asthma, chronic bronchitis, chronic obstructive pulmonary disease, and cystic fibrosis [1]. Various mucins have been named, but their exact physiological functions have yet to be identified. MUC8 has also been known to be expressed in the sinuses of chronic rhinosinusitis patients [2]. The partial cDNA sequence of *MUC8* has been identified by Shankar et al. [3]. For this reason, the mechanisms by which MUC8 affect inflammation in the respiratory track are unknown. Recently, we reported that MUC8 acts as anti-inflammatory

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mucin in reverse using the siRNA-*MUC8* system [4]. In this study, we examined the signal mechanism of adenosine triphosphate (ATP)/P2Y2-induced *MUC8* gene expression in human airway epithelial cells.

Purinergic receptors are ubiquitously expressed in many tissues and contribute to innate and adaptive immunity [5,6]. Of purinergic receptors, P2Y2 is expressed in the apical membrane of airway track cells [7] and is an important physiological receptor for airway inflammation [8]. P2Y2 is a Gαq-coupled receptor involved in intracellular signaling by heterotrimeric G proteins. This physiological mechanism has provided information on characterization of the receptor for ATP-induced *MUC8* gene expression, its Gαq-protein coupling, and secondary messengers of the downstream process after P2Y2 receptor activation. Thus, understanding of intracellular signaling cascades that trigger mucus overproduction/hypersecretion is required for inflammatory control in an affected microenvironment.

Recently, a small molecule signal transducer and activator of transcription 3 (STAT3) inhibitor, C188-9, prevented house dust mite-induced airway remodeling, airway inflammation, and T helper type 2 and 17 (Th2/Th17) cells accumulation [9]. This was not surprising because local blockade of interleukin (IL)-6 decreased STAT3 activation, increased Th17 inflammation, and increased Th2 responses in mice [10,11]. However, the biochemical and physiological mechanisms by which ATP/P2Y2-induced *MUC8* gene expression is affected by IL-6 in respiratory diseases remain unclear.

Methods

1. Materials and cell culture

ATP and 5,15-DPP were purchased from Merck (Darmstadt, Germany). The IL-6 ELISA kit was obtained from R&D Systems (Minneapolis, MN, USA). siRNA specifically targeting P2Y2 was synthesized by Bioneer (Daejeon, Korea). The siRNA sequences of P2Y2 were GAGGAAGGUGGCUUACCAA (dTdT). The human lung mucoepidermoid carcinoma cell line (NCI-H292) was obtained from the American Type Culture Collection (CRL-1848; Manassas, VA, USA). Cells were incubated in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) added with 10% fetal bovine serum in the presence of penicillin/streptomycin at 37°C in a humidified chamber with 5% CO_2.

2. Real-time RT-PCR

Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using a Bio-Rad iQ iCycler Detection System (Bio-Rad Laboratories, Hercules, CA, USA) with iQ SYBR Green SuperMix. The following primers were used: *MUC8*, forward (5'-GACCTGCCCCCATGGAC-3') and reverse (5'-CAGGAGTTCGAGACCAGCCT-3'). β_2M, forward (5'-CGCTCCGTGGCCTTAGC-3') and reverse (5'-GAGTACGCTGGATAGCCTCCA-3'). Reactions were performed in a total volume of 20 μL, which included 10 μL of 2× SYBR Green PCR Master Mix (Thermo Fisher, Waltham, MA, USA), 300 nM of each primer, and 1 μL of previously reverse-transcribed cDNA template. Real-time RT-PCR was performed on a MiniOption Real-time RT-PCR Detection System (Bio-Rad Laboratories). The parameters were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. All reactions were performed in triplicate. The relative quantity of mRNA was obtained using the comparative cycle threshold method and was normalized using β2-microglobulin as an endogenous control [12].

3. Cytokine assay

Cytokine levels were quantified using a Human Cytokine Array Panel A kit (R&D Systems, ARY005B) according to the manufacturer's instructions. Briefly, cells were plated in six-well plates 1 day before transfection with either a construct driving the expression of P2Y2 or P2Y2-specific siRNA using FuGENE 6 (Roche, Indianapolis, IN, USA). Twenty-four hours after transfection, serum-starved cells were treated with ATP for 4 hours. After treatment, supernatants were assayed for cytokine production according to the kit's instructions [13].

4. Calcium colorimetric assay

Ca^{2+} concentration level was quantified using a Calcium Colorimetric assay kit (ab102505; Abcam, Cambridge, UK) according to the manufacturer's instructions. Briefly, cells were plated in six-well plates 1 day before transfection with a construct driving the expression of P2Y2 using FuGENE 6 (Promega, Madison, WI, USA). Twenty-four hours after transfection, serum-starved cells were treated with ATP for 1 hour. The cells were assayed for Ca^{2+} concentration according to the kit's instructions.

5. Statistical analysis

The data are presented as the mean±standard deviation of more than three independent experiments. When appropriate, statistical differences were measured using Wilcoxon Mann-Whitney tests. A *p*-value less than 0.05 was considered statistically significant.

Results

1. ATP activates P2Y2 purinergic receptor to induce *MUC8* gene expression in NCI-H292 cells

We performed real-time RT-PCR to determine whether P2Y2 receptor is essential for ATP-induced *MUC8* gene expression in human airway epithelial (NCI-H292) cells. After transfection with either wild-type P2Y2 or siRNA-P2Y2 construct, ATP was applied for 24 hours (Fig. 1A). The ATP/P2Y2 complex dramatically induced *MUC8* gene expression compared to ATP but not to siRNA-P2Y2. This result suggests that P2Y2 is critical for ATP-induced physiological functioning. ATP is an inducer of inflammation in many tissues in humans. We posit that ATP signaling can breakdown the secretagogues in cells to augment the inflammatory signal by secreting several cytokines/chemokines from the cells. To test that, cytokine array was performed with cell medium (Fig. 1B). Interestingly, IL-6 was secreted out of the cells by the ATP/P2Y2 complex but not by siRNA-P2Y2, suggesting that P2Y2 mediates ATP-induced IL-6 secretion. In addition, IL-6 secretion peaked at 6 hours after ATP treatment and then decreased. These results suggest that ATP/P2Y2 could result in extracellular secretion of IL-6 to induce an inflammatory condition in airway epithelial cells.

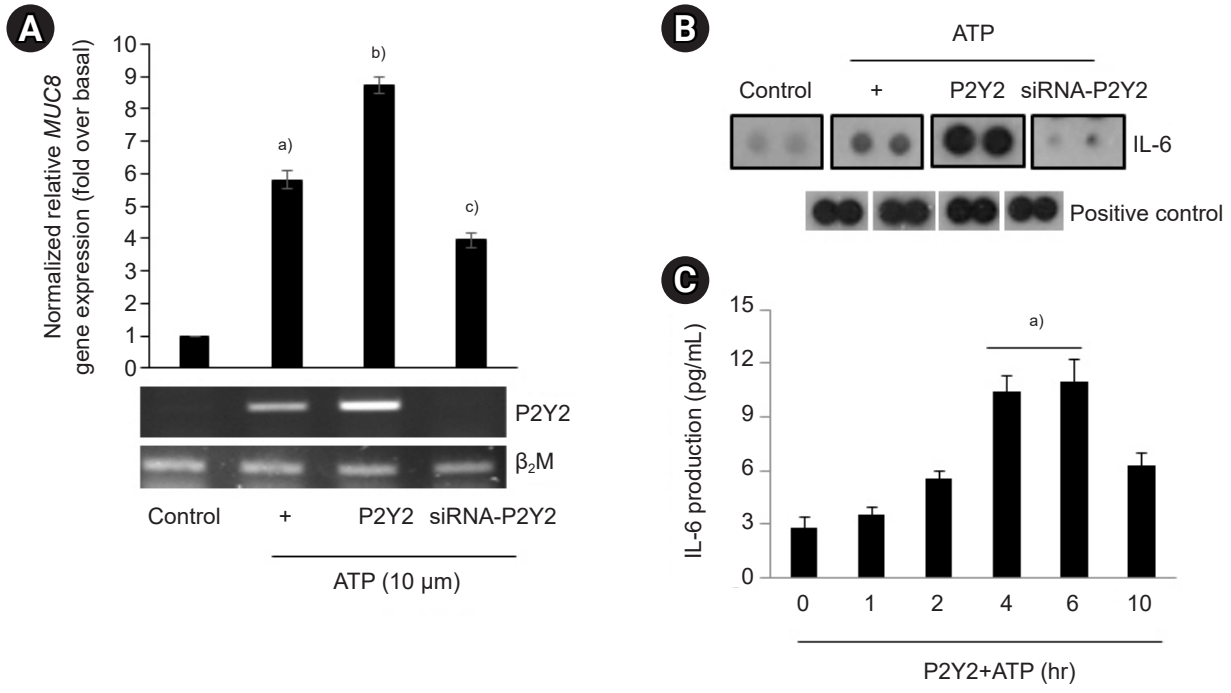

Fig. 1. ATP induces IL-6 secretion in a P2Y2-dependent manner in NCI-H292 cells. (A) Cells were transfected with either wild-type P2Y2 or a siRNA-P2Y2 construct and were then incubated with ATP for 24 hours before the generation of total cell lysates; *MUC8* transcripts were assessed by real-time RT-PCR. (B) A construct expressing wild-type P2Y2 or siRNA-P2Y2 was transiently transfected into NCI-H292 cells. The cells were washed, serum-starved overnight, and treated with ATP for 4 hours for a cytokine assay. The positive control was used as a loading control. (C) After transfection with a construct expressing wild-type P2Y2, NCI-H292 cells were incubated in a time-dependent manner. The supernatants were obtained to perform specific IL-6 ELISA. All figures are representative of three independent experiments. ATP, adenosine triphosphate; IL, interleukin; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay. [a] $p<0.05$ compared to the control; [b] $p<0.05$ compared to ATP only; [c] $p<0.05$ compared to P2Y2-transfected cells.

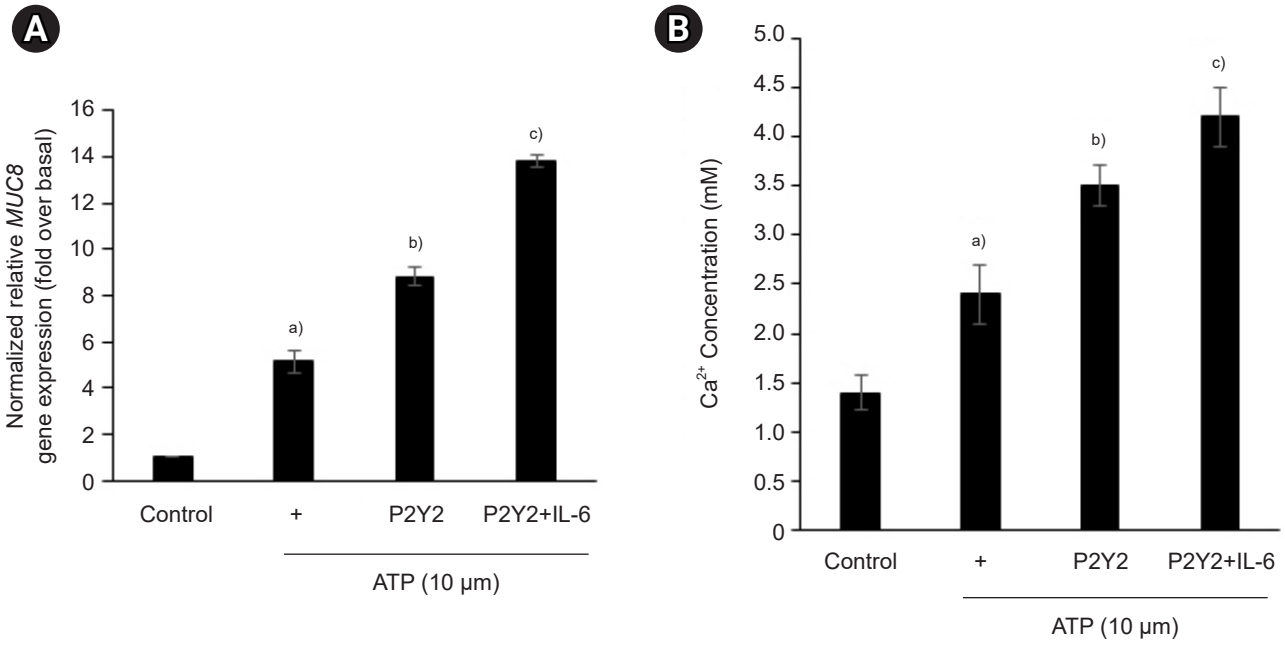

Fig. 2. Cotreatment with ATP and IL-6 dramatically induces *MUC8* gene expression via the Gαq-Ca^{2+} pathway. (A) Cells were transfected with a wild-type P2Y2 construct and were then incubated with ATP (10 μM) and IL-6 (50 ng/mL) for 24 hours before the generation of total cell lysates; *MUC8* transcripts were assessed by real-time RT-PCR. (B) Cells were plated in six-well plates 1 day before transfection with a construct driving the expression of P2Y2. Twenty-four hours after transfection, serum-starved cells were treated with either ATP or ATP/IL-6 for 1 hour. The cells were assayed for Ca^{2+} concentration according to the kit's instructions. All figures are representative of three independent experiments. ATP, adenosine triphosphate; IL, interleukin; RT-PCR, reverse transcription polymerase chain reaction. [a] $p<0.05$ compared to the control, [b] $p<0.05$ compared to ATP only; [c] $p<0.05$ compared to P2Y2-transfected cells.

2. Secreted IL-6 augments ATP/P2Y2-activated [Ca^{2+}] to increase *MUC8* gene expression

To examine whether secreted IL-6 could affect ATP as an essential factor in P2Y2-induced *MUC8* gene expression, extracellular ATP and purified IL-6 were applied to cells transfected with P2Y2 (Fig. 2A). Extracellular IL-6 treatment could strongly increase ATP/P2Y2-treated *MUC8* gene expression compared to ATP/P2Y2 only. Because P2Y2 is a Gαq-coupled receptor, intracellular calcium was measured after treatment with IL-6. As expected, intracellular calcium concentration was increased by extracellular IL-6 treatment (Fig. 2B). These results suggest that either/both autocrine or/and paracrine IL-6 can activate intracellular calcium concentration to increase ATP/P2Y2-induced *MUC8* gene expression in NCI-H292 cells.

3. STAT3 is essential for ATP/P2Y2-induced *MUC8* gene expression after cotreatment with IL-6

To investigate the signaling mechanism of ATP/P2Y2-induced *MUC8* overproduction after cotreatment with IL-6, the specific STAT3 inhibitor 5,15-DPP was utilized (Fig. 3). Because STAT3 can be frequently activated by IL-6, it is a pivotal transcription factor for cell proliferation. Interestingly, 5,15-DPP significantly decreased ATP/P2Y2-induced *MUC8* in a dose-dependent manner after cotreatment with IL-6. This is why IL-6 secretion is critical for airway inflammation in NCI-H292 cells. Although ATP activated Gαq-coupled receptor and secondary messengers like intracellular calcium, STAT3 might play a critical role in this signal complex.

