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Aims and scope

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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.

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Omega-3 fatty acids: promising therapeutic agents for combating kidney injuries

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See "Omega-3 fatty acids upregulate Nrf2 expression and attenuate apoptosis, inflammation, and fibrosis in a rat model of cyclosporine-induced nephropathy" by Ji Young Lee, Young Ki Son, Mi Hwa Lee, Su Mi Lee, Seong Eun Kim, Won Suk An

Cyclosporine (CsA)-induced nephropathy is a debilitating condition characterized by renal dysfunction accompanied by inflammation, apoptosis, fibrosis, and hypoxic injury [1]. Despite extensive research, the influence of omega-3 fatty acids (O-3FA) on nuclear factor erythroid 2-related factor 2 (Nrf2) expression, a key regulator of cellular defense mechanisms, remains unclear [2,3]. However, a recent groundbreaking study sheds light on the potential benefits of O-3FA for attenuating these harmful processes in a rat model of CsA-induced nephropathy [4].

The study divided male Sprague-Dawley rats into three groups: a control group, a group treated with CsA, and a group treated with both CsA and O-3FA. The researchers observed significant kidney function impairment in the CsA-treated rats compared to the control group. Additionally, markers associated with apoptosis, such as caspase-3, caspase-7, and the Bax to Bcl2 ratio, were activated in the CsA-treated group. Remarkably, O-3FA supplementation attenuated these apoptotic activation patterns, indicating its potential anti-apoptotic effects.

Furthermore, the CsA-treated group exhibited increased expression of the inflammatory marker ED-1 and inhibition of the IκB protein. However, O-3FA supplementation effectively mitigated the inflammatory response, as evidenced by the reduced expression of ED-1 and IκB. Furthermore, CsA treatment led to the activation of Smad2/3, Smad4, and transforming growth factor-β1, all associated with renal fibrosis. Nevertheless, O-3FA prevented these activations, highlighting its potential anti-fibrotic properties.

Interestingly, the researchers discovered that Nrf2 expression was decreased in CsA-treated rats, but supplementation with O-3FA significantly increased its expression. This finding suggests that Nrf2 may act as a potential mediator induced by O-3FA supplementation, playing a crucial role in attenuating pro-inflammatory pathways, fibrotic processes, and apoptosis.

The study provides compelling evidence that O-3FA supplementation holds immense promise as a therapeutic intervention in CsA-induced nephropathy. By upregulating Nrf2 expression, O-3FA exhibits notable anti-inflammatory, anti-apoptotic, and anti-fibrotic effects, ultimately protecting the kidneys from damage. However, further investiga-

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tions are necessary to elucidate the intricate crosstalk between Nrf2 expression and the signaling pathways involved in O-3FA treatment.

These findings offer new insights into the potential mechanisms underlying the protective effects of O-3FA against kidney injuries. If translated into clinical practice, O-3FA supplementation could emerge as a valuable adjunct therapy for patients with CsA-induced nephropathy, helping to alleviate their symptoms and enhance renal function. Nonetheless, additional studies are warranted to fully understand the therapeutic potential and optimize the dosing and administration strategies of O-3FA in human subjects.

It would be premature to conclude that the efficacy of O-3FA has been proven based solely on the content of this manuscript. One limitation of this study is that it was conducted on male Sprague-Dawley rats, which may not fully represent the response to O-3FA supplementation in humans. Animal models do not always perfectly reflect human physiology, and there may be species-specific differences in the effects of O-3FA on Nrf2 expression and its associated pathways. Furthermore, the authors acknowledged the need for further studies to elucidate the crosstalk between Nrf2 expression and signals related to O-3FA treatment. This study does not provide a comprehensive understanding of the underlying mechanisms by which O-3FA influences Nrf2 and its downstream effects. Future research is necessary to fully explore and confirm these relationships.

In conclusion, despite some limitations, this study marks a significant advancement in our understanding of the protective effects of O-3FA against CsA-induced nephropathy [5]. With further exploration and clinical validation, O-3FA could become an integral component of the treatment arsenal, offering hope to patients suffering from this challenging kidney condition.

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Author contributions
All work was performed by HJC.

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Do we need Moodle in medical education? A review of its impact and utility

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Various learning management systems (LMSs) are available to facilitate the development, management, and distribution of digital resources for both face-to-face and online instruction. In recent decades, these methods have shown potential for greater efficiency compared to traditional "chalk and talk" approaches. Additionally, they have paved the way for the establishment of ubiquitous learning environments, marking a new era in education. In a trend accelerated by the coronavirus disease 2019 pandemic, LMSs have been increasingly adopted to overcome the restrictions inherent to in-person education. In medical education, LMSs such as Moodle, Canvas, Blackboard Learn, and others have been introduced and used to support teaching, learning, and assessment activities. Of these, Moodle stands out as the most popular choice for many medical schools and institutions, primarily due to its flexibility, functionality, and user-friendliness. The learning environment is gradually transforming from traditional in-person teaching to a hybrid educational approach, driven by the need to fulfill diverse educational demands. Numerous research studies have examined the usability of Moodle in medical education, demonstrating its effectiveness in addressing challenges related to adaptive personalized learning, collaborative learning, blended learning, and more. Consequently, Moodle has emerged as a valuable solution for medical educators seeking a versatile and robust platform to enhance their teaching methodologies. The present review focuses on the practical utilization of Moodle in medical education and the advantages it offers to this field.

Keywords: Adaptive learning; Collaborative learning; Flexibility; Functionality; Learning management system

Introduction

Various learning management systems (LMSs) are available to facilitate the development, management, and distribution of digital resources for both face-to-face and online instruction [1]. In recent decades, these methods have shown potential for greater efficiency compared to traditional "chalk and talk" approaches [2]. Additionally, they have paved the way for the establishment of ubiquitous learning environments, marking a new era in education [3]. In a trend accelerated by the coronavirus disease 2019 (COVID-19) pandemic, LMSs have been increasingly adopted to overcome the restrictions inherent to in-person education [4-6]. In medical education, LMSs such as Moodle (named as a reference to "modular object-oriented dynamic learning environment"), Canvas, Blackboard Learn, and others have been introduced and used to support teaching, learning, and assessment activities [7-12]. Moodle has been reported to be the most popular choice for many medical schools and institutions due to its flexibility, func-

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tionality, and user-friendliness [13-16]. The present review is focused on the practical utilization of Moodle in medical education and the advantages it offers to this field.

Methods

This review is centered on recent studies concerning the use of Moodle in academic settings. Because Moodle's evolving technical features have influenced educational strategy, we also meticulously examined specific Moodle functionalities. While our review adopted a comprehensive approach, covering the full scope of academic subjects and levels, we placed special emphasis on gathering evidence from medical institutions and hospitals. To identify pertinent articles, we examined keywords and themes in educational publications, such as PubMed and Google Scholar. We aimed to understand evolving trends, growing areas of interest, and the trajectory of research emphasis over the years.

Schema of Moodle

Moodle is an open-source LMS that is widely used in educational institutions and organizations around the world [17]. It was developed in 2002 by computer scientist Martin Dougiamas as a digital platform for the creation, delivery, and management of online courses and learning materials [18-20]. Since then, Moodle has undergone numerous transformations to enhance its features, user experience, and functionality. This journey has been marked by functional improvements and essential bug fixes, all tailored to meet the evolving needs of educational practice. As the demand for greater sophistication and conditionality increased, beginning with Moodle 2.0, features were introduced that allowed educators to set criteria for course completion, establish prerequisites, and restrict access to activities [21-26]. With the rise of mobile internet usage, Moodle also began to accommodate different screen sizes and prioritize mobile-friendliness by incorporating so-called bootstrap-based themes, starting with version 2.5 in 2013 [27-31]. In the most recent version, Moodle LMS 4, the platform has been further refined to facilitate more efficient collaborative learning experiences for educators and more intuitive coursework completion for learners, built on a foundation of user-friendly operability [32-34].

With Moodle, educators can create and customize courses, incorporating a variety of multimedia elements such as videos, audio files, and interactive quizzes [35,36]. The system offers a range of tools for course administration, including gradebooks, discussion forums, and assignment submission features [37].

A key aspect of Moodle is its flexibility and adaptability to different learning environments [16]. The software supports a wide range of pedagogical approaches, allowing instructors to design courses that best suit their teaching style and objectives. In addition, Moodle facilitates communication and collaboration among learners and instructors [38]. It offers many collaboration tools, such as messaging features, forums, and Wiki functionality, that promote interaction and knowledge sharing. As an open-source platform, Moodle benefits from a large, active community of developers and users who contribute to its ongoing development and improvement [18]. This community-driven approach fosters innovation and enables the sharing of resources, plugins, and best practices. Overall, Moodle serves as a full-featured LMS that empowers educators to create engaging online learning experiences and support effective teaching and learning.