Discussion

We identified how ATP signaling could induce *MUC8* gene expression via the P2Y2 G-protein coupled receptor receptor in human airway epithelial cells. Though ATP is an inflammation inducer, the precise signaling mechanism of ATP/P2Y2-induced *MUC8* gene expression remains unclear. Interestingly, IL-6 could be secreted extracellularly by the ATP/P2Y2 complex in a time-dependent manner

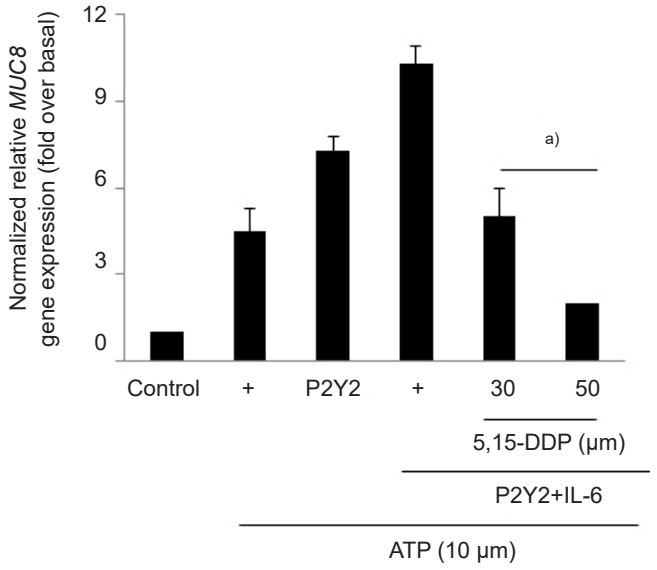

Fig. 3. STAT3 inhibition strongly decreases ATP/P2Y2-induced *MUC8* gene expression. The cells were transfected with a wild-type P2Y2 construct, pre-incubated with 5,15-DPP for 4 hours, and then treated with ATP (10 μM) and IL-6 (50 ng/mL) for 24 hours to generate total cell lysates. Then, *MUC8* transcripts were assessed by real-time RT-PCR. The figure is representative of three independent experiments. STAT3, signal transducer and activator of transcription 3; ATP, adenosine triphosphate; IL, interleukin; RT PCR, reverse transcription polymerase chain reaction. [a)] $p<0.05$ compared to the control.

(Fig. 1C) but not by siRNA-P2Y2. IL-6 is a pro-inflammatory cytokine that induces a number of physiological functions. The IL-6 secreted by the ATP complex was uptaken into nearby cells to transfer inflammatory signals. Surprisingly, IL-6 robustly induced ATP/P2Y2-induced *MUC8* gene expression compared to ATP/P2Y2 treatment (Fig. 2A). Thus, we posited that ATP could transmit inflammatory signals to boost or magnify inflammatory signaling to nearby cells. More interestingly, siRNA-P2Y2 did not induce this phenomenon, because P2Y2 is an essential ATP purinergic receptor in airway epithelial cells. In fact, many cytokines/chemokines were secreted in an ATP-time/dose-dependent manner, including regulated upon activation, normal T cell expressed, and secreted, IL-1α, and IL-1β; however, only IL-6 was affected by siRNA. Thus, we posit that IL-6 plays a critical role(s) in ATP signaling transduction in the airway.

Our previous study tested MUC8 as an anti-inflammatory mucin using siRNA study [4]. After 24 hours of ATP treatment, increased *MUC8* significantly inhibited ATP/P2Y2-dependent inflammatory cytokine production for more than 72 hours. Additional information for full-length cDNA and promoter sequences of *MUC8* is required to support the assumption that MUC8 is an anti-inflammatory mucin.

IL-6 is a small glycoprotein (21 KDa) produced by cells in the innate immune system [14]. Increased level of IL-6 has been frequently observed in asthmatic patients [15]. More importantly, a study examining IL-6 in bronchoalveolar lavage fluid has shown increased level of IL-6 in active asthmatic patients compared with the levels in healthy non-smoker, stable asthmatic, and non-asthmatic patients [16]. Thus, recently, scientists reported that inhibition of STAT3, a major downstream signal of IL-6, significantly decreased lung inflammation, airway remodeling, and inflammatory cell infiltration in asthma [9,17-19]. However, there is little information on the role of STAT3 in mucus hypersecretion and overproduction during airway inflammation.

In summary, our results showed that IL-6 is essential for ATP/P2Y2-induced *MUC8* gene expression in human airway epithelial cells. In addition, activation of STAT3 is mainly related to *MUC8* gene expression through induction of ATP/P2Y2. We suggest that the mucus hypersecretion/overproduction observed during airway inflammation is a consequence of ATP/ P2Y2-induced increase in *MUC8* gene expression within the inflamed microenvironment. Further analysis of the mucus hypersecretion/overproduction induced by various cytokines, bacterial products, or viruses might increase understanding of the pathogenesis of respiratory diseases.

Article information

Conflicts of interest

Kyoung Seob Song is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

Conceptualization: CHK, KSS. Methodology: CHK, KSS. Investigation: CHK, KSS. Funding acquisition: KSS. Writing-original draft: CHK, KSS. Writing - review & editing: CHK, KSS. Approval of final manuscript: all authors.

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Incidence of arterial steno-occlusive disease and related factors in patients undergoing coronary artery bypass graft surgery

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Background: Patients who undergo coronary artery bypass graft (CABG) surgery receive regular physical examinations and medications on an outpatient basis. However, these patients are at risk of developing other vascular diseases, such as postoperative arterial steno-occlusive disease (SOD). This study investigated the incidence of SOD and related factors.
Methods: In total, 246 patients who underwent CABG surgery from January 1, 2017 to December 31, 2021 were investigated. The incidence and risk factors of vascular disease were analyzed by dividing the included patients into SOD and non-SOD groups. Laboratory tests, medical history, surgical information, and family history were investigated through an electronic chart review.
Results: Data from 193 patients who met the criteria were analyzed. SOD occurred in 19.1% of patients, and the cerebral artery (38%) was the most common artery involved, followed by the peripheral artery (32%), the coronary artery (22%), and the retinal artery (8%). Risk factors for the development of SOD included estimated glomerular filtration rate (eGFR; odds ratio [OR]=0.977, p=0.008), cholesterol (OR=1.020, p=0.001), and patients with diabetes complications (OR=5.077, p=0.010). The 3-year cumulative incidence rate was 21.6%, and the risk factors for cumulative occurrence were a low eGFR, elevated cholesterol, and complications of diabetes.
Conclusions: Low eGFR, high cholesterol, and the presence of diabetic complications before CABG surgery may be associated with postoperative vascular disease. In these cases, close monitoring, proper drug administration, and patient warnings may be required.

Keywords: Coronary artery bypass; Steno-occlusive disease; Vascular disease

Introduction

Coronary artery bypass graft (CABG) surgery and percutaneous coronary intervention are two different therapeutic approaches for treating coronary artery disease (CAD) [1-4]. Surgery is usually performed in patients with diseases that involve multiple coronary arteries [5]. CAD is usually caused by atherosclerosis, a buildup of plaque inside the artery walls, which can either affect a single or more blood vessels throughout the body [6]. Although these patients might already have small artery stenosis or occlusion at the time of surgery, a significant portion of patients could

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be diagnosed with newly developed arterial disease after surgery.

After surgery, follow-up is mainly performed on an outpatient basis through medications, blood tests, and physical and radiologic examinations. Arterial disease can have a significant impact on patients and affect their quality of life and can have various clinical manifestations from claudication to stroke and even myocardial infarction.

The prevalence of other vascular diseases found in preoperative examinations has been reported in a number of studies. However, there are few reports on the frequency and risk factors of small artery disease in follow-up of patients after surgery. The purpose of this study was to investigate the frequency and factors related to postoperative arterial steno-occlusive disease (SOD).

Methods

Ethical statements: This study was approved by the Institutional Review Board of the Daegu Catholic University Hospital (IRB No. CR-22-020) with exemption from informed consent. Personal data related to patients' information were used, and personally identifiable information was protected. This study was a retrospective observational study, and this manuscript did not contain personally identifiable information. In addition, all study methods were performed in compliance with relevant guidelines and regulations.

1. Study population

This study included patients who received CABG for CAD from Daegu Catholic University Medical Center from January 1, 2017 to December 31, 2021. Patients who were already suffering from vascular disease at the time of surgery, cases with follow-up loss, or death after surgery were excluded. Data were collected retrospectively, and the patients' medical records were used. We investigated sex, age, and medical history as the baseline characteristics. The type of surgery, the number of anastomosed arterial vessels, and blood test results were also collected. Patients with vascular SOD were selected according to their outpatient treatment. All included patients were distinguished into two groups, namely, SOD and non-SOD groups, and statistical analysis was performed.

2. Disease definition and evaluation

Vascular SOD was defined as a disease group requiring treatment due to symptoms. Cerebrovascular accidents include retinal artery occlusion, peripheral arterial occlusive disease, and disease of native coronary artery or grafted vessels. If examination was necessary, computed tomography, magnetic resonance imaging, and angiograms were performed at the department in charge.

Patients complaining of decreased visual acuity and decreased visual field after surgery were referred to the ophthalmologist in this hospital for a fundus examination. At that time, patients had their best corrected visual acuity and intraocular pressure assessed by the ophthalmologist, who then performed a dilated fundus examination. In cases of suspected retinal vascular occlusive disease, fluorescein angiography was additionally performed to confirm the diagnosis. Patients diagnosed with retinal artery occlusion (central and branch) or ocular ischemic syndrome were included in the study.

3. Operative procedure and follow-up

CABG surgery was performed by median sternotomy after general anesthesia. Off-pump CABG was performed only in cases where aortic cross clamp was difficult to implement due to severe aortic atherosclerosis. On-pump CABG was performed to the remaining patients. Since the conduit was used as a graft, the left internal mammary artery was used as the basis, and great saphenous vein harvesting was performed according to the requirement for additional sites of anastomosis. After surgery, all patients received antiplatelet therapy. After discharge, follow-up was performed at the outpatient clinic every 3–6 months.

4. Statistical analysis

Categorical data are expressed as frequencies and percentages, and continuous data are expressed as mean±standard deviations with ranges. The chi-square test or Fisher exact test was used for categorical data, and the Mann-Whitney test was used for continuous data. Logistic regression analysis was performed to determine the risk factor for SOD. Kaplan-Meier was used to calculate the cumulative incidence. Cox regression analysis was performed to identify the risk factors of the cumulative incidence rate.

Results

1. Basic characteristics

Of the 246 patients initially included, we excluded 53 patients who did not visit the outpatient department within the last 6 months or had already suffered non-coronary artery SOD at the time of surgery.

The basic patient characteristics included in this study are summarized in Table 1. Of a total of 193 patients, 37 patients (19.1%) suffered SOD, and the detailed ratio is shown in Fig. 1. The mean age of the non-disease and SOD groups was 65.7 and 65.2 years, respectively. The proportions of males were 76.3% and 75.7%, respectively, whereas smoking and medical disease history rates were similar in both groups.

2. Operative and laboratory data

Table 2 demonstrates the operative and laboratory data. In both groups, conventional CABG was mainly performed, and the most common number of anastomoses required was three. Although there was no difference in most laboratory findings between the two groups, the estimated glomerular filtration rate (eGFR) was slightly higher in the non-SOD group, whereas cholesterol was higher in the SOD group. However, the differences were not statistically significant.

3. Analysis of the related factors for SOD

Table 3 shows the results of the risk factor analysis for SOD occurrence through multivariate logistic regression analysis. When there was a history of diabetes mellitus (DM) complications, the risk was approximately five times higher, and the *p*-value was 0.01. The higher the cholesterol, the higher the risk, and the higher the eGFR, the lower the risk. Patients with a family history of vascular disease had an approximately 2.7-fold increased risk, but there was no statistical significance.

4. Cumulative incidence of SOD post-CABG

The cumulative incidence rate is shown in Fig. 2. The overall 3-year cumulative incidence rate was 21.6%, but this rate increased to 89.2% in the SOD group. Risk factors for the cumulative occurrence of SOD included eGFR, elevated cholesterol, and history of DM complications.

Discussion

Among the causes of SOD, non-atherosclerosis such as

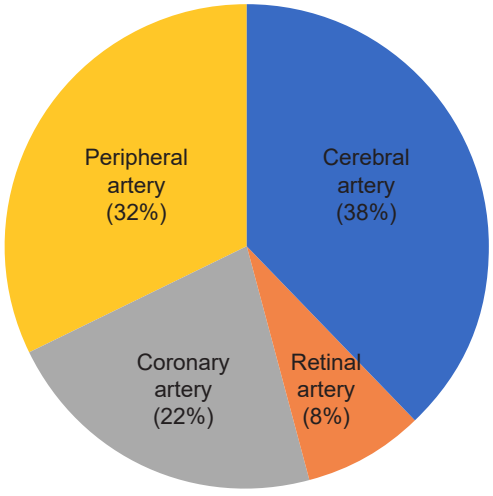

Fig. 1. The distribution of involved sites in cases of steno-occlusive disease in our study. Cerebral artery disease and peripheral artery disease had the highest rates (38% and 32%, respectively). Coronary artery disease involving the graft and native vessels occurred in 22% of cases. Retinal artery disease demonstrated the lowest rate (8%).

Table 1. Basic characteristics of the two groups

Variable	Non-SOD group (n=156)	SOD group (n=37)	*p*-value
Age (yr)	65.7±8.9	65.2±9.8	0.764
Sex (male:female)	119:37	28:9	1.000
Body mass index (kg/m^2)	23.8±2.7	24.0±2.6	0.744
Current smoking	17 (10.9)	8 (21.6)	0.140
Hypertension	67 (42.9)	18 (48.6)	0.657
Diabetes mellitus	33 (21.2)	9 (24.3)	0.843
Hyperlipidemia	26 (16.7)	7 (18.9)	0.933

Values are presented as mean±standard deviation or number (%).
SOD, steno-occlusive disease.

Table 2. Comparison of clinical characteristics before and during surgery between the two groups

Variable	Non-SOD group (n=156)	SOD group (n=37)	*p*-value
CABG (conventional:off-pump)	142:14	33:4	0.975
No. of grafted vessels			0.979
1	12 (7.7)	2 (5.4)	
2	21 (13.5)	6 (16.2)	
3	96 (61.5)	22 (59.5)	
4	19 (12.2)	5 (13.5)	
5	8 (5.1)	2 (5.4)	
Hemoglobin (g/dL)	12.4±1.7	12.3±1.8	0.684
Platelet (10^3/μL)	239.8±64.2	230.4±64.7	0.424
Prothrombin time (sec)	12.3±1.5	12.3±1.4	0.818
Activated prothrombin time (sec)	25.4±2.7	25.7±2.9	0.599
BUN (mg/dL)	16.7±8.4	20.5±13.3	0.104
Creatinine (mg/dL)	0.9±0.3	1.2±1.2	0.157
eGFR (mL/min/1.7 m^2)	82.7±27.6	72.8±30.5	0.056
Cholesterol (mg/dL)	168.7±38.1	182.3±46.8	0.064

Values are presented as number (%) or mean±standard deviation.
SOD, steno-occlusive disease; CABG, coronary artery bypass graft; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

Table 3. Multivariate logistic regression analysis for steno-occlusive disease

Variable	Odds ratio (95% CI)	*p*-value
eGFR	0.977 (0.962–0.992)	0.008
Diabetes mellitus complications	5.077 (1.412–18.252)	0.010
Cholesterol	1.020 (1.008–1.032)	0.001
Vascular disease history	2.776 (0.882–8.736)	0.081

CI, confidence interval; eGFR, estimated glomerular filtration rate.
Model selected by multivariate logistic regression analysis with backward elimination, R^2=0.501 and *P*=0.226 by the Hosmer-Lemeshow test for goodness of fit.

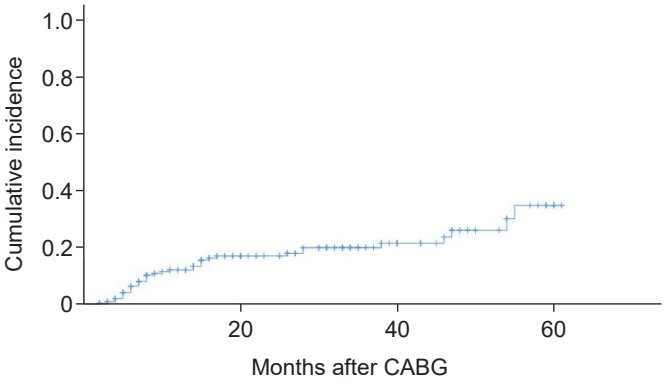

Fig. 2. The cumulative incidence of steno-occlusive disease (SOD) after coronary artery bypass graft (CABG). This figure shows the cumulative incidence of SOD in the study group. The overall 3-year cumulative incidence rate was 21.6%.

vasculitis, Buerger disease, and fibromuscular dysplasia are also known [7-9]. However, none of the patients included in our study were diagnosed with any of the above diseases. It is thought to be mainly caused by atherosclerosis. Atherosclerosis is a systemic disease associated with inflammation [10,11].