Features of Moodle

Moodle offers a variety of features designed to facilitate effective instruction and engage students in the learning process (Fig. 1). The following sections detail several key features [39].

1. Course creation and customization

Using Moodle, educators may create and customize online courses according to their teaching objectives. They can organize course materials into sections, upload files, embed multimedia, and incorporate interactive activities. This functionality enables instructors to design engaging and interactive learning experiences.

2. Discussion forums

Moodle provides discussion forums in which students can communicate with their peers and instructors. These forums foster collaboration, critical thinking, and knowledge sharing among students. Instructors can moderate discussions, pose questions, and encourage active participation.

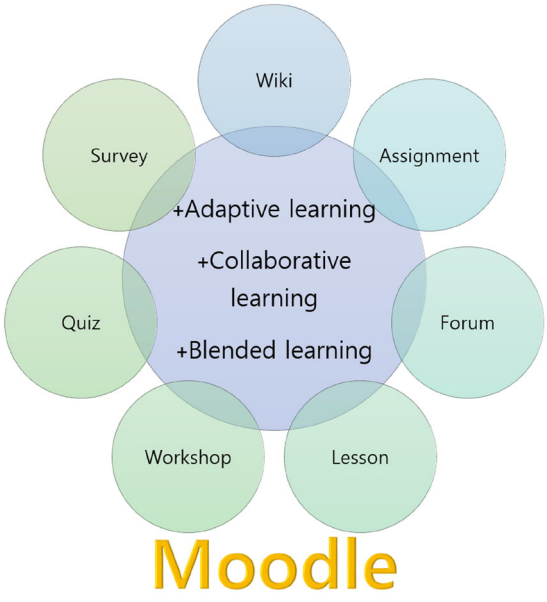

Fig. 1. Key features of Moodle, showcasing its flexibility and adaptability to various learning environments. The Moodle platform offers a wide array of activities, including Wiki pages, assignments, forums, lessons, workshops, quizzes, surveys, and other features to meet the diverse needs of educators and to support various learning approaches, such as adaptive, collaborative, and blended learning.

3. Assignments and assessments

Moodle includes a range of assignment and assessment features. Educators can create various types of assignments, such as essays, quizzes, and projects, and set deadlines for submission. Moodle also includes a gradebook and related tools to facilitate efficient grading and timely provision of feedback.

4. Online quizzes and exams

With Moodle, online quizzes and exams can be built from various question types, including multiple choice, short answer, and matching questions. Instructors can set time limits, randomize questions, and provide immediate feedback to students upon completion. This functionality supports self-assessment and knowledge retention.

5. Communication tools

Moodle includes messaging features, such as private messaging and forums, to facilitate student-instructor communication. This allows students to ask questions, seek clarification, and receive guidance outside of class hours. These tools promote a supportive learning environment and foster a sense of community.

6. Content restriction

Using Moodle, instructors may release course content gradually or sequentially. Students can therefore be guided through a structured learning path while accessing materials in a logical and organized manner. This feature is particularly useful for self-paced or module-based courses.

7. Multimedia integration

Moodle supports the integration of multimedia elements, such as videos, audio files, and interactive simulations. Instructors can enrich their course content with multimedia resources to enhance student engagement and accommodate different learning styles.

8. Mobile accessibility

Moodle is designed to be accessible on various devices, including smartphones and tablets [40]. Its responsive design ensures that students can access course materials, participate in activities, and engage with their learning anytime and anywhere, providing flexibility and convenience. However, smartphone apps exhibit some limitations in operating functions for learning or teaching activities compared to the computer-based version of Moodle [41].

9. Progress tracking and analytics

Moodle provides tools for monitoring student progress, such as activity completion and course completion tracking. Instructors can monitor student engagement, identify areas of improvement, and provide personalized support as needed. Learning analytics features in Moodle also enable instructors to gain insights into student performance and behavior patterns. These features help create an interactive and dynamic learning environment, promote collaboration, and support student-centered learning approaches.

10. Integrations

Moodle is a flexible LMS crafted for seamless integration with numerous external applications and platforms. Its commitment to open design and modular focus have given rise to a plethora of plugins and integrated features.

Through its open application programming interfaces and services, Moodle offers integration opportunities with student information systems, content repositories, and various other tools [42-45].

Practical applications of Moodle

While an online LMS may not be considered a mandatory tool for medical education, demand for computer-assisted LMSs has been rising. Many educators have recognized the value of Moodle's diverse features and have effectively incorporated them for specific purposes. During the COVID-19 pandemic era, educators in an ophthalmology residency program used online learning as a key teaching strategy [46]. Those authors reviewed several commercial and open-source LMS options, including Blackboard, Desire2Learn, Edmodo, ConnectEDU, Moodle, Sakai, edX, and llios. Moodle was selected for implementation due to its cost-effectiveness, feature availability, and compatibility with existing technical infrastructure. The authors concluded that LMS implementation was successful in meeting the needs of faculty and residents. Researchers in a physiology course at the Faculty of Medicine of the University of Montenegro assessed the effect of e-learning on student success in mastering coursework [47]. The authors compared two groups of students: one group who attended the physiology course prior to the implementation of the Moodle platform and another group who attended the course after Moodle had been fully introduced. They concluded that attending face-to-face lectures was associated with better academic performance. However, the introduction of Moodle increased attendance at face-to-face lectures and improved formative and summative scores. This study demonstrated the benefits of blended learning with web-based course management systems like Moodle in medical education.

1. Forum activities

In the Moodle platform, educators can utilize discussion forums for regular dialogue with students [48]. Students can access course materials at their own pace, and the tracking feature of the LMS allows them to diagnose their learning needs. In the Medical School of Universidade Cidade de São Paulo, Brazil, the internship program consists of rotations in several medical specialties and is designed to provide students with practical experience in various healthcare settings. Due to the diverse locations of these settings and the need for effective communication and feedback between students and teaching staff, the Moodle environment was used to facilitate teacher-student communication through the posting of messages and pedagogical information [49]. Additionally, forums have allowed students to maintain much more direct contact when not in class and more easily collaborate on projects despite not being co-located [50,51].

2. Assignment activities

In Moodle, the assignment activity enables educators to collect digital content such as word processor files, spreadsheets, images, and other materials from students and provide grades and feedback. An assignment can be provided to students through various activity modules. In a university hospital, an assignment consisting of essay and quiz activities was provided to teach research ethics to clinical researchers, and the authors concluded that the program was feasible for this purpose [52]. Although a specific module such as an assignment activity can be used to build a course in Moodle, various activity modules or a combination of those activities can also be flexibly selected and applied, depending on the instructor's intention and the educational conditions of the institution.

3. Quiz activities

One of the most frequently used and powerful activities in Moodle is the quiz functionality. This enables educators to generate quizzes containing various question types, such as multiple choice, matching, short answer, and numerical questions. Furthermore, educators can customize the quiz settings in Moodle to promote voluntary self-directed learning [53]. To do this, instructors can set pre-written remarks to appear in response to each presented answer in a quiz, providing the immediate delivery of various pieces of feedback about the student's performance [54,55]. This simultaneous preset feedback can also be utilized as guidance for self-directed learning [19,53].

4. Workshop activities

Peer review is sometimes adopted as a teaching method to foster students' thinking skills through the assessment of their classmates. The Moodle workshop activity for peer assessment has been used to support peer review in educa-

tion [56-59]. In Moodle, educators establish a multi-criterion assessment form, through which students may evaluate one or more of their peers' submissions. In previous research, when the grades assigned by peers were compared with those by instructors, no significant difference was found; consequently, this reduced the workload on the educators as well [60]. This peer feedback activity could be incorporated into online courses to improve students' evaluative judgment [61]. Additionally, it can assist students in improving cognitive schema and strengthen positive attitudes toward discussing and cooperating with peers [62].

5. Lesson activities

The lesson activity module in Moodle is relatively flexible, allowing for the delivery of content and/or practice activities to students, with a composition that can vary in complexity [63]. With this module, educators can choose to craft content pages or instructional activities that offer students multiple paths or options to explore. To boost engagement and comprehension, educators can incorporate diverse question types, including multiple choice, matching, and short answer questions. Based on the student's selected answers and the educator's lesson design, the learning journey may lead the student to the next page, to return to a previous page, or even to take a different route altogether. This adaptability in the learning process ensures a dynamic and personalized learning experience for each student [64].

6. Wiki activities

The Wiki activity module enables participants to contribute to and modify a collection of webpages [65]. Wikis can either be collaborative, meaning that anyone can edit them, or individual, where each user has a Wiki that only that person can edit. One notable characteristic of a Wiki is its capacity to maintain a record of versions of user-created documents [66]. Each contribution made by a user to a Wiki page generates a new version, which is then recorded. As a result, it becomes feasible to track edits, identify the differences between consecutive versions, and even restore previous iterations when needed [67,68]. The tracking capability in a Wiki can enhance the collaborative process, producing a more defined and accurate document focused on a specific topic [69]. In a neuroscience course designed for first-year medical students, a Wiki-based group project was incorporated to assist students in reviewing course content and establishing clinically meaningful connections [70]. A total of 205 master students at a medical school participated in a course that incorporated a flipped-classroom model. In the course of their learning through online and in-class activities, the students were divided into two groups. One group (n=85) completed a group assignment using an educational Wiki, while the other group (n=120) followed a conventional approach. The students in the Wiki group expressed higher satisfaction with the course. Furthermore, both the quantity and the quality of the group assignments among students in the Wiki group surpassed those in the non-Wiki group [71]. These findings confirm that the use of a Wiki-based group assignment effectively enhanced student learning outcomes. Hence, cooperative learning is thought to nurture students' collaborative skills and potentially enhance cognitive outcomes and overall academic performance [72].

7. Survey activities

The survey activity module in Moodle enables educators to collect data from students, providing valuable insights about their classes and facilitating self-reflection on their teaching methods [73]. Educators can design various types of questionnaires in the survey activity module. This module is an extremely valuable tool for researchers and educators alike, as it facilitates investigation through the gathering of insights from students or participants in each course. The tool also empowers researchers and educators to analyze the collected data effectively.

Current trends in the use of Moodle

As continuous innovations in e-learning technologies point towards an educational revolution, facilitating individualized learning experiences and enriching learners' interactions with others [74], Moodle is increasingly utilized as a platform for adaptive and collaborative learning [75]. In an innovative approach, the Internal Medicine Residency Program at the Cleveland Clinic assessed the medical knowledge of its residents before clinical rotations. Trainees were given access to an online, adaptive spaced-education module to augment their medical knowledge before embarking on demanding outpatient clinical rotations. The effectiveness of this approach was demonstrated [76]. In higher education, online collaborative learning has incor-

porated a diverse set of tools, including various Moodle activities such as forums, Wikis, workshops, and assignments, to foster cooperative learning experiences [77,78]. During the COVID-19 pandemic, courses on the Moodle platform served as alternatives to in-person curricula for medical student education [79]. Driven by the pandemic, some universities in the United States have introduced multi-institutional online didactic programs that have transformed the conventional resident teaching model from isolated institutional knowledge hubs into a collective nationwide learning repository [80].

Blended learning that combines the Moodle platform with in-person traditional teaching has also shown a positive impact on students' knowledge, attitudes, and practices [81]. In a study conducted at the Faculty of Medicine, University of Pristina, Kosovska Mitrovica, third-year medical students participated in an assessment of the effectiveness of problem-based learning (PBL) modules integrated into blended learning courses on medical statistics [82]. A blended learning course on medical statistics and informatics was structured on the Moodle platform and comprised 15 theoretical lecture classes, 30 practical exercise classes, and 15 other classes involving online readings or seminars. The presented PBL modules were readily applicable to existing medical statistics courses developed on the Moodle platform, allowing for seamless implementation and integration.

In a study evaluating hybrid teaching and assessment approaches in anatomy courses, the researchers contrasted student performance in both the theoretical and practical segments of two initial anatomy courses across three semesters [83]. During the spring 2019 term, students experienced traditional, face-to-face classroom instruction and examinations for both components. In contrast, the spring 2020 semester began with conventional face-to-face methods, but a swift conversion occurred mid-term to online instruction and examinations. This abrupt transition to a digital format corresponded with elevated average scores and reduced score variance in both theoretical and hands-on assessments. However, in the spring 2021 term, when a wholly online approach was implemented, the average scores dropped. The results of this study indicate that blended teaching approaches may be as effective as conventional in-person instruction. However, for optimal results, careful planning and preparation are crucial.

The unique benefits provided by online learning environments, which can be lacking in traditional in-person instruction, are anticipated to fuel the growing adoption of blended learning in real-life educational settings. Consequently, demand is rising for a well-designed and effective LMS to meet these evolving requirements, and Moodle has been demonstrated to effectively fulfill these needs.

Limitations of Moodle

Despite these promising findings, Moodle faces some unresolved issues. Firstly, the platform may pose challenges for technology-challenged educators due to its somewhat confusing interface [84]. Consequently, educators may require prior knowledge or experience to effectively build a course within Moodle. Secondly, despite Moodle being a free open-source learning platform, the installation and support process may not be as user-friendly as desired [85]. While some companies offer assistance to institutions or individuals in setting up and maintaining the Moodle platform, the technical specifications for installation are relatively rigid, and the process lacks user-friendliness and intuitiveness. Thirdly, concerns have been raised regarding security vulnerabilities such as SQL injection risks, which could potentially grant an attacker unauthorized access and control over a database server [86]. It is imperative to promptly address security issues to maximize the safety and reliability of the Moodle platform.

Conclusion

Among the wide array of LMS options in medical education, Moodle has emerged as a preferred choice based on its remarkable functionality and cost-effectiveness. Over time, the learning environment has undergone a gradual shift from traditional in-person teaching to a hybrid educational approach, both in response to challenges like the COVID-19 pandemic and to accommodate diverse educational requirements. Various research studies analyzing the usability of Moodle in medical education have demonstrated its effectiveness in addressing issues related to adaptive learning, collaborative learning, and blended learning, among others. As a result, Moodle has been shown to be a valuable solution for medical educators seeking a versatile and robust platform to enhance their teaching methodologies.

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Gut microbiota and nonalcoholic fatty liver disease

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The gut microbiota has been reported to exert a significant influence on various physiological responses of hosts. Extensive evidence has recently emerged linking metabolic and cardiovascular disorders to the gut microbiota. Nonalcoholic fatty liver disease (NAFLD) is the most common underlying metabolic disorder, and its prevalence is increasing worldwide. In this study, we aim to review the relationship between the gut microbiota and NAFLD, and explore the potential of the gut microbiota as a novel target for NAFLD treatment.

Keywords: Gut microbiome; Gut microbiota; Non-alcoholic fatty liver disease; Steatohepatitis

Introduction

The gut microbiota in adults is known to comprise between 10 and 100 trillion microorganisms, a quantity that is more than 10-fold the number of human cells [1]. Moreover, the collective genomes of the gut microbiota are 100 to 150 times greater than that of the human genome [2]. Several metagenomic studies have suggested a correlation between the quantity or diversity of genes in the gut microbiota and the health of the host [3-5]. The gut microbiota has evolved alongside human evolution and has been found to significantly influence various physiological responses of the host. Notably, recent studies have demonstrated that alterations in the gut microbial composition are associated with various metabolic diseases, including obesity [6], nonalcoholic fatty liver disease (NAFLD) [7], type 2 diabetes [8], and cardiovascular disease [9-11].

More than one-third of the global population is affected by NAFLD, and the prevalence of this condition has significantly increased [12]. NAFLD induces insulin resistance and generates numerous inflammatory cytokines, bile acid, and cholesterol. Collectively, these factors can lead to type 2 diabetes, and conversely, type 2 diabetes can exacerbate NAFLD [13-17]. Furthermore, NAFLD is a significant contributor to the onset of cardiovascular diseases [18,19]. NAFLD can progress to liver inflammation and hepatocyte damage, resulting in nonalcoholic steatohepatitis (NASH). In some patients, NASH can cause slow, progressive, and severe liver damage, including fibrosis and ultimately, liver cirrhosis (LC). Therefore, NAFLD acts as a fundamental underlying condition contributing to various metabolic disorders.

In this article, we aim to review the relationship between the gut microbiota and NAFLD, as established by various

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studies to date. We also explore the potential of the gut microbiota as a novel therapeutic target for NAFLD in the future.

Gut microbiota and NAFLD: pathogenic mechanisms

Dysbiosis of the gut microbiota is recognized as a cause of NAFLD and NASH. Various environmental factors influence this dysbiosis. Notably, a diet high in fat and fructose, extensive exposure to medications such as antibiotics and proton pump inhibitors, and various food preservatives are identified as significant environmental contributors to gut microbiota dysbiosis [20-24]. Fructose, which is a monosaccharide naturally found in fruits and honey, has been demonstrated to play a substantial role in the pathogenesis of NAFLD and NASH in both preclinical and clinical studies [25-28], and it is known to induce gut microbiota dysbiosis [29,30]. In addition to its impact on gut microbiota, fructose also has direct, detrimental effects on the liver. Its unique metabolic pathway leads to ATP depletion, uric acid generation, mitochondrial dysfunction, de novo lipogenesis, and the inhibition of beta-fatty oxidation [31-35].