The majority of patients treated with CABG suffer from multivascular coronary artery stenosis or occlusion. In addition to the coronary arteries, these patients are likely to suffer from similar arterial disease throughout the body.

The prevalence of arterial disease at the time of CABG surgery has been previously reported in the literature [12,13]. In particular, carotid artery stenosis has been detected in a significant number of patients during preoperative CABG examinations, and thus, it is controversial whether to perform simultaneous or staged operation [14,15].

Nonetheless, the incidence of vascular disease after surgery has not been reported in the literature. In our study, we investigated the pattern of disease development in other blood vessels throughout the body in patients who had already developed CAD and had undergone CABG surgery.

In this study, patients' data were followed for a period of 5 years after CABG surgery. Among them, 19.1% of patients developed SOD after surgery. Head vessels were the most common, followed by peripheral arterial occlusive disease and coronary artery. One issue that might influence the incidence of these findings is that the examinations started

and a positive diagnosis was reached as soon as the patient started to complain of symptoms related to vascular disease.

In our study, we found that the risk factors that may influence the development of new vascular SOD after surgery in CABG patients were low eGFR and complications of DM, high cholesterol, and a family history of vascular disease.

To our knowledge, this is the first study providing results from patients who have undergone CABG. Therefore, commonly known risk factors for SOD of blood vessels are listed. Risk factors for cerebral artery disease are hypertension, smoking, DM, and hyperlipidemia [16-19], whereas risk factors for peripheral arterial disease include DM, hyperlipidemia, arterial hypertension, platelet activity, and smoking [20-22]. Our study reveals that the most important concerns for CABG patients is the onset of symptoms due to graft patency problems or native vessel disease progression after surgery. Risk factors for these important events include sex, hypertension, hyperlipidemia, DM, and chronic kidney disease [23-25].

The overall 3-year cumulative incidence rate was 21.6%, and the risk factors for the cumulative incidence were low eGFR, high cholesterol, and DM complications.

According to this study, a significant number of patients who received CABG may suffer from SOD in other arteries. In particular, patients with low eGFR, patients with DM complications, and patients with poor cholesterol control may develop vascular disease. Therefore, it is necessary to assess the patients' kidney function and cholesterol levels through periodic blood tests at outpatient clinics. Patients with high cholesterol should be prescribed with a lipid-lowering agent, whereas patients with poor cholesterol control should be referred to a related internal medicine clinic to receive appropriate treatment.

A limitation of this study is that it is a retrospective single-center study. Furthermore, there was no detailed patient information regarding any concomitant use of medications, such as antiplatelet drugs or hyperlipidemia drugs, during this study. Therefore, it is unknown how or whether these medications have affected the clinical results.

In conclusion, patients with low eGFR, high cholesterol, or diabetic complications, who have previously undergone CABG surgery, are particularly at increased risk of developing other vascular diseases. Therefore, it is necessary for these patients to receive the appropriate medication in the outpatient department and to have a close referral system with the relevant department.

Article information

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: GWL. Data curation: SGK. Formal analysis: CHL. Investigation: SGK. Methodology: GWL, CHL. Project administration: CHL. Resources: GWL. Software: CHL. Supervision: CHL. Validation: CHL. Visualization: SGK, LGW, CHL. Writing - original draft: SGK. Writing - review & editing: CHL.

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A prospective study of the correlation between hepatic fibrosis and noninvasively measured fibrosis markers including serum M2BPGi and acoustic radiation force impulse elastography

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Background: Mac-2 binding protein glycosylation isomer (M2BPGi) was introduced as a noninvasively measurable serologic marker for liver fibrosis. Acoustic radiation force impulse imaging (ARFI) elastography is another noninvasive method of measuring hepatic fibrosis. There are limited data about the correlations between histologic fibrosis grade and noninvasively measured markers, including M2BPGi and ARFI.

Methods: This prospective study was conducted among patients admitted consecutively for liver resection, cholecystectomy, or liver biopsy. ARFI elastography, serum M2BPGi levels, and the aspartate aminotransferase to platelet ratio index (APRI) score were evaluated before histologic evaluation. Histologic interpretation was performed by a single pathologist using the METAVIR scoring system.

Results: In patients with high METAVIR scores, M2BPGi levels and ARFI values showed statistically significant differences between patients with fibrosis and those without fibrosis. In 41 patients with hepatocellular carcinoma, as METAVIR scores increased, M2BPGi levels also tended to increase (p=0.161). ARFI values changed significantly as METAVIR scores increased (p=0.039). In 33 patients without hepatocellular carcinoma, as METAVIR scores increased, M2BPGi levels significantly increased (p=0.040). ARFI values also changed significantly as METAVIR scores increased (p=0.033). M2BPGi levels were significantly correlated with ARFI values (r=0.604, p<0.001), and APRI values (r=0.704, p<0.001), respectively.

Conclusions: Serum M2BPGi levels increased with liver fibrosis severity and could be a good marker for diagnosing advanced hepatic fibrosis regardless of the cause of liver disease.

Keywords: Elastography; Histology; Liver fibrosis; M2BPGi

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Introduction

The most important pathologic finding of chronic liver disease is liver fibrosis, which can progress to decompensated liver cirrhosis or hepatocellular carcinoma (HCC). Various kinds of surrogate markers for liver fibrosis have been developed in clinical fields [1,2]. In practice, commonly used indirect markers of liver fibrosis include the aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis-4 [3,4]. Liver fibrosis is traditionally diagnosed with liver biopsy, imaging, and surrogate biomarkers [1,5]. Among them, liver biopsy is the gold standard for diagnosis of liver fibrosis based on histopathological grade [6,7], but the information that can be obtained from a liver biopsy can be inaccurate because of insufficient biopsy tissue and focal sampling [5]. For histological diagnosis, the opinion of an experienced pathologist is very important because differences in diagnosis can occur between observers.

Acoustic radiation force impulse imaging (ARFI) elastography uses ultrasound to evaluate the stiffness of liver tissue. ARFI imaging has been demonstrated in many clinical settings, including hepatobiliary patients [8]. Currently, ARFI elastography is well known as a noninvasive modality to detect hepatic fibrosis mainly in patients with chronic viral hepatitis [9].

In addition, Mac-2 binding protein glycosylation isomer (M2BPGi) has been introduced as a noninvasive serologic marker for liver fibrosis [10,11]. Numerous studies have shown that M2BPGi can predict against liver fibrosis in various liver diseases, such as viral hepatitis, nonalcoholic fatty liver disease, autoimmune hepatitis, primary biliary cirrhosis, and biliary atresia [12].

This prospective study aimed to evaluate the performance of noninvasive hepatic fibrosis markers, including M2BPGi and ARFI point shear wave elastography result, and to compare it with the liver histologic fibrosis grade.

Methods

Ethical statements: This study design was approved by the Institutional Review Board of Kosin University Gospel Hospital (KUGH 2015-06-105). All patients gave written consent for histologic evaluation of the liver at the time of initial consent for study participation.

1. Study protocol

This study was performed prospectively in patients admitted consecutively for liver resection, cholecystectomy, or liver biopsy from September 2015 to April 2020. ARFI elastography, serum M2BPGi measurement, and APRI testing were performed before histologic evaluation.

2. Measurement of M2BPGi

Preoperative serum samples were collected before histologic evaluation. Serum M2BPGi was measured at baseline using an automated immunoanalyzer (HISCL-800; Sysmex, Kobe, Japan). The measured result was presented as a cutoff index calculated as follows:

$$\text{WFA-M2BP}=(\text{WFA-M2BPsample}-\text{WFA-M2BPnc})/(\text{WFA-M2BPpc}-\text{WFA-M2BPnc}),$$

where WFA-M2BPsample is the measured value of the patient serum sample; WFA-M2BPnc is the negative control value; and WFA-M2BPpc is the positive control provided by the manufacturer.

3. ARFI imaging

Liver stiffness was measured using an ARFI elastography machine (ACUSON S3000; Siemens, Munich, Germany) at the Liver Clinic of Kosin University Gospel Hospital. For the ARFI examination of the liver, the patient lay in a supine position with abduction of the right arm. The liver was evaluated with grayscale ultrasound before ARFI, which was performed at 2–3 cm below the liver capsule, away from large vessels. For each patient, the ARFI value was obtained by repeating measurement more than three times, and then the average value was used in this study.

4. Histologic assessment of hepatic fibrosis

All liver histology interpretations were performed by a single pathologist well experienced in hepatobiliary pathology. A tissue sample was stained and scored for degree of fibrosis according to the METAVIR scoring system, which specifies a fibrosis score from 0–4 points; no portal fibrosis (stage F0), portal fibrosis without septa (stage F1), portal fibrosis with few septa (stage F2), septal fibrosis without cirrhosis (stage F3), and cirrhosis (stage F4) [13].

5. Laboratory examination

Laboratory data were collected at the time of the initial admission to the hospital. Complete blood count results, including hemoglobin concentration, white blood cell count, platelet count, and prothrombin time, and blood chemistry data were recorded. The APRI score was calculated using laboratory data as follows [14]:

$$\text{APRI}=([\text{AST/upper limit of the normal range of AST}]\times 100)/\text{platelet}\times 10^{9}/\text{L}$$

6. Statistical analysis

Demographic data and baseline characteristics are presented as mean and standard deviation values for continuous variables. To compare the three or four groups according to histologic fibrosis grade, one-way analysis of variance or the Kruskal-Wallis test was performed with continuous variables, and linear-by-linear association was performed with categorical variables. Statistical significance was determined as $p<0.05$ using SPSS software version 23 (IBM Corp., Armonk, NY, USA).

Results

1. Baseline characteristics

A total of 74 patients were included in this study. The mean age was 59.2±9.0 years, and 55 of the patients (74.3%) were male. The mean body weight was 65.2±11.0 kg, 19 patients (25.7%) had diabetes, and 26 patients (35.1%) had hypertension. Hepatitis B surface antigen positivity was found in 37 patients (50.0%), and anti-hepatitis C virus positivity was found in nine (12.2%). Thirteen patients had alcoholic liver disease, and 41 (55.4%) had HCC. Liver tissue was acquired through liver resection (68.9%), metastasectomy (8.1%), cholecystectomy (5.4%), liver transplantation (9.5%), and needle biopsy (8.1%). Histologic fibrosis grades were identified as F0/1 (35.1%), F2 (14.9%), F3 (12.2%), and F4 (37.8%) based on the METAVIR system (Table 1).

2. M2BPGi and ARFI correlated with METAVIR score

In all 74 patients, as the METAVIR score increased, there was a statistically significant difference between histologic fibrosis and both the M2BPGi (Fig. 1A) and ARFI (Fig. 1B) values. The M2BPGi result showed no statistical difference depending on the cause of liver disease, including hepatitis B virus, hepatitis C virus, or alcohol consumption ($p=0.884$). The APRI value also showed no significant difference according to the cause of liver disease ($p=0.066$) (Table 1).

1) M2BPGi and ARFI correlated with METAVIR score in HCC

Forty-one patients were diagnosed with HCC. As the METAVIR score increased, the M2BPGi level also tended to increase, but there was no statistical significance ($p=0.161$) (Fig. 2A). However, the ARFI value elevated significantly as the METAVIR score increased ($p=0.039$) (Fig. 2B).

2) M2BPGi and ARFI correlated with METAVIR score without HCC

Thirty-three patients did not have HCC. As the METAVIR score increased, the M2BPGi level also significantly increased ($p=0.040$) (Fig. 3A), and the ARFI value elevated significantly ($p=0.033$) (Fig. 3B).

3. Correlation among noninvasive fibrosis markers

The M2BPGi level was found to have a significant correlation with the ARFI value ($r=0.604$, $p<0.001$) (Fig. 4A) and the APRI value ($r=0.704$, $p<0.001$) (Fig. 4B).

Discussion

This prospective study confirmed a significant relationship between hepatic fibrosis grade and serum M2BPGi. Even in HCC patients, the M2BPGi level showed a tendency to infer hepatic fibrosis. M2BPGi showed a significant association with both the ARFI value as an elastography marker and APRI as a serologic marker.

M2BPGi, a secreted glycoprotein present in the extracellular matrix, correlates with liver fibrosis [15]. M2BPGi was chosen as a biomarker of liver fibrosis using serum from chronic hepatitis C patients, in whom it is currently used in hepatic fibrosis evaluation [16,17]. In addition, the M2BPGi level in patients with chronic hepatitis B increased as liver fibrosis progresses. However, the M2BPGi level in chronic hepatitis B patients was slightly lower than that in chronic hepatitis C patients [18]. In addition, the M2BPGi level for diagnosing liver fibrosis in nonalcoholic fatty liver disease patients was lower than that in chronic hepatitis C patients. Therefore, it is necessary to consider the cause of the underlying liver disease when interpreting the M2BPGi results [19]. According to the results of the present study, the M2B-

Table 1. Baseline characteristics, M2BPGi levels, and laboratory data of patients by cause of liver disease

Characteristics	All (n=74)	Cause of disease				*p*-value
		HBV (n=37)	HCV (n=9)	Alcohol (n=13)	Unknown (n=15)	
Age (yr), median (IQR)	58.5 (54.0–66.0)	57.0 (52.5–63.0)	57.0 (55.5–62.5)	59.0 (52.0–67.5)	65.0 (55.0–71.0)	0.246
Sex, no. (%)						0.002
Male	55 (74.3)	32 (86.5)	7 (77.8)	10 (76.9)	6 (40.0)	
Female	19 (25.7)	5 (13.5)	2 (22.2)	3 (23.1)	9 (60.0)	
Body scale, median (IQR)						
Body weight (kg)	64.80 (57.20–70.60)	68.00 (58.25–75.20)	60.40 (55.80–77.10)	64.00 (54.75–68.50)	64.80 (54.00–69.60)	0.490
Height (cm)	165.4 (159.7–168.0)	166.0 (160.4–169.5)	166.7 (160.5–169.7)	165.0 (158.0–169.0)	160.0 (155.1–165.0)	0.103
Co-morbidity, no. (%)						
Diabetes mellitus	19 (25.7)	7 (18.9)	4 (44.4)	4 (30.8)	4 (26.7)	0.452
Hypertension	26 (35.1)	11 (29.7)	3 (33.3)	6 (46.2)	6 (40.0)	0.331
Hepatocellular carcinoma, no. (%)	41 (55.4)	28 (75.7)	8 (88.9)	4 (30.8)	1 (6.7)	<0.001
Noninvasive marker, median (IQR)						
ARFI	1.50 (1.25–1.88)	1.52 (1.39–1.84)	1.82 (1.59–2.46)	1.44 (1.24–2.11)	1.23 (1.12–1.50)	0.011
M2BPGi	1.12 (0.62–1.99)	1.08 (0.68–1.84)	1.26 (0.53–7.59)	1.12 (0.42–2.33)	1.21 (0.54–2.25)	0.884
APRI	23.86 (13.53–51.44)	28.69 (15.97–56.07)	36.59 (15.31–81–81)	16.24 (9.99–29.20)	16.49 (8.82–45.00)	0.066
Tissue acquisition, no. (%)						0.183
Liver resection	51 (68.9)	27 (73.0)	8 (88.9)	8 (61.5)	8 (53.3)	
Cholecystectomy	4 (5.4)	3 (8.1)	0	0	1 (6.7)	
Metasectomy	6 (8.1)	0	1 (11.1)	1 (7.7)	4 (26.7)	
Liver transplantation	7 (9.5)	5 (13.5)	0	2 (15.4)	0	
Needle biopsy	6 (8.1)	2 (5.4)	0	2 (15.4)	2 (13.3)	
METAVIR						0.001
F 0/1	26 (35.1)	8 (21.6)	1 (11.1)	6 (46.2)	11 (73.3)	
F2	11 (14.9)	6 (16.2)	1 (11.1)	2 (15.4)	2 (13.3)	
F3	9 (12.2)	7 (18.9)	0	1 (7.7)	1 (6.7)	
F4	28 (37.8)	16 (43.2)	7 (77.8)	4 (30.8)	1 (6.7)	
Laboratory result, median (IQR)						
WBC ($\times 10^3/\mu L$)	5.08 (4.00–6.19)	4.82 (3.59–6.04)	4.45 (2.94–5.09)	5.96 (5.22–7.45)	5.18 (4.41–6.98)	0.038
Platelet ($\times 10^3/\mu L$)	157.11 (111.50–214.75)	131.00 (111.00–183.50)	116.00 (71.50–141.00)	234.00 (142.50–282.50)	204.00 (116.00–247.00)	0.004
PT (INR)	1.03 (0.98–1.11)	1.02 (0.99–1.17)	1.03 (0.94–1.08)	1.02 (0.97–1.35)	1.03 (0.97–1.09)	0.844
Protein total (g/dL)	7.10 (6.70–7.50)	7.15 (6.80–7.50)	7.30 (7.10–7.85)	6.90 (6.55–7.35)	7.10 (6.60–7.70)	0.317
Albumin (g/dL)	4.10 (3.80–4.42)	4.30 (3.85–4.45)	4.00 (3.65–4.55)	3.80 (3.75–4.30)	4.10 (3.70–4.50)	0.526
Total bilirubin (mg/dL)	0.83 (0.60–1.31)	0.93 (0.66–1.49)	0.75 (0.60–1.17)	0.76 (0.56–3.57)	0.67 (0.47–0.90)	0.316
Direct bilirubin (mg/dL)	0.31 (0.21–0.50)	0.34 (0.23–0.53)	0.34 (0.25–0.38)	0.25 (0.20–1.71)	0.27 (0.19–0.43)	0.423
AST (U/L)	35.00 (23.75–54.50)	35.00 (27.00–55.50)	36.00 (22.05–60.50)	38.00 (22.50–64.00)	31.00 (20.00–45.00)	0.862
ALT (U/L)	26.00 (16.75–38.25)	28.00 (19.00–40.50)	23.00 (15.50–28.50)	17.00 (12.00–42.50)	26.00 (16.00–39.00)	0.335
Creatinine (mg/dL)	0.69 (0.57–0.87)	0.74 (0.63–0.86)	0.79 (0.59–0.90)	0.68 (0.58–0.92)	0.56 (0.47–0.90)	0.281
hs-CRP (mg/dL)	0.13 (0.03–0.90)	0.13 (0.02–0.51)	0.03 (0.02–0.18)	0.83 (0.16–2.63)	0.10 (0.04–0.97)	0.023