Small internal bacterial overgrowth and gut leakiness play a key role in the occurrence and progression of NAFLD (Fig. 1). Small internal bacterial overgrowth is commonly triggered by an imbalance in gut microbiota [36]. This imbalance leads to an increase in various microbial components and metabolites, including ethanol, lipopolysaccharide, trimethylamine, short-chain fatty acids, and microbial DNA. These elements, along with intestinal mucosal lesions, contribute to increased gut permeability [37,38]. Gut leakiness can also result from dysfunction in the structures of the intestinal barrier. Proteins in the tight junctions serve as crucial mucosal barriers that prevent bacterial translocation. When these proteins are damaged, the translocation of microbial metabolic products, such as lipopolysaccharide, into the bloodstream is increased. This process induces a state of endotoxemia, triggering inflammation in the liver [37,38]. The endotoxemia caused by increased intestinal permeability and the subsequent translocation to the liver are critical factors in the development of NAFLD (Fig. 1).

The gut-liver axis refers to the communication between the gut and the liver. This communication is bidirectional and occurs through the biliary tract, portal vein, and systemic circulation (Fig 1). Endotoxins that reach the liver via the portal vein interact with receptors such as Toll-like receptors 4 or 9 (TLR4 or TLR9). TLR4 is found on the cell membranes of hepatocytes and immune cells, specifically Kupffer cells. TLR4 facilitates the activation of molecules such as NF-κB, which in turn activate inflammatory cytokines [39,40]. The biliary tract plays a pivotal role in enabling bidirectional communication between the liver and

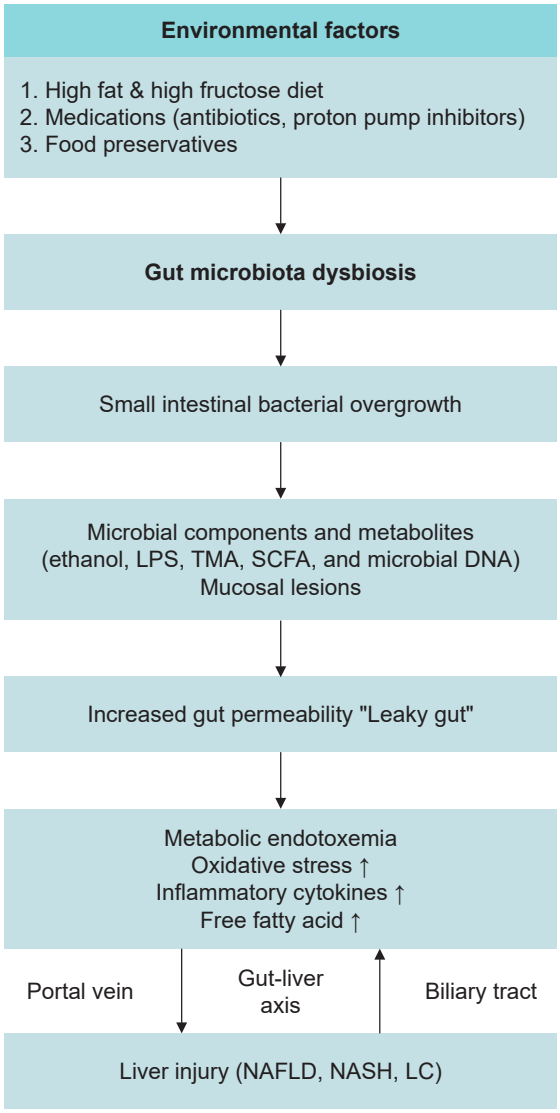

Fig. 1. Summary of the mechanisms through which gut microbiota dysbiosis leads to NAFLD progression. LPS, lipopolysaccharide; TMA, trimethylamine; SCFA, short-chain fatty acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; LC, liver cirrhosis.

the intestine. Substances derived from the liver significantly influence both the composition of the gut microbiota and the integrity of the gut barrier [41]. These processes can aggravate liver damage. NAFLD, NASH, and LC are often viewed as a continuum, with shared pathways influenced by the gut microbiota. However, there is still a substantial gap in research concerning the specific mechanisms by which the gut microbiota uniquely contributes to the progression from NAFLD to NASH and LC. Future research should aim to uncover these unique mechanisms for each condition and gain a better understanding of how the gut microbiota may impact these processes. In conclusion, maintaining the integrity of tight junctions and inhibiting gut microbiota dysbiosis could be an effective strategy for preventing or treating NAFLD and other gut-related diseases.

Keystone species of gut microbiota

Patients with NAFLD have been found to exhibit alterations in their gut microbiota compared to healthy individuals. Notably, the gut microbiota signatures associated with NAFLD include an increase in the Proteobacteria phylum, the Enterobacteriaceae family, and the *Escherichia*, *Bacteroides*, *Dorea*, and *Peptoniphilus* genera. Conversely, there is a decrease in the Rikenellaceae and Ruminococcaceae families, and the *Faecalibacterium*, *Coprococcus*, *Anaerosporobacter*, and *Eubacterium* genera [42-46]. However, interventional clinical studies to determine whether these specific species cause NAFLD, in order to establish causality, are not feasible due to observations from several studies that these species change following bariatric metabolic surgery [47-49].

Certain specific species have been utilized in the treatment of NAFLD, and the results have shown promise in improving the condition [50,51]. The most used probiotics belong to the *Lactobacillus* genus, as follows: *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* [52-57]. Others include *Bifidobacteria* and *Streptococcus thermophiles* [58]. Numerous studies have recently explored the combination of multiple species of probiotics [59-61].

Gut microbiota and NAFLD: therapeutic interventions

Numerous studies have targeted the gut microbiota for therapeutic and preventative interventions. These interventions encompass probiotics, prebiotics, synbiotic supplements, and fecal microbiota transplantation (FMT). In the context of NAFLD treatment, research has been conducted to alter the gut microbiota composition and reestablish balance through the administration of probiotics, prebiotics, and synbiotic supplements. Probiotics are specific species that could offer beneficial effects. Prebiotics have recently been defined as indigestible dietary components that selectively stimulate the growth and activity of beneficial gut bacteria. This definition has been broadened to include not only indigestible carbohydrates such as fructooligosaccharides, galactooligosaccharides, and trans-galactooligosaccharides, but also other substances like polyunsaturated fatty acids and polyphenols that can modulate the gut microbiota [62,63]. Synbiotics are defined as a mixture of probiotics and prebiotics.

Animal studies have shown that probiotics can slow the progression of NAFLD [64,65]. Furthermore, a meta-analysis of clinical studies, in which patients with NAFLD were treated with probiotics, revealed significant reductions in alanine aminotransferase, aspartate aminotransferase, and total cholesterol within the probiotics group [66,67]. Although the number of patients included in these studies is limited, making it challenging to evaluate any actual changes in the composition of the intestinal microflora posttreatment, probiotics, prebiotics, and synbiotic supplements are associated with minimal side effects. Therefore, the results of future research are eagerly anticipated. A randomized controlled study of FMT, where fecal bacteria from healthy individuals are transplanted into NAFLD patients, has also been recently published [68]. The group that underwent FMT showed improved intestinal permeability. However, there was no observed difference in insulin resistance or intrahepatic fat [68]. In the same study, while an increase in bacterial diversity was noted, there were no definitive changes in the composition of the microbiota. This lack of change in microbiota composition may be due to the administration of FMT into the duodenum. Stool specimen analysis may not accurately reflect changes in the microbiome of the small intestine or the proximal colon. Conversely, another clinical trial where FMT was administered via colonoscopy demonstrated changes in both the composition of the microbiota and fatty liver post-FMT [69]. Therefore, further research is needed to explore the therapeutic

effects of FMT in patients with NAFLD.

Another promising area for therapeutic intervention lies in factors associated with bile acid metabolism. Bile acids serve to prevent intestinal bacterial overgrowth, both directly and indirectly. Obeticholic acid (OCA), a potent activator of the farnesoid X receptor, has been shown to improve hepatic steatosis and fibrosis in animal studies [70]. Furthermore, OCA has been found to reduce bacterial translocation and improve gut microbiota dysbiosis in rats with LC [71]. A phase 3 clinical trial with OCA demonstrated a protective effect against fibrosis, as confirmed by biopsy [72]. However, despite these promising results, numerous patients have reported unusual observations, such as dermatological manifestations, during clinical trials. Consequently, the applicability of OCA to patients remains unconfirmed and is a subject of ongoing debate. Fibroblast growth factor 19 is a gut hormone that plays a major role in regulating bile acid metabolism [73,74]. The fibroblast growth factor 19 analog NGM282, which regulates bile acid synthesis and glucose homeostasis, has been shown to reduce hepatic steatosis in patients with NASH. A phase 2 study of NAFLD with NGM282, published in 2018, revealed that the treatment group experienced significant reductions in intrahepatic fat, fibrosis-related markers, and intrahepatic fat content within 12 weeks [75]. Regarding drug side effects, only mild symptoms such as digestive discomfort and pain at the injection site have been reported [75]. Further research results on the use of NGM282 to treat NAFLD and NASH are expected in the future.

Conclusion

Gut microbiota dysbiosis resulting from various environmental factors causes NAFLD. There have been attempts to identify treatment targets for NAFLD through studies on the mechanisms through which gut dysbiosis causes NAFLD. Moreover, numerous studies have shown improvements in NAFLD by directly restoring the composition of intestinal microbiota through probiotics, prebiotics, synbiotics, and FMT. In the future, studies exploring how the gut microbiome could be targeted for the treatment of NAFLD are expected.

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Application of Raman spectroscopy in breast cancer surgery

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The incidence of breast cancer is increasing worldwide. As cancer screening has become more widespread, the rate of early breast cancer detection has increased and treatment methods have changed. Partial mastectomy is performed more often than total mastectomy for the surgical treatment of early breast cancer, and sentinel lymph node biopsy plays an important role. A high level of accuracy is necessary for the intraoperative examination of surgical margins and sentinel lymph nodes to identify malignancies. Therefore, several examination techniques, including Raman spectroscopy, that replace or supplement the currently used frozen-section methods are being studied. Raman spectroscopy has the ability to diagnose cancer in normal tissue by providing in real time a chemical fingerprint that can be used to differentiate between cells and tissues. Numerous studies have investigated the utilization of Raman spectroscopy to identify cancer in the margins of resected tissues and sentinel lymph nodes during breast cancer surgery, showing the potential of this technique for clinical applications. This article introduces and reviews the research on Raman spectroscopy for breast cancer surgery.

Keywords: Breast neoplasms; Raman spectroscopy; Sentinel lymph node biopsy; Spectrum analysis

Introduction

Breast cancer is currently the most commonly diagnosed cancer in women, accounting for a quarter of all cancer cases [1]. A total of 2.3 million new cases of breast cancer were diagnosed in 2020, accounting for one in eight newly diagnosed cancers [2]. The incidence of breast cancer is rising not only in the United States and Europe but also in Asia [3]. With the development of screening using mammography and ultrasound, and the development of various treatment methods, the treatment results for breast cancer are becoming more outstanding [4]. Among all breast cancer diagnoses, the proportion of early-stage breast cancers continues to increase. Accordingly, the surgical methods for breast cancer are constantly changing. Modified radical mastectomy was the standard for surgical treatment of breast cancer. However, with the introduction of partial mastectomy accompanied by radiation, the surgical treatment of breast cancer has undergone great changes [5,6]. In addition, with the introduction of sentinel lymph node biopsy (SLNB) into breast cancer surgery by Giuliano et al. [7], many patients with early breast cancer skipped axillary lymphatic dissection, thereby reducing the incidence of lymphedema. Such changes in breast cancer surgery require higher levels of accuracy and safety. In the process of confirming the resection margin and sentinel lymph nodes by frozen section

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examination during surgery, a certain false-negative rate is reported in the pathological examination [8,9]. If the frozen biopsy result is negative, but cancer cells are found in the final biopsy report, the patient may experience the inconvenience of having to undergo reoperation to remove any cancer cells that may remain in the body. The reoperation rate has been reported up to 50% depending on the study [10-12]. It follows that optical technologies have been developed for accurate diagnosis of sentinel lymph node and tumor margins such as diffuse reflectance spectroscopy, fluorescence spectroscopy, and photoacoustic spectroscopy [13-18]. Especially Raman spectroscopy is an excellent technique for material analysis due to its high molecular specificity [19], various studies have been conducted on the evaluation of sentinel lymph node and tumor margin in breast cancer [20-24]. But Raman scattering has disadvantages that are difficult to apply clinically, such as low signal-to-noise ratio and exacerbated by fluorescence interference [25], and long measurement time [26]. Nonetheless, recent advances such as high-efficiency laser sources, low-noise detectors, effective filters, and high-efficiency optics have greatly improved this applicability [27,28].

Background of Raman spectroscopy

1. Raman spectroscopy system

Raman spectroscopy was first observed experimentally in 1928 [29]; however, because of the rare occurrence of Raman scattering, which only occurs with a probability of 1 in approximately 10^8, it was difficult to observe [30,31]. Recent advancements in technology have enabled real-time observation using Raman spectroscopy, leading to its widespread application in the clinical field. Raman spectroscopy is a powerful technique for the spectroscopy of vibrations produced by the interacting energy in materials, including cells and tissues. It allows the identification and analysis of the molecular structure, symmetry, electronic environment, and composition of a material, providing a chemical fingerprint that can be used to distinguish between cells and tissues [32-37]. Diseases, particularly cancer, alter the chemical fingerprints of tissues. Raman spectroscopy has the potential to differentiate between diseased and normal tissues. However, it requires a large amount of trained reference data and an accurate analysis model [32,34].

To explain the Raman scattering phenomenon, it is necessary to first describe Rayleigh scattering. Various interactions, such as absorption, reflection, and scattering, occur when light interacts with a material. Scattering refers to the deviation of light from its original path and its propagation in different directions. Rayleigh scattering occurs when the energies of the incident light and light emitted in different directions are equal. This type of scattering is also known as elastic scattering, owing to its characteristic nature. However, there are cases in which the scattered light possesses more or less energy than the original energy. For example, a portion of the incident energy may be utilized for the vibrational motion of atoms or the rotational motion of molecules, whereas the remaining energy is scattered as light. In this scenario, only energy lower than the incident energy is emitted, resulting in the emission of light with relatively longer wavelengths compared to Rayleigh scattering. This process is referred to as Stokes Raman scattering. Conversely, when the material is already in a high-energy state upon receiving light, more energy is emitted than incident energy. Consequently, the wavelength of the scattered light shortens; this phenomenon is known as anti-Stokes Raman scattering (Fig. 1) [38].

2. Raman spectra

The incident photons interact with molecules in the tissue. Rayleigh occurs when the energy of the scattered photon is equal to that of the incident photon. The rare occurrence of a difference in energy between a scattered photon and an incident photon is called inelastic scattering and is known as the Raman effect. Only one photon in 10^8 undergoes the Raman effect [30,39].

A laser is used to provide high-quality monochromatic light and induce Raman scattering. To collect light that reacts with the tissue, appropriate optics must be configured in the optical path. Recent developments in Raman detectors include the use of highly sensitive detectors and gratings. A schematic of the Raman spectroscopy system is shown in Fig. 2.

In general, Raman spectra have characteristic chemical fingerprints that depend on the wavelength of the incident laser, molecular composition, and bonding form. Raman spectroscopic techniques have been developed in several types. Spontaneous Raman spectroscopy, when used in combination with a fiber probe or microscope, is characterized by being label-free, noninvasive, and nonde-

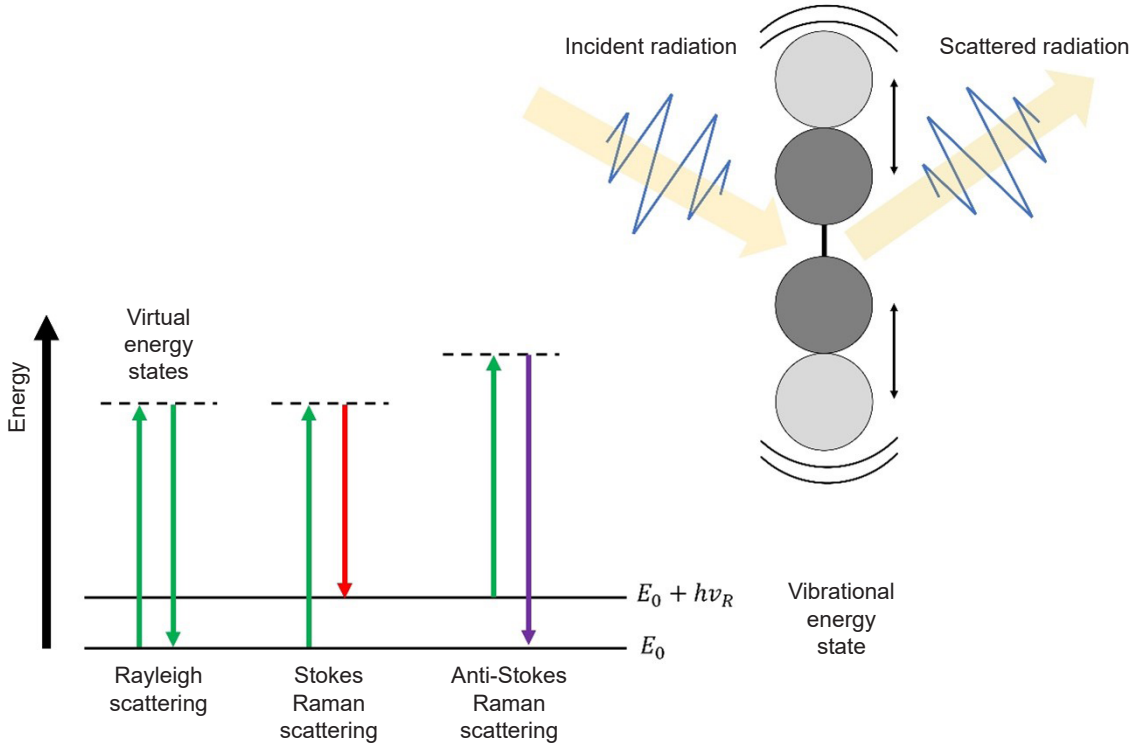

Fig. 1. Jablonski diagram illustrating the energy transition for Rayleigh and Raman scattering. In Raman scattering, energy transition is defined when there is an energy difference of hv_R from Rayleigh scattering. This energy arises from the vibrations of atoms or the rotational motion of molecules.