M2BPGi, Mac-2 binding protein glycosylation isomer; HBV, hepatitis B virus; HCV, hepatitis C virus ; IQR, interquartile range; ARFI, acoustic radiation force impulse; APRI, aspartate aminotransferase to platelet ratio index; WBC, white blood cell; PT (INR), prothrombin time (international normalized ratio); AST, aspartate aminotransferase; ALT, alanine aminotransferase; hs-CRP, high-sensitivity C-reactive protein.

PGi level does not appear to change significantly depending on the cause of liver disease. It was also revealed to be able to predict hepatic fibrosis without being significantly affected by the presence or absence of HCC. This result will be helpful in evaluating the degree of hepatic fibrosis using M2BPGi easily and conveniently in clinical practice.

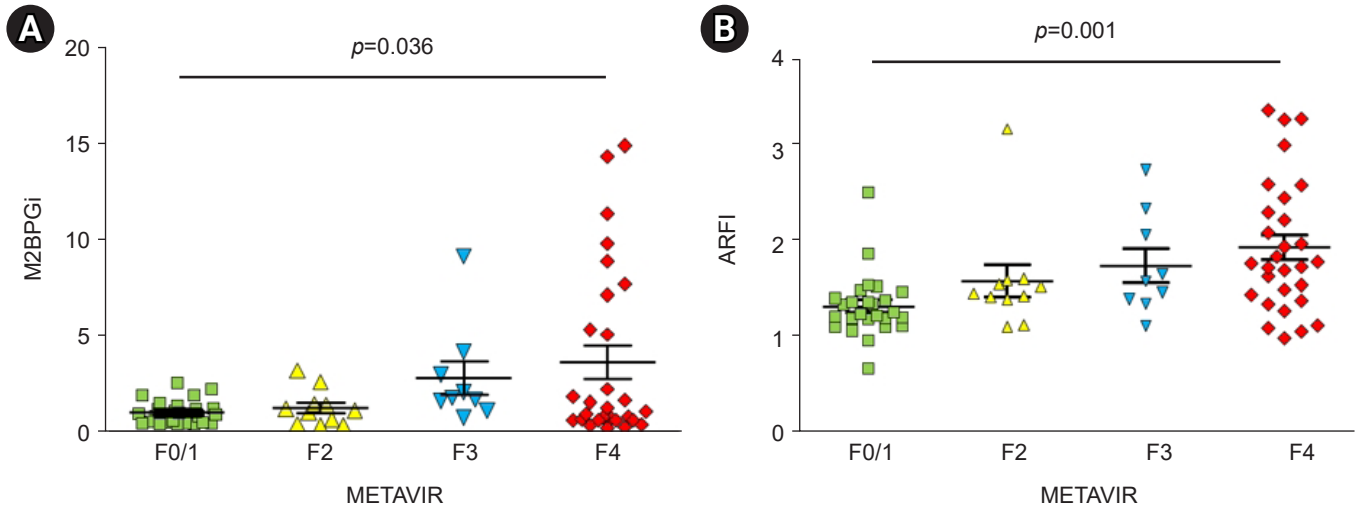

Fig. 1. M2BPGi levels and ARFI values correlated with METAVIR scores in all patients. As METAVIR scores increased, there were statistically significant differences in M2BPGi (A) and ARFI (B) values. M2BPGi, Mac-2 binding protein glycosylation isomer; ARFI, acoustic radiation force impulse imaging.

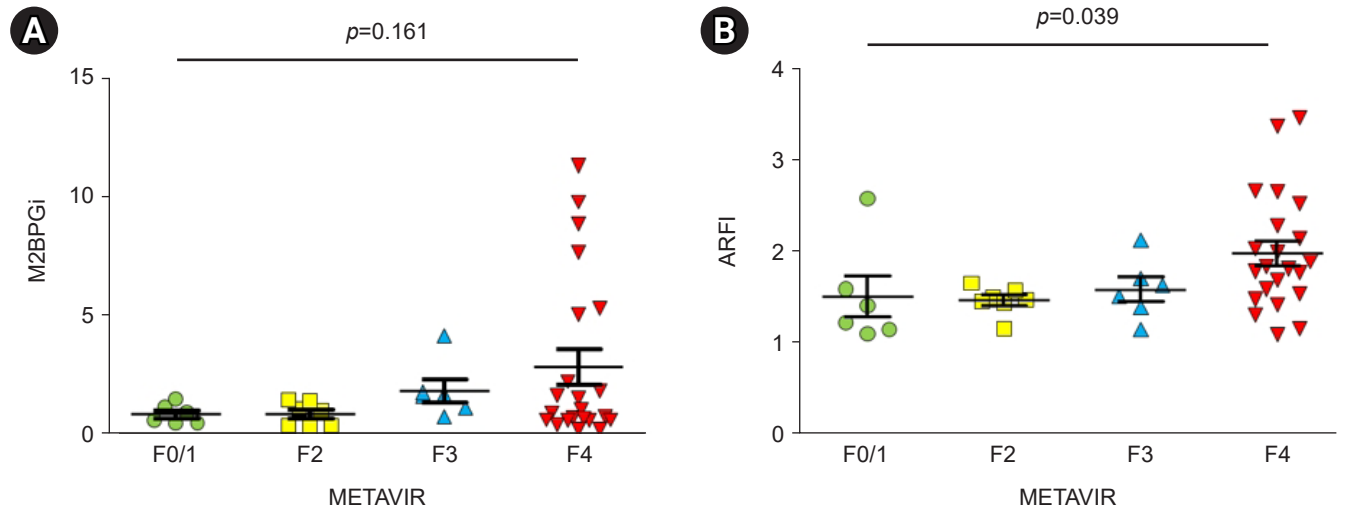

Fig. 2. M2BPGi levels and ARFI values correlated with METAVIR scores in hepatocellular carcinoma. (A) As METAVIR scores increased, M2BPGi levels tended to increase, but without statistical significance. (B) ARFI values changed significantly as METAVIR scores increased. M2BPGi, Mac-2 binding protein glycosylation isomer; ARFI, acoustic radiation force impulse imaging.

ARFI elastography uses ultrasound to visualize liver stiffness. In many studies, ARFI elastography was capable of detecting hepatic fibrosis, especially in patients with chronic viral hepatitis [9]. Recently, a meta-analysis of noninvasive imaging modalities in four ARFI elastography studies (6 cohorts) with 486 patients [20-23] revealed that the overall sensitivity and specificity were 0.92 (95% confidence interval, 0.81–0.97) and 0.72 (95% confidence interval, 0.62–0.81), respectively [24]. In addition, APRI is an indirect marker of hepatic fibrosis based on routine laboratory examinations. In a meta-analysis of 40 studies, investigators concluded that an APRI score >1.0 point had a sensitivity of 76% and a specificity of 72% for predicting advanced hepatic fibrosis [25]. In our study, M2BPGi level showed a significant correlation with both the APRI and ARFI values. In particular, M2BPGi level had a high correlation with noninvasive markers even when analyzed separately based on the presence or absence of HCC.

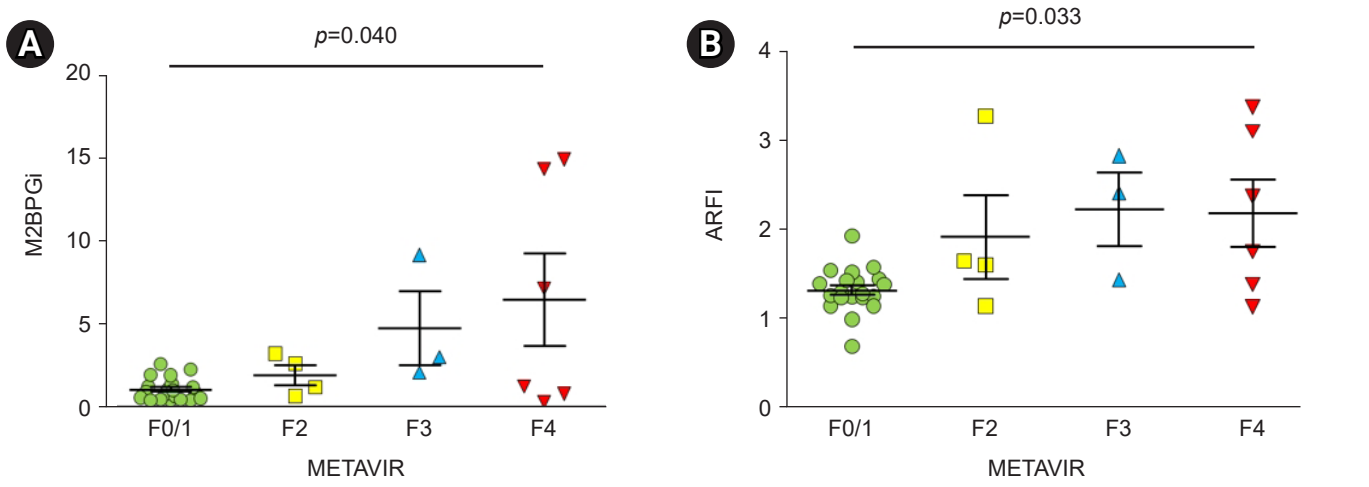

Fig. 3. M2BPGi levels and ARFI values correlated with METAVIR scores in the absence of hepatocellular carcinoma. (A) As METAVIR scores increased, M2BPGi levels significantly increased, as did (B) ARFI values. M2BPGi, Mac-2 binding protein glycosylation isomer; ARFI, acoustic radiation force impulse imaging.

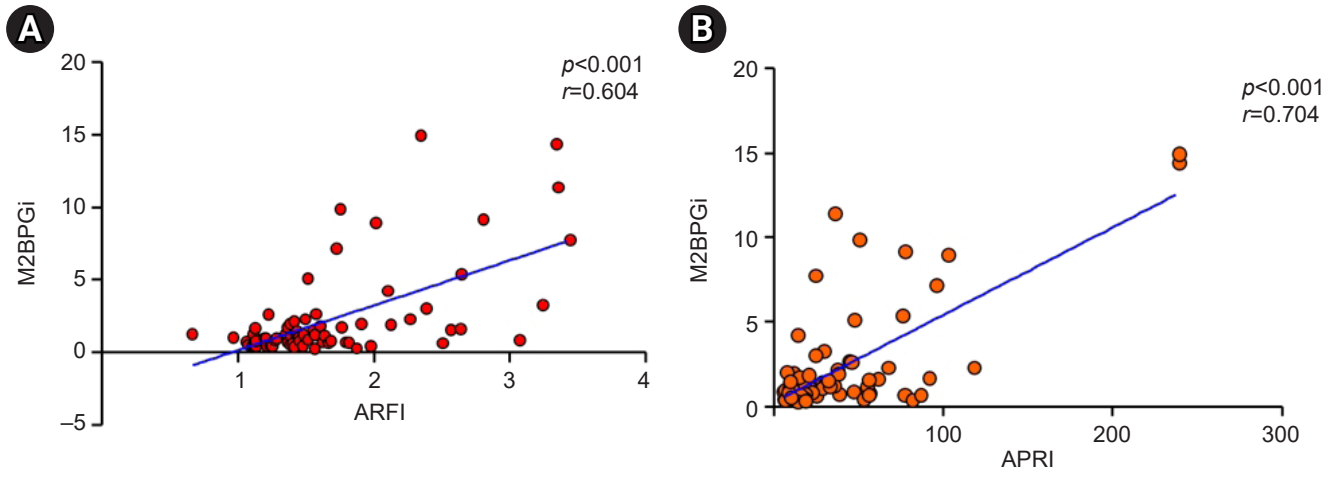

Fig. 4. Correlations among noninvasive fibrosis markers. (A) M2BPGi levels showed a significant correlation with ARFI values and (B) with APRI values. M2BPGi, Mac-2 binding protein glycosylation isomer; ARFI, acoustic radiation force impulse imaging; APRI, aspartate aminotransferase to platelet ratio index.

The main limitations of this study are its relatively small sample size (n=74) and lack of prognostic analysis for predicting deterioration of liver function or development of HCC. In addition, elastography study was performed with ARFI not FibroScan, which is more highly validated in real life. However, this study prospectively analyzed the correlation between liver histology and M2BPGi level and will be of great help in clinical practice. In conclusion, the serum M2BPGi level increased with liver fibrosis severity and could be a good marker for diagnosing advanced hepatic fibrosis regardless of the cause of liver disease.

Article information

Conflicts of interest

Hyunyong Hwang and Young Il Choi are editorial board members of the journal but were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

Conceptualization: KIS, HH. Data curation: BCY, HHM, YIC, DHS, MY. Formal analysis: KIS, HH. Funding acquisition: KIS, HH. Investigation: BCY, HHM, YIC, DHS, MY. Methodology: KIS, HH, BCY, HHM, YIC. Project administration: HH. Resources: HH. Supervision: BCY, DHS, MY. Visualization: KIS, HH. Writing - original draft: KIS. Writing - review & editing: HH. Approval of final manuscript: all authors.

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Performance comparison between Elecsys Anti-SARS-CoV-2 and Anti-SARS-CoV-2 S and Atellica IM SARS-CoV-2 Total and SARS-CoV-2 IgG assays

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Background: Although serological severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) tests from several manufacturers have been introduced in South Korea and some are commercially available, the performance of these test kits has not yet been sufficiently validated. Therefore, we compared the performance of Elecsys Anti-SARS-CoV-2 (ACOV2) and Anti-SARS-CoV-2 S (ACOV2S) and Atellica IM SARS-CoV-2 Total (COV2T) and SARS-CoV-2 IgG (sCOVG) serological tests in this study.

Methods: A total of 186 patient samples were used. For each test, we analyzed the positive rate of serological antibody tests, precision, linearity, and agreement among the four assays.