structive. Resonance Raman spectroscopy, which matches the excitation wavelength to the electron resonance of molecules, increases the signal-to-noise ratio by 10^3 to 10^5. Surface-enhanced Raman spectroscopy applied to rough metal surfaces results in a 10^6-fold increase in the signal-to-noise ratio and has been applied to cell-based assays and immunoassays. Spatially offset Raman spectroscopy technology, which collects diffusely scattered photons, can acquire information even in relatively thick tissues and has been applied to cancer detection in breast tissue [30].

The Raman spectrum provides the fingerprint of a material; however, it is not possible to directly interpret the composition of the material from this chemical fingerprint. A database of reference spectra is required to use Raman spectroscopy in the analysis of materials. A large number of Raman spectra have been published for this purpose [40-42]. Also, simulations and deep learning continue to be studied [39,43]. Unlike single materials, human tissues are a complex assembly of various molecular structures. Raman spectra of protein structures in human tissues are continuously being studied [44,45].

Raman spectroscopy of breast cancer surgery

1. Frozen section analysis in breast cancer surgery

Partial mastectomy combined with postoperative radiation therapy has become the gold standard treatment for patients with early-stage breast cancer, offering equivalent survival and improved quality of life compared to patients undergoing total mastectomy [46,47]. Complete resection of tumors is essential for partial mastectomy to reduce the recurrence rate after surgical treatment [48,49]. During partial mastectomy, the surgeon may request rapid pathological information of marginal status and determine whether additional resection is required. SLNB is also important for the surgical treatment of early breast cancer. The surgeon checks the progress of the disease through SLNB during

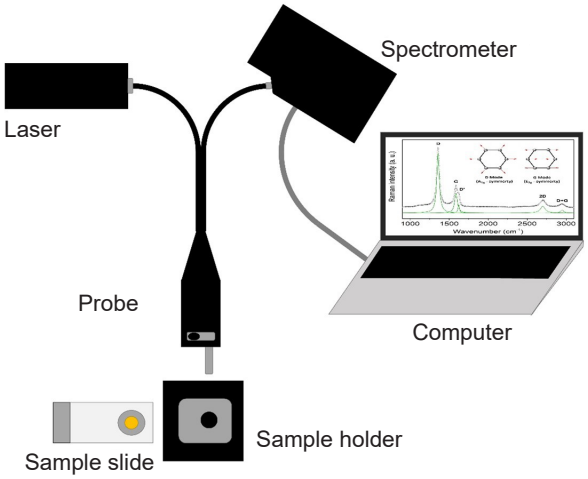

Fig. 2. Monochromatic excitation light generated by a laser source passing through a narrow-band filter within a probe. After interacting with the sample, the laser light re-enters the probe. When the Raman operation is activated, the incident light is directed to an optical system and transmitted to a spectrometer. The spectrometer's grating separates the collected light, which is then detected and analyzed through software. A sample holder can be used to minimize ambient light noise.

surgery and makes a dicision of the surgical scope of the axillary region. As the rate of early breast cancer increases, the need for a method that can quickly confirm pathological results during surgery has increased. Currently, frozen section analysis is the most frequently performed method for confirming the pathological results of intraoperative biopsies. When a surgeon sends a tissue that needs to be inspected during surgery to a pathologist, the tissue is analyzed through frozen sectioning and notified of the result, which reduces the frequency of reoperations that occur after surgery [50,51]. However, This method has limitation in sensitivity. Studies analyzing marginal frozen sections obtained from partial mastectomy have reported sensitivities of 77%–81% [52,53]. In SLNB, the sensitivity of macro-metastasis and micro-metastasis was different. The sensitivity for diagnosis of macro-metastasis was over 90%, but the sensitivity for diagnosis of micro-metastasis was reported to be 30%–40% [8,54-56]. If the results of frozen section analysis confirmed during surgery and the final biopsy results confirmed after surgery are different, the patient may experience the inconvenience of having to repeat the operation, which leads to an increase in complications, hospitalization days, and medical expenses [57-60].

2. Raman spectroscopy of surgical margins

In 2006, a study that implemented Raman spectroscopy of breast tissue in an *in vivo* environment was reported for the first time. Haka et al. [61] obtained and analyzed 31 Raman spectra from nine patients who underwent partial mastectomy. In that study, cancer tissues were accurately distinguished from normal and benign tissues using Raman spectroscopy. These researchers later reported a negative predictive value of 99% using 129 tissue samples in a new prospective study [62]. However, to confirm the presence or absence of cancerous tissue on the surgical cut surface using Raman spectroscopy in actual clinical practice, an accurate location must be specified, and the single-point method using a probe causes sampling errors. To solve this problem, Zhang et al. [63] conducted a comparative study using Raman spectral mapping. A total of 53 sets of mapping data and 2,597 Raman spectra were analyzed and compared, and the data obtained using the mapping technology displayed excellent diagnostic performance. Raman microspectroscopy studies have also been reported. Raman microspectroscopy makes diagnosis without staining based on the morphological and biochemical contrast between normal and tumor tissue. Kong et al. [64] reported that using Raman microspectroscopy to detect invasive ductal carcinoma within breast tissue with 95.6% sensitivity and 96.2% specificity. Zhang et al. [65] reported characterization of biochemical properties and structural alterations of breast cancer tissues at various TNM stages and grades by Raman microspectroscopy. Early Raman microspectroscopy studies had limitations in that the scanning method used to construct Raman spectral images for tumor diagnosis was very slow [66]. However, with the development of various technologies, such as the use of selective sampling based on integrated autofluorescence imaging, the possibility of its clinical application as an intraoperative method has been demonstrated [64,67,68].

3. Raman spectroscopy of SLNB

Raman spectroscopy is noninvasive and can provide detailed chemical information about tissue, thereby making it a very suitable test to check the status of the sentinel lymph nodes in real time during surgery. In 2003, Smith et al. [69]

first identified axillary lymph nodes in breast cancer using Raman spectroscopy. After that, Horsnell et al. [70] reported a sensitivity of 81% and specificity of 97% using the method of examining 10 points in the lymph node. Unfortunately, studies using Raman spectroscopy as a diagnostic tool for sentinel lymph node evaluation have not yet been conducted. Most studies were limited to small sample sizes and were laboratory-based. However, recently, studies using new technologies, such as a tissue mapping protocol obtained by analyzing the spectra of each cell [22] and studies using a nontoxic Raman nanoparticle tracer [21] have been reported, confirming the possibility that sentinel lymph node diagnosis through Raman spectroscopy can be used in clinical practice.

Conclusions

Research on Raman spectroscopy has been conducted in various fields, ranging from basic to clinical applications. The high sensitivity of Raman spectroscopy was previously regarded as a disadvantage that made it difficult to apply in clinical practice; however, these limitations are now being overcome by the incorporation of various technologies and the development of spectrum analysis. Studies analyzing Raman spectra to identify cancerous tissue at the surgical margin and lymph node during breast cancer surgery are ongoing, and the positive results of the studies show the possibility of supplementing the frozen section method. In the surgical treatment of breast cancer, if it becomes possible to distinguish malignant tissue from normal tissue *in vivo* using Raman spectroscopy, unnecessary surgical biopsies during surgery will be reduced.

Article information

Conflicts of interest

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

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Omega-3 fatty acids upregulate Nrf2 expression and attenuate apoptosis, inflammation, and fibrosis in a rat model of cyclosporine-induced nephropathy

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Background: Cyclosporine A (CsA)-induced kidney injury is characterized by renal impairment with inflammatory cell infiltrations, apoptosis, fibrosis, and hypoxic injury. It is not clear whether omega-3 fatty acids (O-3 FAs), which have anti-inflammatory and antioxidant roles, affect nuclear factor erythroid 2-related factor 2 (Nrf2) expression. The aim of this study was to investigate whether O-3 FAs affect Nrf2 expression and exert anti-inflammatory, anti-apoptotic, and anti-fibrotic effects in CsA-induced nephropathy.