Results: The positive rates of COV2T, sCOVG, and ACOV2S were high (81.7%–89.2%) in total, with those for ACOV2S being the highest, while those of ACOV2 were as low as 44.6%. This may be related to the high completion rate of vaccination in Korea. The repeatability and within-laboratory coefficients of variation were within the claimed allowable imprecision; however, further research is needed to establish an allowable imprecision at low concentrations. COV2T showed a linear fit, whereas sCOVG and ACOV2S were appropriately modeled with a nonlinear fit. Good agreement was found among COV2T, sCOVG, and ACOV2S; however, the agreement between ACOV2 and any one of the other methods was poor.

Conclusions: Considering the different antigens used in serological SARS-CoV-2 antibody assays, the performance of the tested assays is thought to show no significant difference for the qualitative detection of antibodies to SARS-CoV-2.

Keywords: COVID-19; Nucleocapsid proteins; Quantitative evaluations; SARS-CoV-2; Serological test

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been in the spotlight as the causative agent of coronavirus disease 2019 (COVID-19) since pneumonia patients were confirmed to be infected with a novel coronavirus in December 2019 [1,2]. The manifestations of SARS-CoV-2 infection can be various, spanning from an asymptomatic status to lethal complications [3,4]. As COVID-19 has been declared a global pandemic and spreads rapidly, accurate diagnosis is very important to control the pandemic.

Although a variety of laboratory tests are used for a dif-

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ferential diagnosis, real-time reverse transcription-PCR (RT-PCR) has been considered the gold standard to identify SARS-CoV-2 in clinical practice [5-7]. On the other hand, in serological testing, such as testing for SARS-CoV-2, antibody tests have been known to raise heterogeneity issues and limit identifying SARS-CoV-2 in the early stages of illness [8]. Antibody tests are primarily used to determine if a person has previously been infected with COVID-19 [9].

SARS-CoV-2 has four main structural proteins; spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [10]. It has been reported that the S protein binds to the angiotensin-converting enzyme 2 (ACE2) to enter the host cell present on the surface of alveolar type II cells of the lung and epithelial cells of the oral mucosa [11-13]. The S protein has a subunit S1 harboring receptor-binding domains (RBD) which interacts with ACE2 [14,15]. These proteins, especially the S and N proteins, have been used as target antigens for the development of serological SARS-CoV-2 detection tests [16].

The antigens of Atellica IM SARS-CoV-2 Total (COV2T) and SARS-CoV-2 Immunoglobulin (Ig) G (sCOVG) antibody tests (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) target the S protein, while Elecsys Anti-SARS-CoV-2 (ACOV2) and Anti-SARS-CoV-2 S (ACOV2S) antibody tests (Roche Diagnostics International, Rotkreuz, Switzerland) target the N and S proteins, respectively. Although serological SARS-CoV-2 tests from several manufacturers have been introduced in South Korea and some of them are commercially available, the performance of each test kit has not yet been sufficiently validated.

Therefore, we compared the performance of ACOV2, ACOV2S, COV2T, and sCOVG serological tests in this study.

Methods

Ethical statements: This study was approved by the Institutional Review Board of the Kosin University Gospel Hospital and informed consent was waived (KUGH 2022-03-038).

1. Preparation of patient samples

We collected leftover serum samples from 186 admitted patients in our hospital. After completing the requested tests from the inpatient clinics, each sample leftover was divided into new tubes and given a new code for the analyses.

2. Serological testing of antibodies to SARS-CoV-2

We used ACOV2 (Roche Diagnostics International), ACOV2S (Roche Diagnostics International), COV2T (Siemens Healthcare Diagnostics Inc.) and sCOVG (Siemens Healthcare Diagnostics Inc.) for the detection of antibodies to SARS-CoV-2. The sCOVG and ACOV2S test kits are immunoassays for the *in vitro* quantitative detection of IgG and total antibodies to SARS-CoV-2 in human serum and plasma, respectively, while the ACOV2 test qualitatively detects total antibodies to the SARS-CoV-2. The COV2T test is for the *in vitro* qualitative and semi-quantitative detection of total antibodies, including IgG and IgM antibodies, to SARS-CoV-2. The COV2T, sCOVG, and ACOV2S assays each use recombinant proteins representing the RBD of the S antigen in a sandwich assay format. On the other hand, an antibody to the N recombinant protein is used in the ACOV2 assay [16,17].

In this study, the COV2T and sCOVG tests and the ACOV2 and ACOV2S tests were performed on an Atellica IM automated analyzer (Siemens Healthcare Diagnostics Inc.) and a Cobas 8000 e801 automated analyzer (Roche Diagnostics International), respectively.

3. Positive rate of serological tests in enrolled patients

The enrolled patient group was divided into SARS-CoV-2 RT-PCR positive and negative groups. We calculated the percentage of serological SARS-CoV-2 positive test patients in two PCR testing groups and in the total patients group.

4. Precision analysis

Precision was determined according to the protocols from Clinical and Laboratory Standards Institute (CLSI) EP15-A3 [18]. Quality control materials were used in the precision evaluation. These controls consisted of two concentrations (low and high). The tests were performed at five runs per day for 5 days to calculate the repeatability and within-laboratory precision.

5. Linearity analysis

The protocol from CLSI EP06-A was used for linearity analysis [19]. We prepared two sets of five samples with equally spaced concentrations for the experiments. High and low concentrations of serum samples were prepared and they were mixed proportionally at 0:4, 1:3, 2:2, 3:1, and 4:0. We randomly measured the concentrations of the samples

twice. The results were interpreted using the flowchart of EP06-A, which is based on a polynomial evaluation [19].

6. Agreement evaluation

To analyze the correlation between the assays, we used Pearson's correlation coefficients and Passing-Bablok regression for the slope and intercept. Bland-Altman analysis was performed for the bias (mean difference) and 95% limits of agreement. Concordance rates and κ-values values were determined for the agreements between the methods based on the qualitative test results (positive vs. negative). Each serological SARS-CoV-2 antibody test used its own cutoff value that was claimed by its manufacturer to interpret the test results as positive or negative. The cutoffs for ACOV2, ACOV2S, COV2T, and sCOVG are 0.80 U/mL, 1.0 cutoff index (COI), 1.00 index, and 1.00 index (U/mL), respectively. When the measured serological test values were equal to or greater than the cutoff values, positive tests were reported.

7. Statistical analysis

The precision, linearity, Passing-Bablok regression, and Bland-Altman analyses were performed using Analyze-it for Microsoft Excel version 6.01.1 (Analyze-it Software Ltd., Leeds, UK). The remaining statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Pearson correlation was used for the correlation between methods and kappa statistics were used for the agreement analysis. The κ-values are interpreted as follows: 0.00–0.20 for slight agreement; 0.21–0.40 for fair agreement; 0.41–0.60 for moderate agreement; 0.61–0.80 for substantial agreement; and 0.81–1.00 for almost perfect agreement [20]. The differences were considered statistically significant at a $p<0.05$.

Results

We prospectively analyzed the serum samples from 186 patients (66.2±15.2 years, male/female ratio=1.33), including 115 SARS-CoV-2 RT-PCR positive and 71 PCR negative patients. The 186 tested samples had a sampling date of 5 days or earlier after PCR testing.

1. Positive rate of serological tests in enrolled patients

The patient group was divided into 115 SARS-CoV-2 positive PCR and 71 negative PCR groups. The positive rates of COV2T and ACOV2S were the highest in SARS-CoV-2 RT-PCR positive and negative groups (87.0% and 94.4%, respectively) (Table 1).

ACOV2S showed the highest positive rate (89.2%) in total, while the positive rates of ACOV2 were the lowest in SARS-CoV-2 RT-PCR positive and negative groups and in total (43.5%, 46.5%, and 44.6%, respectively).

2. Precision analysis

The repeatability and within-laboratory precision of COV2T, sCOVG, ACOV2, and ACOV2S tests are shown in Table 2. The repeatability and within-laboratory precision of the low concentration material of COV2T and ACOV2S are not presented because all values were below the measuring range (<0.6 U/mL and <0.4 U/mL, respectively). The CVs in the samples of low and high concentration materials ranged from 2.4% to 92.2% and from 1.0% to 2.5%, respectively.

3. Linearity analysis

Since ACOV2 is an immunoassay for the *in vitro* qualitative detection of antibodies to SARS-CoV-2, linearity analysis was not performed. The measuring intervals of COV2T, sCOVG, and ACOV2S were 0.64–73.56 U/mL, 0.53–141.15 U/mL, and 0.46– 174.33 U/mL, respectively. COV2T showed excellent linearity within each analytical measurement range (Fig. 1). As analyzed using the flowchart of CLSI EP06-A, no 2nd or 3rd order polynomial fits were statistically better than a linear fit at the 5% significance concen-

Table 1. Positive rates of serological tests in SARS-CoV-2 RT-PCR positive and negative patients

Test	Result	No.	Positive rate of serological test (%)			
			COV2T	sCOVG	ACOV2	ACOV2S
SARS-CoV-2 RT-PCR	Positive	115	87.0	79.1	43.5	86.1
	Negative	71	90.1	85.9	46.5	94.4
Total		186	88.2	81.7	44.6	89.2

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, reverse transcription polymerase chain reaction; COV2T, Atellica IM SARS-CoV-2 Total; sCOVG, Atellica IM SARS-CoV-2 IgG; ACOV2, Elecsys Anti-SARS-CoV-2; ACOV2S, Elecsys Anti-SARS-CoV-2 S.

Table 2. Repeatability and within-laboratory precisions of 4 SARS-CoV-2 assays

Test	Sample	Repeatability				Within-laboratory precision			
		Low		High		Low		High	
		Mean±SD (U/mL)	CV (%)	Mean±SD (U/mL)	CV (%)	Mean±SD (U/mL)	CV (%)	Mean±SD (U/mL)	CV (%)
COV2T	QC material	<0.6	-[a]	3.236±0.067	2.1	<0.6	-[a]	3.236±0.067	2.1
sCOVG	QC material	0.019±0.018	92.2	3.748±0.067	1.8	0.019±0.018	92.2	3.748±0.069	1.8
ACOV2	QC material	0.089±0.002[b]	2.4	3.518±0.036	1.0	0.089±0.003	2.9	3.518±0.063	1.8
ACOV2S	QC material	<0.4	-[a]	7.275±0.092	1.3	<0.4	-[a]	7.275±0.181	2.5

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; CV, coefficient of variation; COV2T, Atellica IM SARS-CoV-2 Total; sCOVG, Atellica IM SARS-CoV-2 IgG; ACOV2, Elecsys Anti-SARS-CoV-2; ACOV2S, Elecsys Anti-SARS-CoV-2 S; QC, quality control.

a)SDs and CVs could not be determined because the low QC material in the COV2T and ACOV2S had concentrations below the measuring range (<0.6 U/mL and <0.4 U/mL, respectively) throughout the experiment.

b)The results of ACOV2 were presented as a cutoff index.

tration for COV2T. However, the 2nd order polynomial fits were statically better than a linear fit for sCOVG (p=0.048) and ACOV2S (p=0.001).

4. Agreement study

In Table 3, the correlation between sCOVG and ACOV2S was the best among the compared methods (r=0.833, p<0.001). The measured values of ACOV2S were greater than those of sCOVG (slope=142.7, intercept=–33.84) and the difference between the two paired methods proportionally increased with increasing concentrations (Fig. 2). COV2T was moderately correlated with sCOVG and ACOV2S (r=0.496, p<0.001 and r=0.413, p<0.001, respectively) (Table 3). The difference between ACOV2S and COV2T measurements showed a proportional increase to the average of the paired methods (Fig. 2). A proportional increase in the difference between COV2T and sCOVG measurements was observed. However, the two-pronged measurements were plotted against the average measurements over concentrations of around 40,000 U/mL (Fig. 2).

The concordance rate between methods was highest between COV2T and ACOV2S (96.8%), where the agreement was almost perfect (κ=0.839, p<0.001) (Table 4). Substantial agreements were observed between COV2T and sCOVG and between sCOVG and ACOV2S (r=0.750, p<0.001 and r=0.700, p<0.001, respectively). The concordance rate and agreement between ACOV2 and either one of the other methods were relatively worse compared to those between other combinations of compared groups (concordance rate and κ-value: 51.5% and 0.096, 53.2% and 0.124, and 51.1% and 0.098 between COV2T and ACOV2, sCOVG and ACOV2, and ACOV2 and ACOV2S, respectively).

Discussion

After all, clinical complications and prognosis are the main concerns in SARS-CoV-2 patients. Therefore, accurate measurement of antibodies to SARS-CoV-2 should help to predict and monitor the clinical manifestations during treatments. Unfortunately, several medications have been introduced worldwide, however, the validity of the treatments seems to be not perfectly proven yet [21-23]. This would make serological antibody tests more valuable for the better prognosis of patients. From this perspective, the performance comparison of this study would provide some insight into serological SARS-CoV-2 antibody tests.

When this study began, a majority of Koreans had completed vaccination for COVID-19 and every admitting patient had to be tested with SARS-CoV-2 RT-PCR to identify if a patient was currently infected with SARS-CoV-2; omicron was the dominant coronavirus variant in South Korea [24]. As a result, almost all enrolled patients were assumed to have experienced either real coronavirus infections or vaccination before admission. Although we collected samples from patients with SARS-CoV-2 positive and negative RT-PCR results, the positive rate of the serological test was highest in the negative RT-PCR group (94.4%) (Table 1). The positive rates of serological tests spanned from 79.1% to 94.4% and those of ACOV2 were relatively low (43.5%–46.5%). Considering that ACOV2 uses a nucleocapsid protein as an antigen and most vaccines used in Korea for inoculation were targeting spike protein in SARS-CoV-2 [25,26], it is assumed that more than half of enrolled patients had not recently been exposed to SARS-CoV-2 and that it is likely that the high positive rates in serological tests were

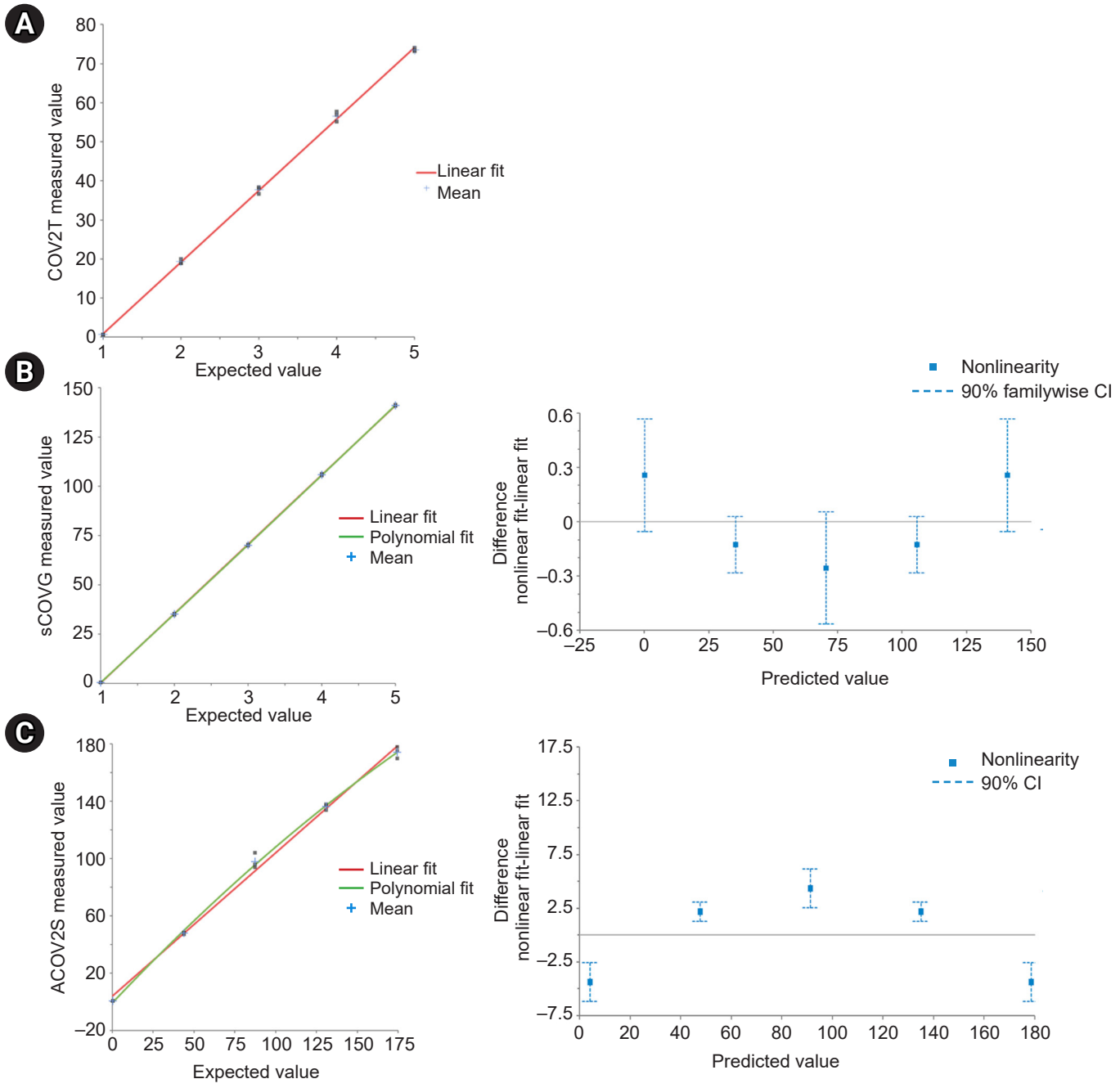

Fig. 1. Linearity of COV2T, sCOVG, and ACOV2S using Cobas 8000 e801 and Atellica IM automated analyzers. The COV2T (A) and sCOVG (B) were tested on an Atellica IM automated analyzer, the ACOV2S (C) being tested on a Cobas 8000 e801 automated analyzer. The COV2T showed excellent linearity within each analytical measurement range, therefore, a difference plot was not presented. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COV2T, Atellica IM SARS-CoV-2 Total; sCOVG, Atellica IM SARS-CoV-2 IgG; ACOV2S, Elecsys Anti-SARS-CoV-2 S.

caused by vaccinations. Practically, the seroprevalence of N-specific antibodies has been reported to remain as low as 1.59% after a vaccine campaign in Japan [27].

In Table 2, repeatability and within-laboratory coefficient of variations (CVs) are within the allowable imprecision claimed by manufacturers, except for those in the low concentration control in the sCOVG antibody test. The upper repeatability and within-laboratory CVs claimed by

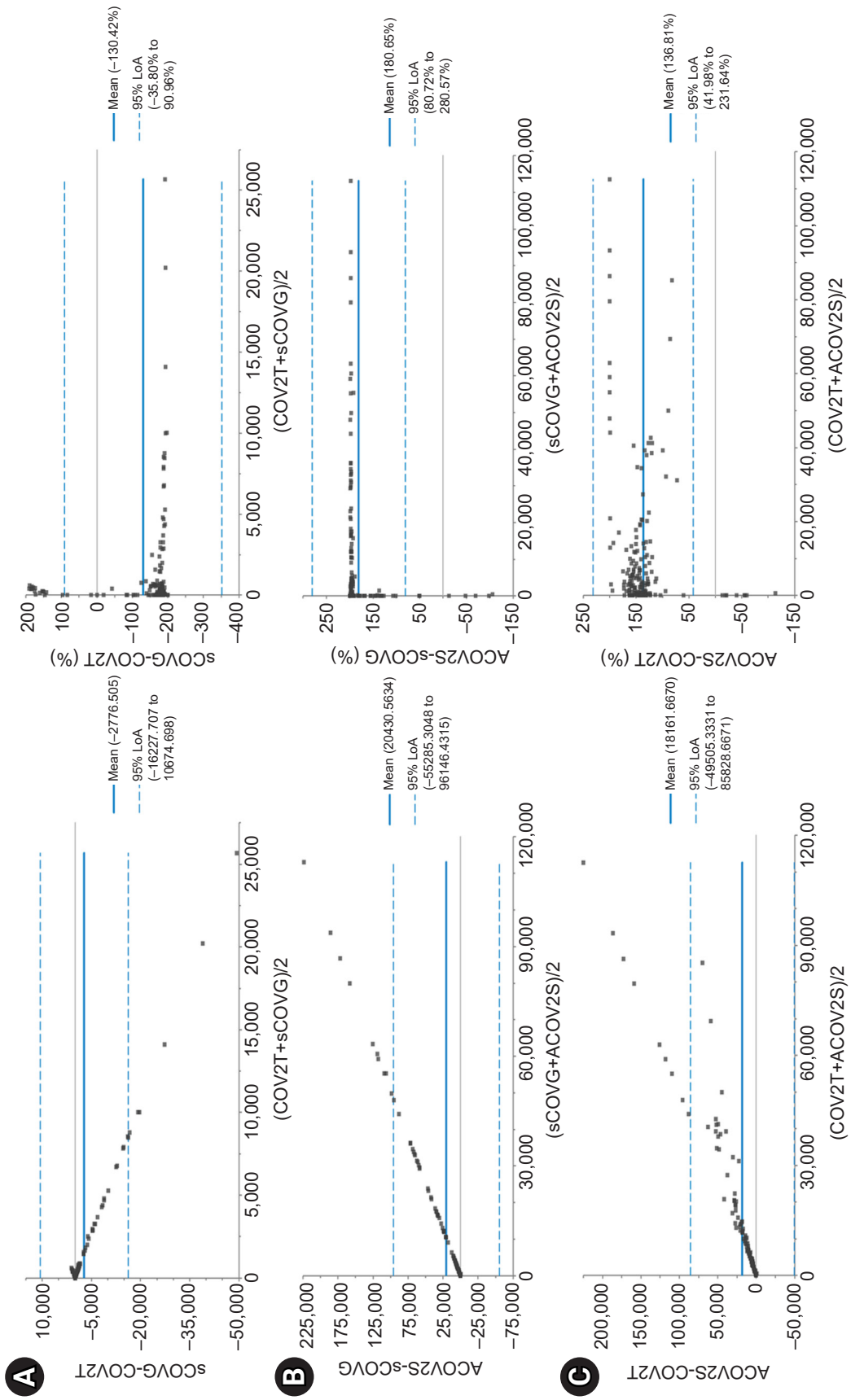

Fig. 2. Bland-Altman plot for ACOV2S, COV2T and sCOVG. (A-C) Differences are presented as U/mL (left column) and percentage (right column) for each Bland-Altman plot. The mean presents the mean difference between x and y, and the 95% LoA lines indicate the mean difference ±1.96 standard deviation. sCOVG, Atellica IM SARS-CoV-2 IgG; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COV2T, Atellica IM SARS-CoV-2 Total; ACOV2S, Elecsys Anti-SARS-CoV-2 S; LoA, limits of agreement.

Table 3. Passing-Bablok and Pearson correlation coefficients among COV2T, sCOVG, and ACOV2S

Method x	Method y	Slope	Intercept (U/mL)	$r^{a)}$
COV2T	sCOVG	0.041	1.795	0.496
sCOVG	ACOV2S	142.7	−33.840	0.833
ACOV2S	COV2T	6.352	−4.449	0.413

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COV2T, Atellica IM SARS-CoV-2 Total; sCOVG, Atellica IM SARS-CoV-2 IgG; ACOV2S, Elecsys Anti-SARS-CoV-2 S; *r*, Pearson correlation coefficient.
a) $p<0.001$.

Table 4. Concordance rates and agreements among 4 SARS-CoV-2 assays

Comparing group	Concordance rate (%)	Agreement (κ)	*p*-value
COV2T vs. sCOVG	93.5	0.750	<0.001
COV2T vs. ACOV2	51.5	0.096	0.028
COV2T vs. ACOV2S	96.8	0.839	<0.001
sCOVG vs. ACOV2	53.2	0.124	0.018
sCOVG vs. ACOV2S	92.5	0.700	<0.001
ACOV2 vs. ACOV2S	51.1	0.098	0.019

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COV2T, Atellica IM SARS-CoV-2 Total; sCOVG, Atellica IM SARS-CoV-2 IgG; ACOV2, Elecsys Anti-SARS-CoV-2; ACOV2S, Elecsys Anti-SARS-CoV-2 S.

the manufacturer were 12.0% and 15% within the 0.80–2.00 U/mL concentration interval, respectively. The mean of low concentration material was 0.019 U/mL in this study, which is at least a 42.1 times lower concentration. In a previous study, materials over 2.0 U/mL were used for imprecision and the CVs were lower than the manufacturer's claim [28]. Therefore, further evaluation needs to be performed to establish the imprecision at lower concentrations.

Although nonlinearity was determined in sCOVG and ACOV2S, the degree of nonlinearity of these two methods seemed visually different. The sCOVG test was relatively more linear than the ACOV2S test. When the difference plot was recalculated again when setting the allowable nonlinearity to 15%, which is claimed by manufacturers across the measuring interval, the concentration of nonlinearity was at the left end in both methods. After removing the left endpoint, the degree of nonlinearity was alleviated. As a result, the remaining 4 points were located within the allowable nonlinearity interval and the *p*-value of the 2nd order polynomial equation increased from 0.001 to 0.018 for the ACOV2S test (data not shown), which indicates that the degree of nonlinearity was more alleviated than before. Likewise, after removing left endpoint of the sCOVG test, the *p*-value of the 2nd order polynomial equation was changed from 0.048 to 0.747 (data not shown), which is more appropriate for a linear fit. Instead of establishing linearity, the range of the measuring interval should be reduced. Although COV2T has been claimed to be a semi-quantitative test, its linearity was good.

In the Passing-Bablok analysis, the slopes of regression lines were 0.041 and 142.7 between COV2T and sCOVG and between sCOVG and ACOV2S, respectively. This indicates that the absolute concentration of reported test results should be greatly different depending on the methods. Although the compared three methods detect different antibodies, test harmonization issues need to be raised. Significant differences between plasma- and serum-based SARS-CoV-2 antibody tests have already been reported [29].

The correlation and agreement were best between sCOVG and ACOV2S antibody tests (r=0.833, κ=0.839, $p<0.001$) (Tables 2, 3). Although the cutoffs of positive for anti-SARS-CoV-S in sCOVG and ACOV2S are not markedly different (0.80 U/mL and 1.0 U/mL, respectively), the difference in the absolute concentration between them showed a proportionally increasing trend as the concentration of antibodies increased. This might happen because constant CV% was affected across the measuring interval, which would show a linear slope. This could be caused by a calibration error in one method [30,31]. Since the manifestation of a regression line between ACOV2S and COV2T is different from the other paired methods, it is not easy to confirm if this is due to a proportional constant error, such as a calibration error.

Serological SARS-CoV-2 antibody tests require a careful interpretation in clinical practice because the sensitivity of the serological tests varies depending on the days after disease onset [32]. The cumulative seroprevalence for IgM and IgG increased from 44% and 56% on day 7 after symptom onset to over 95% on day 20 for IgM and day 16 for IgG, respectively [33]. The enrolled patient samples did not contain clinical information except for the age, sex, and SARS-CoV-2 RT-PCR results in this study. Furthermore, the majority of admitted patients had already completed vaccination when samples were obtained from patients. Therefore, the diagnostic validity of the assays could not be evaluated due to the lack of linked clinical information.

In conclusion, the positive rates of COV2T, sCOVG, and ACOV2S were as high as 81.7%–89.2% in total, and that for ACOV2S was the highest, while those of ACOV2 were as low as 44.6%. This may be related to the high completion rate of vaccination in Korea. The precision results were allowable based on the claimed allowable imprecision, however, further study needs to be conducted to establish the allowable imprecision at a lower concentration level. COV2T showed a linear fit, on the other hand, sCOVG and ACOV2S were appropriately modeled with a nonlinear fit. The agreements among COV2T, sCOVG, and ACOV2S were good, however, those between ACOV2 and either one of the other methods were poor, which is assumed to be due to the different antigens in methods. Considering the different adopted antigens that the serological SARS-CoV-2 antibody assays use, the performance of tested assays is thought to show no significant difference for the qualitative detection of antibodies to SARS-CoV-2.

Article information

Conflicts of interest

Seri Jeong and Hyunyong Hwang are editorial board members of the journal but were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this paper were reported.

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Author contributions

Conceptualization: HH. Data curation: SJ, YRH, HH. Formal analysis: HH. Funding acquisition: HH. Investigation: SJ, YRH. Methodology: HH. Project administration: HH. Supervision: HH. Validation: HH. Visualization: SJ, YRH. Writing - original draft: SJ, YRH. Writing - review & editing: HH. Approval of final manuscript: all authors.

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Case report

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Overcoming high pre-transplant isoagglutinin titers using high-dose intravenous immunoglobulin, salvage plasmapheresis, and booster rituximab without splenectomy in ABO-incompatible living donor liver transplantation: a case report

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High pre-transplant isoagglutinin is a risk factor for antibody-mediated rejection in ABO-incompatible living donor liver transplantation. A 55-year-old man with alcoholic liver cirrhosis underwent ABO-incompatible living donor liver transplantation. The initial isoagglutinin immunoglobulin G titer was 1:1,024. Despite five sessions of plasmapheresis, the isoagglutinin titer was not significantly reduced (from 1:1,024 to 1:512). We decided to perform 11 plasmaphereses and proceed with liver transplantation regardless of the isoagglutinin titer (1:128 at transplantation day). Instead, we planned to administer 0.5 g/kg intravenous immunoglobulin and booster rituximab (200 mg) after transplant. On postoperative day 6, the isoagglutinin titer increased from 1:32 to 1:64, and the patient received plasmapheresis twice. The patient maintained stable liver function without evidence of further complications or rejection. The high-dose intravenous immunoglobulin, salvage plasmapheresis, and booster rituximab protocol might be able to overcome a pre-transplant high isoagglutinin titer in ABO-incompatible living donor liver transplantation without splenectomy.

Keywords: Blood group incompatibility; Liver transplantation; Living donor; Rituximab; Splenectomy

Introduction

The incompatibility of ABO blood type between donor and recipient is one of the significant barriers to liver transplantation (LT) [1]. Many transplant centers have proposed Various desensitization protocols to overcome the barrier of ABO incompatibility [2]. Plasmapheresis (PP), splenectomy, intravenous immunoglobulin (IVIG) administration, aggressive immunosuppressive protocols, and intrahepatic portal and arterial infusions of anti-inflammatory drugs, such as rituximab, have all been utilized to improve the outcome of ABO-incompatible (ABOi) living donor liver transplant (LDLT) [3]. These strategies have three main objectives: (1) suppression of B-cell activity, (2) attenuation of local inflammation, and (3) pre-transplant reduction of isoagglutinin (IA) titers. Since the introduction of rituximab, the graft survival of ABOi-LDLT has improved dramatically by the depletion of CD20 positive B cells [4]. Moreover, the

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effectiveness of local graft infusion therapy with anti-inflammatory drugs such as prostaglandin E1, methylprednisolone, and gabexate mesylate is no longer effective in the era of rituximab [5]. Meanwhile, the role of pre-transplant ABO antibody titer in antibody-mediated rejection (AMR) is controversial in ABOi-LDLT [4]. Although the strategy to reduce IA titers has been considered to play a critical role in the success of ABOi-LDLT, recent studies proposed a simplified protocol without PP. However, those studies only included patients with a low baseline IA titer [2]. There is no established protocol or cutoff value for patients with a high titer or who do not respond to PP before LT. Here, we report a successful ABOi-LDLT in a recipient with high pre-transplant IA titer using our modified protocol.

Case

Ethical statements: This study was approved by the Institutional Ethics Committee of Kosin University Gospel Hospital (2022-03-005). The informed consent was waived because this design is a retrospective study.

The patient was a 55-year-old man with alcoholic liver cirrhosis. He underwent a transjugular intrahepatic portosystemic shunt procedure due to recurrent episodes of esophageal variceal bleeding. Three months later, the patient underwent gastric varix embolization because of gastric variceal bleeding. Since then, endoscopic sclerotherapy has been performed because of gastric varix. Subsequently, ascites gradually developed, and his son donated his liver. His son's blood type (B+) was not compatible with the patient's (O+); therefore, we planned ABOi-LDLT. The patient's Child-Pugh score and model for end-stage liver disease score were C11 and 24, respectively. Initial titers of immunoglobulin G and immunoglobulin M were 1:1,054 and 1:256, respectively.