Methods: Male Sprague-Dawley rats were divided into control, CsA-treated, and CsA-treated with O-3 FA groups. Nrf2 expression was measured by Western blots and immunohistochemical staining.

Results: Kidney function was impaired in the CsA-treated rats compared to the controls. Caspase-3 and caspase-7 were activated in the CsA-treated group, and the Bax/Bcl2 ratio was higher. O-3 FAs attenuated these apoptosis-related changes. ED-1 and inhibition of kappa B (IκB) protein expression were significantly upregulated in the CsA-treated group. Compared to the control group, O-3 FA supplementation attenuated the increased expression of ED-1 and IκB related to inflammation. Smad2/3, Smad4, and transforming growth factor-β1 were activated in the CsA group, and O-3 FA treatment prevented these changes related to renal fibrosis. The expression of Nrf2 was reduced in CsA-treated rats, but Nrf-2 was increased by O-3 FA treatment.

Conclusions: We suggest that Nrf2 is a potential mediator induced by O-3 FA supplementation and that it attenuates pro-inflammatory pathways, fibrotic processes, and apoptosis. Further studies are needed to elucidate the crosstalk between Nrf2 expression and signals related to O-3 FA treatment.

Keywords: Apoptosis; Fibrosis; Inflammation; Nuclear factor erythroid 2-related factor 2; Omega-3 fatty acid

Introduction

Cyclosporine A (CsA), a calcineurin inhibitor, is an important immunosuppressant used in organ transplantation and autoimmune diseases. It is clinically limited by its nephrotoxicity, which is characterized by a decreased glomerular filtration rate, inducing progressive irreversible renal structural damage, tubulointerstitial inflammation, and striped fibrosis [1]. Recent studies have revealed roles for CsA-induced oxidative stress and apoptosis in causing kidney

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damage [2,3]. Therefore, it is important to prevent acute CsA nephropathy and retard the progression of chronic CsA nephropathy.

Nuclear factor erythroid 2-related factor 2 (Nrf2) serves as a key transcriptional regulator of the basal and inducible expression of many genes encoding detoxification enzymes, antioxidant proteins, and other stress-response mediators [4]. Previous studies have reported the role of Nrf2 in modulating the expression of cytoprotective genes in the kidney through *in vivo* and *in vitro* studies [5,6]. A loss of Nrf2 activity may exacerbate oxidative and inflammatory damage, which is linked to oxidative stress and inflammatory diseases, such as atherosclerosis, diabetes, and rheumatoid arthritis [7-10].

Omega-3 fatty acids (O-3 FAs) comprise a family of polyunsaturated FAs that consist of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA and/or EPA have been reported to reduce plasma triglyceride (TG) levels [11] and improve vascular compliance and vasodilatation to stabilize atherosclerotic plaques [12,13] and cardiovascular events [14,15]. A randomized, double-blind study of dietary fish oil supplementation after renal transplantation reported a beneficial effect on renal hemodynamics and blood pressure and significantly fewer rejection episodes in the fish oil group than in the control group [16]. Furthermore, there was a trend toward increased graft survival [16]. Sakai et al. [17] reported that EPA and DHA have upregulated the Nrf2-mediated antioxidant response, attenuating DNA damage induced by oxidative stress in vascular endothelial cells. Only a few studies have reported that O-3 FA affected Nrf-2 expression [17-20]. In this study, we investigate whether O-3 FA has anti-inflammatory, anti-apoptotic, and anti-fibrotic activities in a rat model of CsA-induced nephropathy. In addition, we evaluated the impact of O-3 FA on the Nrf2 expression in this model.

Methods

Ethical statements: The animal procedures were authorized by the Institutional Animal Care Committee of Dong-A University (DIACUC-14-29).

1. Study design

This study included 18 adult male Sprague-Dawley rats weighing 250 to 280 g. The rats were kept in cages that controlled temperature and light. They were fed a low-salt diet (0.05% sodium; Teklad Premier) and tap water.

Rats were divided randomly into three groups. The control group (n=6) was administered saline (1 mL/kg/day) via subcutaneous injection and saline (1 mL/kg/day) by gastric gavage for 4 weeks, the CsA-treated group (n=6) was administered CsA (15 mg/kg/day) via subcutaneous injection and saline (1 mL/kg/day) by gastric gavage for 4 weeks, and the CsA+O-3 FA group (n=6) was administered CsA via subcutaneous injection and O-3 FA (300 mg/kg/day) via gastric gavage for 4 weeks. CsA (Chong Kun Dang Pharm.) was dissolved to a concentration of 15 mg/mL in normal saline, and the dose and administration route were determined based on a previous study of chronic CsA nephropathy [21]. O-3 FA was administered via a gastight micro-syringe (Gastight; Hamilton) and its dose was determined in a previous study [22]. Rats fed in pairs and monitored their daily body weight.

Blood samples were taken from the heart on the day of euthanasia. Whole blood CsA levels were gauged by a monoclonal radioimmunoassay (Incstar Corp.). Blood urea nitrogen and serum creatinine levels were quantified via an automatic analyzer (Roche), and total cholesterol and TG levels were estimated using a colorimetric test kit (Asan Pharmaceutical Co.).

2. Histopathologic evaluation

Rat kidney tissues were cleansed with heparinized saline and fixed in a periodate-lysine-paraformaldehyde solution, and wax-embedded kidney tissues were cut into 4 μm sections. Kidney sections were stained with periodic acid-Schiff. The kidney lesions, including vacuolization of the tubular cells, tubular atrophy and dilatation, and inflammatory cellular infiltration and interstitial fibrosis, were viewed by an Aperio ScanScope (Aperio Technologies).

3. Western blot analysis

Proteins were extracted from kidney tissues using lysis buffer (pH 7.6, including 300 mM NaCl, 50 mM Tris-Cl, 0.5% Triton X-100, and protease inhibitor cocktail). The protein concentrations of the lysates were determined using a Bradford protein assay kit (Bio-Rad). Protein lysates were separated using 7.5% to 15% SDS/PAGE gels and then transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech). After blocking with 1% skim milk,

membranes were incubated with primary antibodies overnight at 4 ˚C. Antibodies against transforming growth factor-β1 (TGF-β1), Smad2/3, Smad4, Bcl-2, Bax, and inhibitor of kappa B (IκB) were acquired from Santa Cruz Biotechnology. Rabbit polyclonal anti-caspase 3 and caspase 7 antibodies were supplied from Cell Signaling Technology. Antibodies against Nrf2 were supplied from Abcam. Antibodies against ED-1 and β-actin were supplied from Serotec and Sigma-Aldrich. Membranes were sequentially incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Immunostaining with antibodies was carried out using the Super Signal West Pico (Thermo Fisher Scientific) enhanced chemiluminescence substance and founded with LAS-3000 Plus (Fuji Photo Film). The response was quantified and regularized to the β-actin control band using ImageJ version 1.48q.

4. Immunohistochemical staining

To perform immunohistochemical staining for Nrf2, the kidney sections were transferred into a Tris-EDTA buffer solution (pH 9.0; DAKO). Slides were microwaved at medium power for 7 minutes to retrieve the antigens. To block the endogenous peroxidase activity, 0.3% H_2O_2 in distilled water was applied to the tissue sections for 20 minutes and incubated for 30 minutes at 37 °C with 5% normal goat serum. The slides were incubated overnight at 4 °C with anti-Nrf2 antibody and additionally incubated with secondary antibody for 1 hour at 37 °C. The slides were then incubated in 3,3-diaminobenzidine with H_2O_2 substrate and hematoxylin. The negative control group was stained with a buffer solution instead of the primary antibody under the same circumstances. Results were verified by means of an Aperio ScanScope slide scanner.

5. Statistical analysis

Data were expressed as means±standard deviation and analyzed using SPSS 18.0 software (SPSS Inc.). Mann-Whitney *U* test and Kruskal-Wallis test were performed to compare the two groups and the three groups, respectively. A *p*-value of <0.05 was considered significant.

Results

1. Biochemical data

Results obtained at week 4 are summarized in Table 1. The body weights of each group were at the start of the study. The final body weights of the rats in the CsA-treated and CsA+O-3 FA-treated groups were significantly decreased compared to the control group. At week 4, the serum creatinine and blood urea nitrogen were increased in the CsA-treated group compared to the control group. The CsA+O-3 FA-treated group showed serum creatinine levels similar to those of the CsA-treated group. The total cholesterol and TG levels were also meaningfully raised in the CsA-treated group compared to the control group, but there was no discernible difference between the two groups. The serum cyclosporine levels in the CsA and CsA+O-3 FA groups were over 2,000 ng/mL.