We administered 633 mg of rituximab (375 mg/m^2 body surface area [BSA]) 3 weeks before the scheduled transplantation date, and the patient had no specific adverse reaction. We planned five sessions of PP with 5% albumin and two sessions of PP with fresh frozen plasma (FFP) as per our standard ABOi-LDLT protocol (Fig. 1). The patient's estimated plasma volume was 3,300 mL; 1.0 estimated plasma volume was exchanged as one session of PP. PP

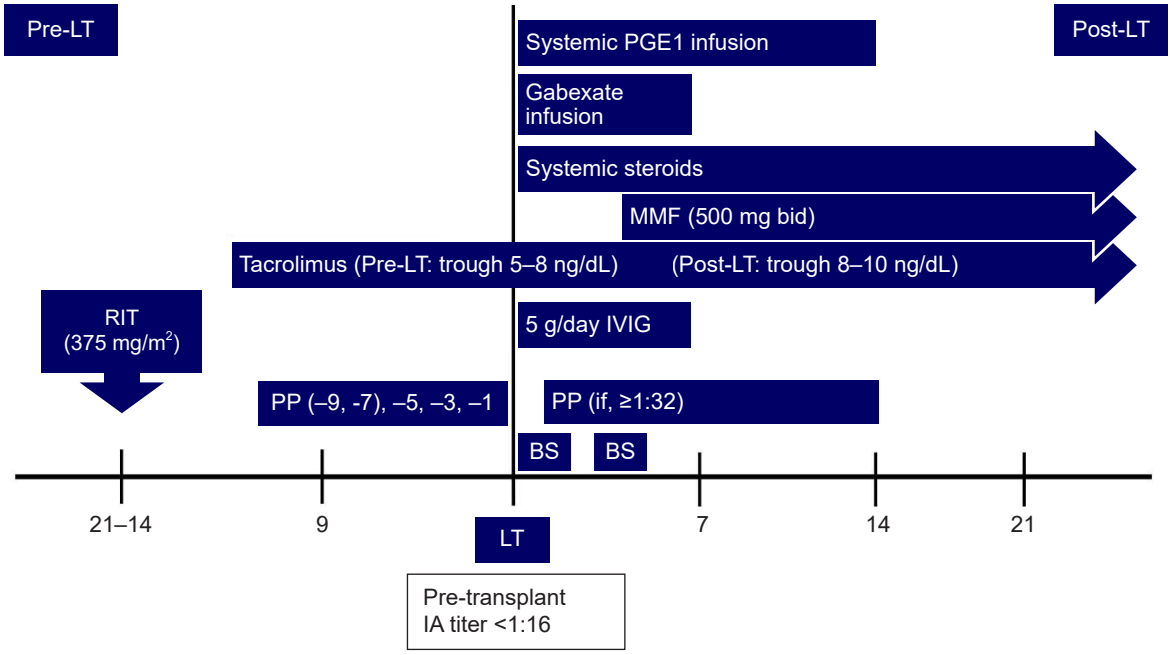

Fig. 1. Desensitization protocol for ABO-incompatible living donor liver transplantation. LT, liver transplantation; RIT, rituximab; PP, plasmapheresis; PGE1, prostaglandin E1; MMF, mycophenolate mofetil; IVIG, intravenous immunoglobulin; BS, basiliximab; IA, isoagglutinin.

began a week after rituximab administration. Despite the 1st five PPs, the reduction in the IA titer was insignificant (from 1:1,024 to 1:512). We decided to postpone the LT and planned an additional PP. However, thrombocytopenia worsened after the 7th PP session, international normalized ratio increased to 3 or more, and bleeding occurred at the PP catheter insertion site. Therefore, the patient had to receive FFP transfusion, and it was determined that the IA titer goal of our protocol could not be reached. We decided to perform three more daily PPs with FFP and proceed with LT regardless of the IA titer. Instead, 0.5 g/kg IVIG from the anhepatic phase to postoperative day (POD) 5, booster rituximab 200 mg on POD 2, and salvage PP were planned after transplantation. The IA titer measured on the day of LT was 1:128 (Fig. 2).

The right liver (802 g) was donated, and the graft-to-recipient weight ratio was 1.20%. Two right inferior hepatic veins were made into one and connected to the inferior vena cava. V5 and V8, which are venous branches from the middle hepatic vein to the segments, were reconstructed using a cryopreserved iliac vein and anastomosed to the common trunk of the middle and left hepatic veins. The right anterior bile duct and right posterior bile duct were separated. Two bile openings were made into one hole and anastomosed to the common hepatic duct using an external bile stent.

On the 1st day after LT, bleeding around the adrenal gland was confirmed on computed tomography and did not resolve by intervention. Re-operation for bleeding control was performed via laparotomy. After this operation, the pulmonary edema became severe, and continuous renal replacement therapy was needed until POD 10.

We measured the cell count and percentage of CD19/20 B cells as markers serially before and after LT (Table 1). Before transplantation, the cell count and percentage of CD19/20 B cells were close to nadir. IA titer was maintained as 1:32 until POD 4. On POD 5, the IA titer increased to 1:64, and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) rose more than twice as much as the previous day to 151/194. Therefore, two sessions of PPs were performed, IA titer decreased to 1:32, the AST/ALT was stabilized, and bile was well-drained through the external bile stent after that. The IA titer increased to 1:64 on POD 13, but we did not

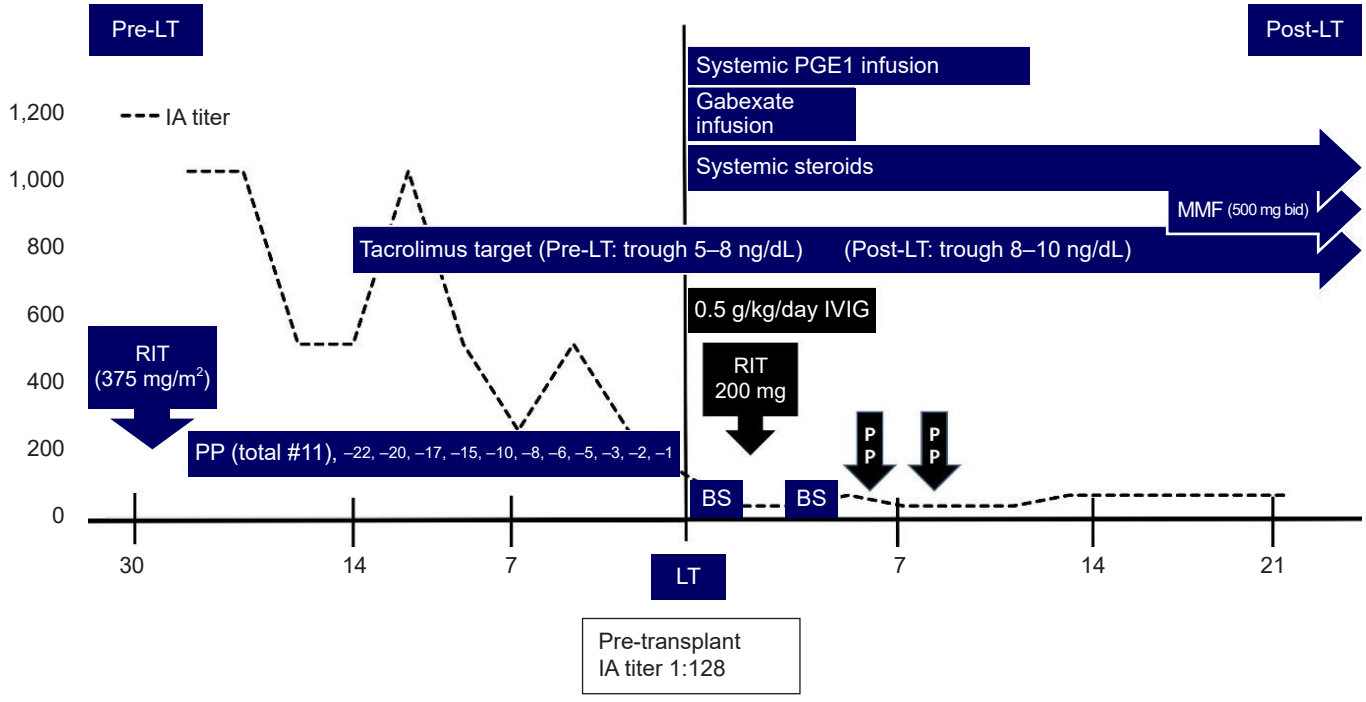

Fig. 2. Desensitization management and IA titers in high-IA-titer ABO-incompatible living donor liver transplantation. LT, liver transplantation; IA, isoagglutinin; RIT, rituximab; PP, plasmapheresis; PGE1, prostaglandin E1; MMF, mycophenolate mofetil; IVIG, intravenous immunoglobulin; BS, basiliximab.

Table 1. Changes in WBC and lymphocyte counts and CD19+ B cell and CD20+ B cell distribution according to rituximab administration

Transplant day		WBC (count/μL)	Lymphocytes (count/μL)	CD19 (count/μL)	CD20 (count/μL)
Pre-transplant day 28	1st RIT	3,650	495 (13.56)	36 (7.26)	33 (6.70)
Pre-transplant day 22		3,130	419 (13.39)	1 (0.20)	0
Pre-transplant day 3		4,840	541 (11.70)	1 (0.01)	0
Post-transplant day 2	2nd RIT	3,280	22 (0.66)	1 (3.23)	0
Post-transplant day 11		2,940	88 (2.96)	0	0
Post-transplant day 31		3,240	37 (1.13)	0	0
Post-transplant day 240		3,430	368 (10.74)	4 (11.02)	39 (10.52)

The values in parentheses are the percentages of lymphocyte, CD19, and C20 to white blood cells count.
RIT, rituximab; WBC, white blood cell.

perform additional PPs because it was determined that graft was accommodated in the recipient and resistant to AMR. The total bilirubin level was 3.37 mg/dL on POD 1 and then steadily declined to 0.94 mg/dL on POD 29 at discharge. Cytomegalovirus polymerase chain reaction DNA was detected in the blood on POD 19; it was treated with ganciclovir, and no other infections occurred. Four months after the transplant, the external biliary stent was removed, the patient maintained stable liver function without evidence of infection, vascular or biliary complication, and rejection.

Discussion

Pre-transplant high IA titer is a risk factor for postoperative AMR, biliary complications, and hepatic necrosis [6]. In most desensitization protocols, including pre-transplant PP, rituximab, IVIG, splenectomy, the pre-transplant IA titer target is at least 1:64 with or without PP. However, there is no established protocol in cases where the pre-transplant IA titer is very high, the titer does not fall enough after PP, or PP cannot be performed due to allergic reactions or other reasons. Several case reports have introduced their experiences and protocols in patients with pre-transplant high IA titers.

Lee et al. [4] reported three cases of successful ABOi-LDLT in patients with failed PP. They used a single dose of rituximab pre-transplant and post-transplant high-dose IVIG (0.8 g/kg from the anhepatic phase to 2 days after LT). They performed a splenectomy during LT to eradicate remnant antibody-producing plasma cells that PP cannot eliminate. Saitoh et al. [7] reported a very high pre-transplant IA titer of 1:4,096. They administered rituximab twice (500 mg of rituximab 2 weeks before LT and 300 mg of rituximab 1 day before LT), splenectomy, and pre-transplant and post-transplant PP without IVIG. Ikegami et al. [8] used high-dose IVIG (0.6 g/kg) and PP in a patient with rebound IA titer to 1:2,048 after LT with splenectomy.

In all reported cases, concomitant splenectomy was performed during LT, but splenectomy was not performed in our patient. Splenectomy in a patient with splenomegaly and developed collaterals takes a long operation time and carries the risk of massive bleeding [9]. In addition, splenectomy increases the risk of post-transplant infection and portal vein thrombosis, pancreatic fistula, and sepsis in patients undergoing heavy immunosuppression and PP [10]. Meanwhile, it is thought that rituximab can replace splenectomy as a chemical splenectomy. Rituximab has been proven to deplete CD20 positive B cells from the circulation and lymphoid tissues, including the spleen, within 72 hours [11]. However, despite the use of rituximab in the cases as mentioned above reports, the reason for splenectomy is to remove remnant antibody-producing plasma cells that cannot be eliminated by rituximab and PP [4].

Instead of splenectomy, we chose 200 mg of booster rituximab on POD 2 in this patient. B cells become activated only due to sensitization by ABO histo-group antigens on the vascular endothelial cells of the graft. Some B cells may escape rituximab depletion preoperatively and become activated B cells producing antibodies after transplantation [12]. Therefore, it may be beneficial that rituximab is administered postoperatively, especially in AMR-high risk patients, to deplete activated B cells completely.

In ABOi-LDLT, the most common strategy is to administer a single dose of rituximab (375 mg/m^2 BSA or less) 1–4 weeks before ABOi-LDLT [13]. However, the optimal dosage and timing of rituximab in ABOi LT remains controversial. A

375 mg/m^2 BSA dosage was adopted from lymphoma treatment protocols. Egawa et al. [14] considered that a dosage of <300 mg is probably insufficient. Furthermore, administering multiple doses of rituximab has been shown to increase the incidence of fungal infections and cytomegalovirus disease significantly [15].

IVIG is another immunologic strategy for ABOi-LDLT. The proposed mechanisms include the blockade of Fc receptors on mononuclear phagocytes, direct antibody neutralization, inhibition of CD19 expression on activated B cells, suppression of complement, and suppression of T cells [2]. We administered 0.5 g/kg/day of IVIG to this patient for 6 days from the anhepatic phase. A Japanese multicenter study reported that administration of IVIG did not decrease the incidence of AMR significantly [15]. However, IVIG and PP are used as rescue treatments in severe AMR [8]. IVIG might have worked on for B lymphocytes may have survived in the lymph node under rituximab therapy or may have been newly differentiated [16]. It is thought that further research on IVIG's optimal dose, timing, and frequency is needed.

The most critical factor for preventing AMR in recipients undergoing ABOi-LDLT is the suppression of de novo antibodies. If the baseline titer before LT is high, the titer does not respond to PP, or PP cannot be performed, the protocol of well- combined high-dose IVIG, salvage PP, booster rituximab without splenectomy might be considered to be able to overcome ABOi-LDLT.

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Conflicts of interest

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Author contributions

All the work was done by HHM.

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Case report

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Tracheostomy with aortic debranching: a case report

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Percutaneous dilatational tracheostomy (PDT) is preferred over conventional surgical tracheostomy for prolonged airway protection and mechanical ventilation. However, despite its advantages, severe PDT-related complications have been reported, including catastrophic hemorrhage from common carotid artery laceration and innominate artery pseudo-aneurysm. PDT can typically be applied to the trachea, which is the focal point for the transverse course of great vessels (e.g., the anomalous brachiocephalic trunk, which overlaps with the targeted lesion anteriorly); therefore, to improve patient outcomes, an alternative method using aortic debranching may be considered.

Keywords: Aorta; Case reports; Tracheostomy

Introduction

The anatomical structures of the aortic arch branches (supra-aortic branches) should be considered during percutaneous dilatational tracheostomy (PDT) to prevent accidental vascular injury and associated hemorrhage [1]. Clinicians need to be aware of arterial deviations such as a high aortic arch, wide brachiocephalic trunk (BCT) that overlaps with the cervical trachea, the pretracheal course of the right subclavian artery (SCA) and common carotid artery (CCA), and unusual origins of branches of thyroid arteries [2].

Case

Ethical statements: This study was approved by the Institutional Ethics Committee of Eulji University (EMC 2021-08-005). The informed consent is waived for this case study.

A 72-year-old woman presented as a consult with our team for a tracheostomy. She had a history of prolonged mechanical ventilator support for 1 month because ventilator weaning was not available. The patient had a recent percutaneous coronary intervention for a non-ST elevated myocardial infarction via the left circumflex artery culprit and heart failure. Her past history included right-sided weakness after a cerebrovascular accident with the right middle cerebral artery infarction that had persisted for 7 years, myotonic dystrophy for 3 years and bronchial asthma. Prior to the tracheostomy, we detected a pulsating portion on the anterior surface of the neck, which was targeted for tracheostomy, and a computed tomography (CT) scan showed a high riding anomalous BCT that crossed the tracheostomy target in the midline on the trachea (Fig. 1).

A literature review indicated that a transverse course of BCT or aberrant right SCA precluded longer tracheostomy cannulation and could result in tracheo-brachiocephalic

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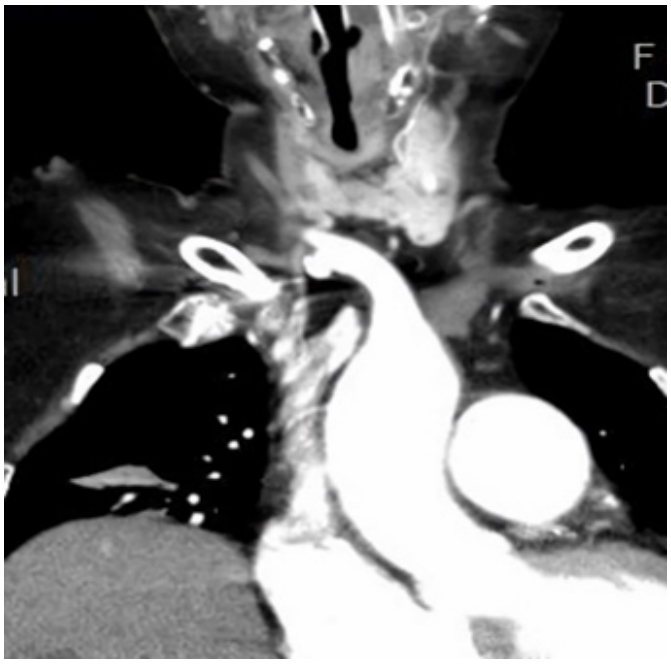

Fig. 1. A computed tomography scan showed a high-riding anomalous brachiocephalic trunk that crossed the tracheostomy target in the midline on the trachea.

fistula. Therefore, aortic debranching followed by PDT can present as a challenging case for an aortic surgeon.

Under general endotracheal anesthesia, we applied a reversed T-shaped partial upper sternotomy from the manubrium to the second intercostal body of the sternum and separated the right supraclavicular incision to optimize exposure of the ascending aorta, aortic arch, and bifurcation of the BCT (Fig. 2A). After systemic heparinization, both sides of BCT were clamped and then divided after bifurcation. The proximal origin of BCT was oversewn and then the distal portion of the SCA and CCA were anastomosed with 10-mm and 8-mm straight Hemashield grafts, which were collagen-impregnated woven double velour polyester grafts (Hemashield Platinum; Maquet Cardiovascular, LLC, Wayne, NJ, USA). The graft from the CCA was anastomosed with the other graft from the SCA as a Y graft using an end-to-side approach. A Y graft produced at our institution was anastomosed with the ascending aorta with an end-to-side approach under a side bite for partial clamping (Fig. 2B). The postoperative wound (Fig. 3A) was examined and determined to be clean and the CT scan indicated that no additional vasculature crossed over the PDT target (Fig. 3B).

At postoperative day 12, we performed percutaneous di-

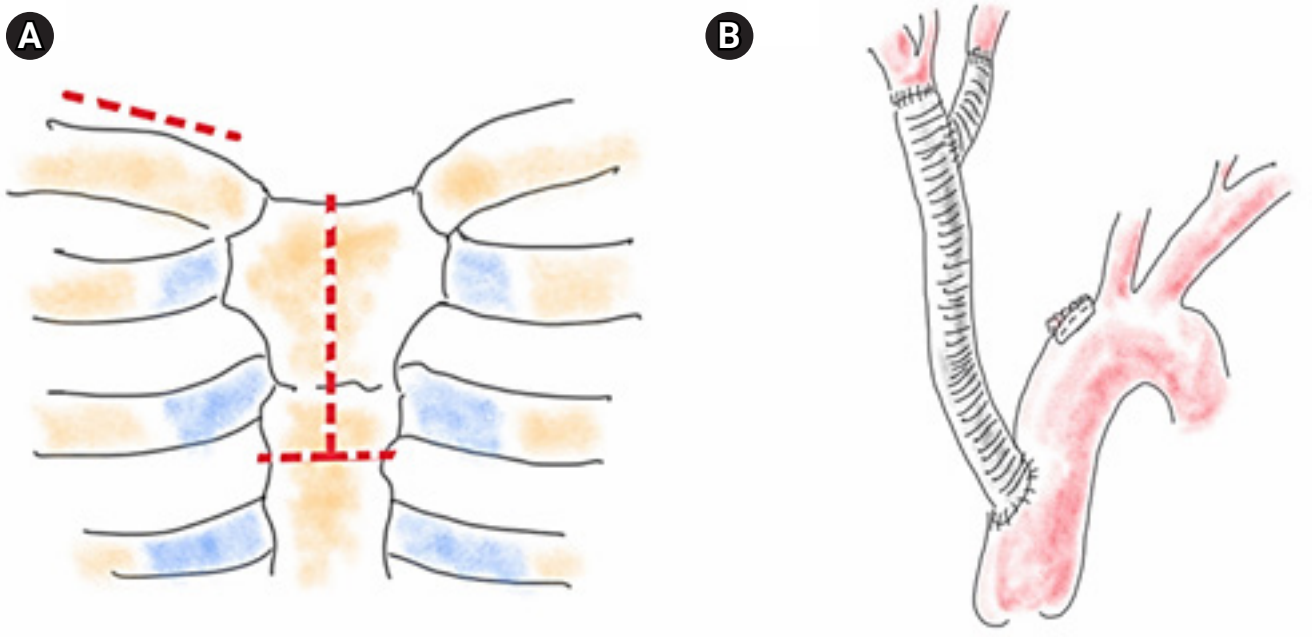

Fig. 2. Aortic debranching technique. (A) Reverse T-shaped partial sternotomy and a separate right supraclavicular incision. (B) Post-anastomotic configuration of the reconstructed brachiocephalic trunk using a Y graft that was made at our institution and comprised 10-mm and 8-mm Hemashield grafts for the right subclavian artery and common carotid artery, respectively, after debranching.

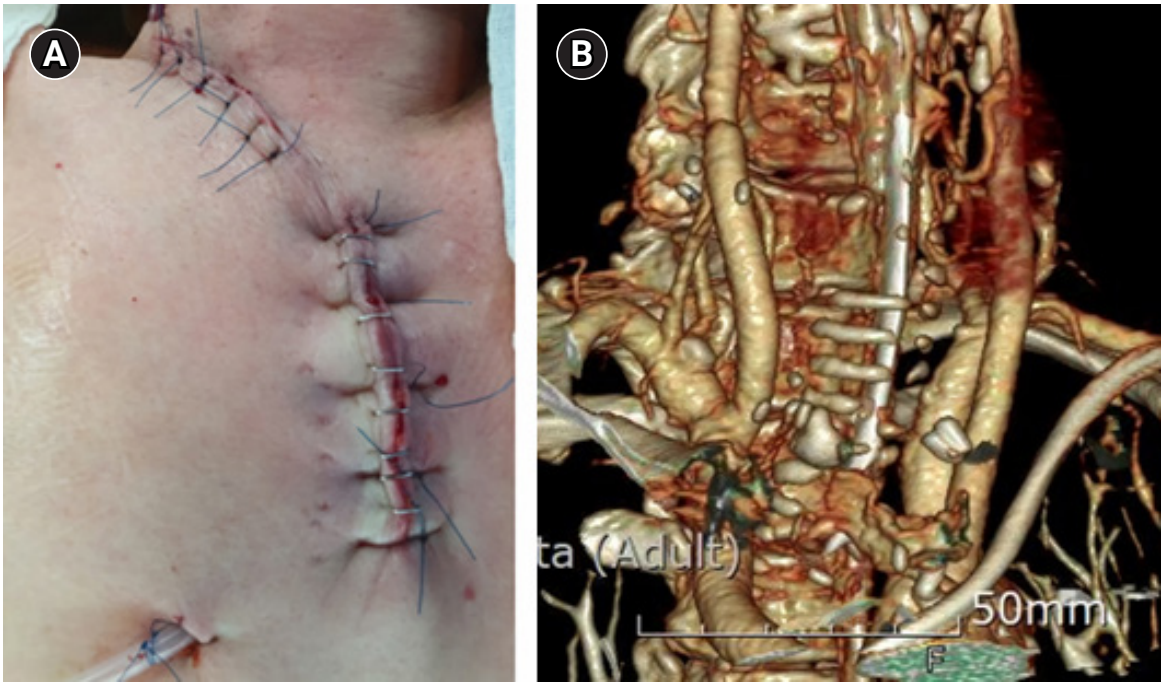

Fig. 3. Postoperative status. (A) Wound and (B) computed tomography scan.

lational tracheostomy, which was uneventful and without wound infection, after the debranching of the anomalous BCT had healed. Unfortunately, the patient succumbed to sepsis with pneumonia after three months of prolonged mechanical ventilation via PDT.

Discussion

The anatomical structures of the aortic arch branches (supra-aortic branches) should be considered during PDT to prevent accidental vascular injury and associated hemorrhage PDT [1]. Clinicians need to be aware of arterial deviations such as a high aortic arch, wide BCT that overlaps with the cervical trachea, the pretracheal course of the right SCA and CCA, and unusual origins of branches of thyroid arteries [2].

To avoid injuries of vessels in the low neck, it is important for physicians to consider anatomic variations that can present in individual patients. Vascular variations such as a cervical aortic arch, an accessory inferior thyroidal artery, and high riding BCT in the pretracheal position could be detected by handheld Doppler before tracheostomy [2]. In addition to anatomic variations in the level and size of the arch vessels, maximal neck extension can elevate a normal BCT or arch [3]. Reduced neck extension can reduce injury to vessels that are lower in the neck [4].

In patients with aortic anomalies, a "debranching technique" can be applied as an alternative approach for tracheostomy without any vascular complications. This approach can be considered as an alternative method to prevent vascular complications during tracheostomy as well as for applying long-term mechanical ventilation for patients that cannot be weaned from mechanical ventilation.

In practice, patients are frequently identified with a portion of a major vessel that is anterior to the trachea at sites where a tracheostomy might be performed. Therefore, physicians must be cautious when performing a percutaneous dilational tracheostomy. Debranching techniques can be used for aortic surgeries and also for tracheostomy in patients with any major vessels that overlap with the trachea at the suprasternal notch.

Article information

Conflicts of interest

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Conceptualization: JHC, HL. Visualization: JHC, HL. Writing - original draft: JHC, HL. Writing - review & editing: JHC, HL. Approval of final manuscript: JHC, HL.

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- Methodology
- Project administration
- Resources
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- Supervision
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- Writing – review and editing

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A clinical trial defined as "any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome" should be registered with the primary registry prior to publication. The KMJ accepts registration in any of the primary registries that participate in the WHO International Clinical Trials Portal (https://www.who.int/clinical-trials-registry-platform), NIH ClinicalTrials.gov (http://www.clinicaltrials.gov), ISRCTN Resister (www.ISRCTN.org), or the Clinical Research Information Service (CRIS), Korea CDC (https://cris.nih.go.kr/cris/index.jsp). The clinical trial registration number shall appear at the end of the abstract.

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The KMJ accepts the "ICMJE Recommendations for Data Sharing Statement Policy." All manuscripts reporting clinical trial results from July 1, 2018 should submit a data-sharing statement following the ICMJE guidelines. Authors can refer to the editorial, "Data Sharing Statements for Clinical Trials: A Requirement of the International Committee

of Medical Journal Editors," in JKMS Vol. 32, No. 7:1051-1053 (http://crossmark.crossref.org/dialog/?doi=10.3346/jkms.2017.32.7.1051&domain=pdf&date_stamp=2017-06-05).

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Copies of written informed consent and institutional review board (IRB) approval for clinical research (original article and case report) should be kept. Authors should include the IRB approval number in the manuscript. If necessary, the editor or reviewers may request copies of these documents to resolve questions about IRB approval and study conduct. For studies involving human subjects, the method through which informed consent was obtained from the participants must be stated in the Methods section.

Statement on human and animal rights

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- Every manuscript should be written in English.
- The main document, including manuscript text and tables, should be prepared in MS Word.
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- All manuscript pages are to be numbered consecutively, beginning with the abstract as page 1. Neither the authors' names nor their affiliations should appear on the manuscript pages.
- Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. A spelled-out term followed by the abbreviation in parentheses should be used on first mention unless the abbreviation is widely recognized (e.g., UN for United Nations).
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The abstract should briefly describe the content of the manuscript in a structured format within 250 words. The abstract should be structured as follows; Background, Methods, Results, and Conclusion. Initialisms, acronyms and informal abbreviations should be avoided where possible; if needed, they should be kept to an absolute minimum and accompanied by proper definitions. Three to five keywords should be listed below the Abstract on the Abstract page. For the selection of keywords, refer to Medical Subject Headings (MeSH) in PubMed, or http://www.nlm.nih.gov/mesh/MBrowser.html.

Main text

- Introduction

A brief background, references to the most pertinent papers general enough to inform readers, and the relevant findings of others should be included. It is recommended that the introduction include a "General and specific background," "Debating issue," and "Specific purpose of this study."

- Methods

The explanation of the experimental methods used should be concise and sufficient for repetition by other qualified investigators. Procedures that have been published previously should not be described in detail. However, new or significant modifications of previously published procedures require full descriptions. Clinical studies or

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(For clinical study)

The present study protocol was reviewed and approved by the Institutional Review Board of xxx XXXXXX of the University College of Medicine (approval No. 2018001). Informed consent was submitted by all subjects when they were enrolled.

(For animal study)

The procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee at xxxXXXXX University (approval No. 2018002).

(For clinical trials)

This was a randomized clinical trial at the second phase, registered at the Clinical Research Information Service (CRIS, http://cris.nih.go.kr), number KCT0002018.

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The data should be interpreted concisely without repeating materials already presented in the Results section. A summary or conclusion should be included at the end of this section. We recommend authors describe the clinical or biomedical significance of the study. Speculation is permitted but must be clearly supported by the results or literature published.

Acknowledgments

The authors can list the names of persons who helped plan or conduct the study but are not eligible authors in this section. Funding sources, which are supplied on the title page, should not be duplicated in this section.

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References should be numbered in the order they appear in the text and should not exceed 40 in number. Citation of references in the text should be identified with Arabic numerals in square brackets (for example, ... the leading cause of death in Korea [1,2] and preceding any punctuation). References should be listed in the order they are cited in the text, with consecutive numbers in this section. The style for citing papers in periodicals is: last name and initials of all authors, full title of article, journal name abbreviated in accordance with the PubMed style, year, volume, issue number and first and last page numbers. The style for a chapter of a book is: author and title of the chapter, editor of the book, title of the book, edition, volume, place (city), publisher, year, and first and last page numbers. Up to six authors can be listed in a reference; the names of all authors after the first 6 should be abbreviated to "et al." The use of software to manage references (e.g., EndNote) is recommended.

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   Verbalis JG. Renal physiology of nocturia. Neurourol Urodyn 2014;33(Suppl 1):S6-9.
2. Book
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3. Chapter in a book
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Case reports will be published only in exceptional circumstances, if they illustrate a rare occurrence of clinical importance. The manuscript should be in the following sequence: title page, abstract (no more than 250 words) and three to five keywords (as in MeSH), introduction, case(s), discussion, acknowledgments, references (no more than 20), tables, and figure legends. The manuscript should have no more than six tables or figures in total. For case reports involving humans, whether informed consent for publication of clinical data was obtained from the study participants must be stated in the case description section.

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Abstract (words)	250	250	250	NR
Text (words)[a]	NL	6,000	1,500	1,000
References	40[b]	100	20	20
Tables and Figures	NL	NL	6	3

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☐ **Sequence:** Title page, Conflict of interest, All sources of funding, Author contributions, Abstract and keywords, Main text, (Acknowledgments), References, Tables, and Figure legends. Numbered pages and lines (from Abstract page).

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Three to five keywords as in MeSH

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