2. Renal pathology

Compared to the control group, the CsA-treated group revealed vacuolization of tubular cells, tubular atrophy, and interstitial fibrosis in the periodic acid-Schiff-stained specimens (Fig. 1). The rats given O-3 FA supplementation had fewer tubulointerstitial alterations than the group treated

Table 1. Biochemical characteristics in each group

Characteristic	Control (n=6)	Cyclosporine (n=6)	Cyclosporine with omega-3 fatty acids (n=6)	*p*-value
Final body weight (g)	433.67±8.62	335.81±1.82*	344.06±9.80*	<0.001
Blood urea nitrogen (mg/dL)	16.15±2.78	56.08±15.60*	50.53±7.50*	<0.001
Creatinine (mg/dL)	0.40±0.03	0.55±0.12*	0.54±0.29*	-
Cyclosporine (ng/mL)	-	2,732±198	2,587±320	0.469
Total cholesterol (mg/dL)	67.44±16.33	108.50±3.83*	92.50±9.88*	<0.001
Triglyceride (mg/dL)	111.9±40.8	349.8±122.4*	305.3±43.6*	<0.001

Values are presented as means±standard deviation.
*p<0.05 compared to the control group.

Fig. 1. Morphological changes in the kidneys in the three groups. Periodic acid-Schiff-stained sections were examined using a light microscope (original magnification, ×200). Treatment of rats with cyclosporine for 4 weeks induced interstitial fibrosis, inflammatory cell infiltration, and tubular atrophy (B) compared to the controls (A). Omega-3 fatty acid supplementation decreased the pathologic changes (C).

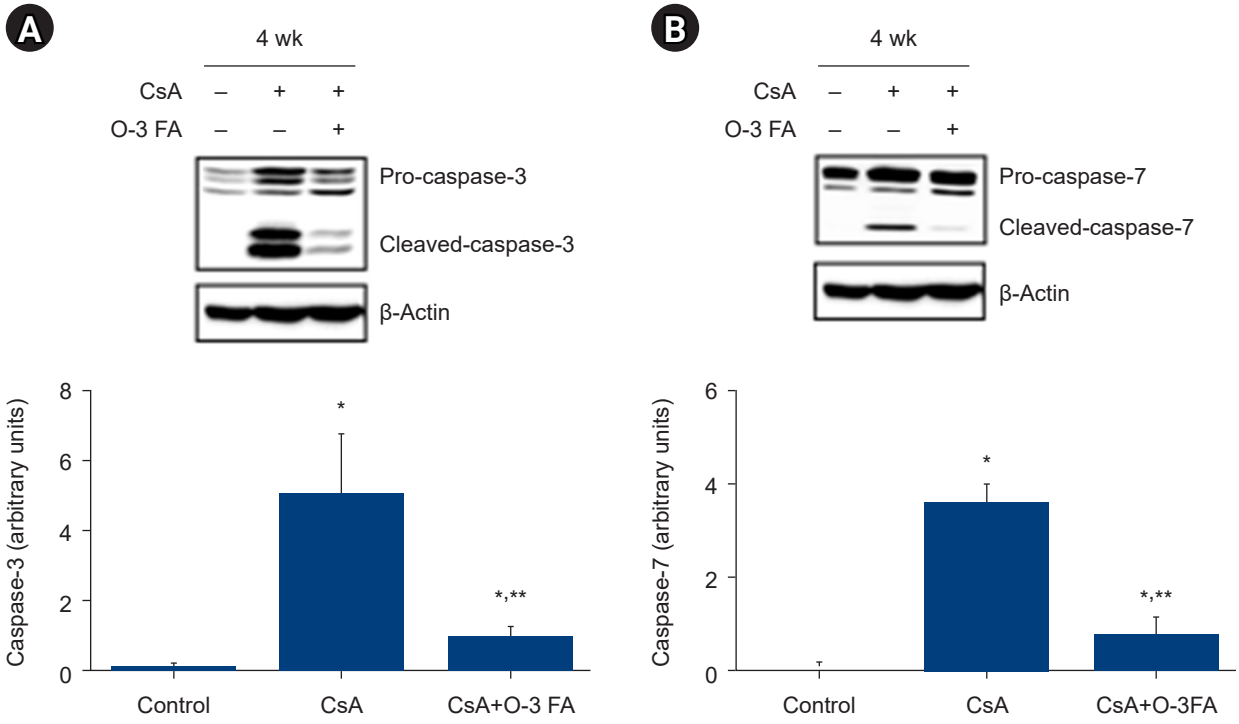

Fig. 2. Expression of caspase-3 (A) and caspase-7 (B) in the kidneys of cyclosporine A (CsA)-induced rats detected by Western blots. Compared to the control group, increased expression of caspase-3 and caspase-7 was shown in the kidneys of the CsA group, and omega-3 fatty acid (O-3 FA) supplementation reversed these effects. *p<0.05, compared to control, **p<0.05, compared to the CsA group.

with CsA alone.

3. Apoptosis data

Caspase-3 and caspase-7 expression were activated in the CsA nephropathy, and O-3 FA supplementation attenuated the activation of these effector caspases in CsA nephropathy (Fig. 2). The ratio of Bax and Bcl-2 was higher in the CsA group and was attenuated by O-3 FA in the CsA+O-3 FA-treated group (Fig. 3).

4. Inflammation and renal fibrosis

ED-1 and IκB protein expression were activated in the CsA-treated group compared to the control group. O-3 FA supplementation attenuated the increased ED-1 and IκB

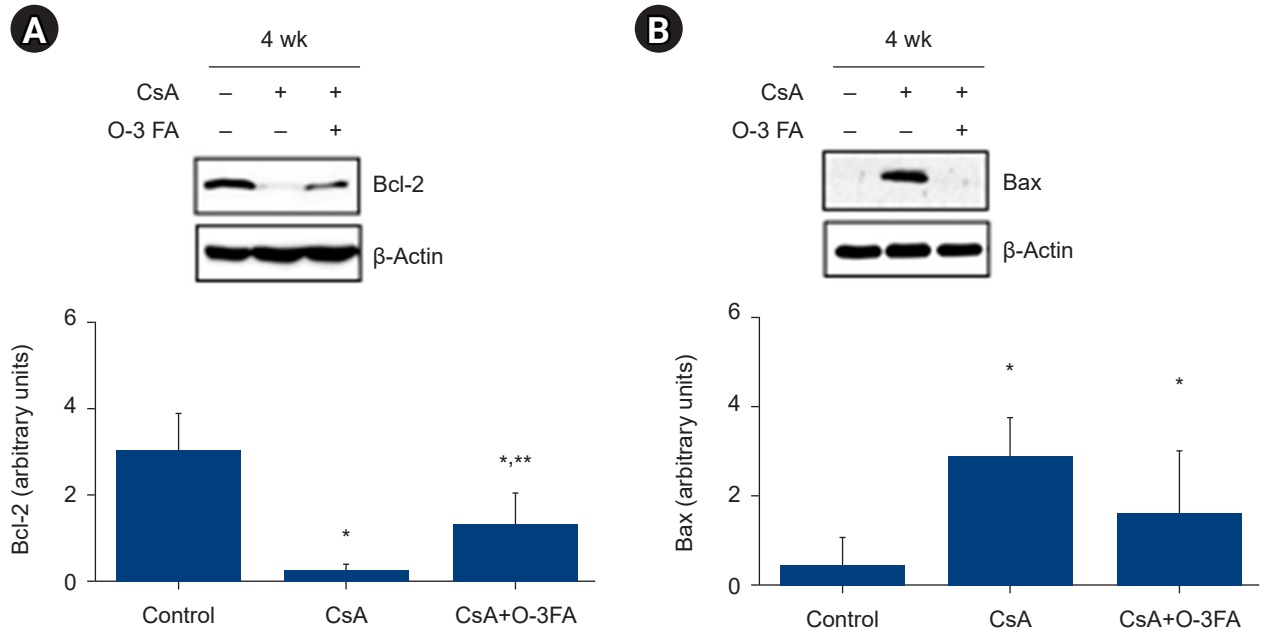

Fig. 3. Bax and Bcl-2 expression in the kidneys of cyclosporine A (CsA)-induced rats. Compared to the control group, CsA treatment increased the expression of pro-apoptotic Bax (A) and attenuated the expression of anti-apoptotic Bcl-2 (B). Omega-3 fatty acid (O-3 FA) supplementation attenuated the upregulation of Bax and increased that of the anti-apoptotic protein Bcl-2. *p<0.05 compared to control, **p<0.05 compared to the CsA group.

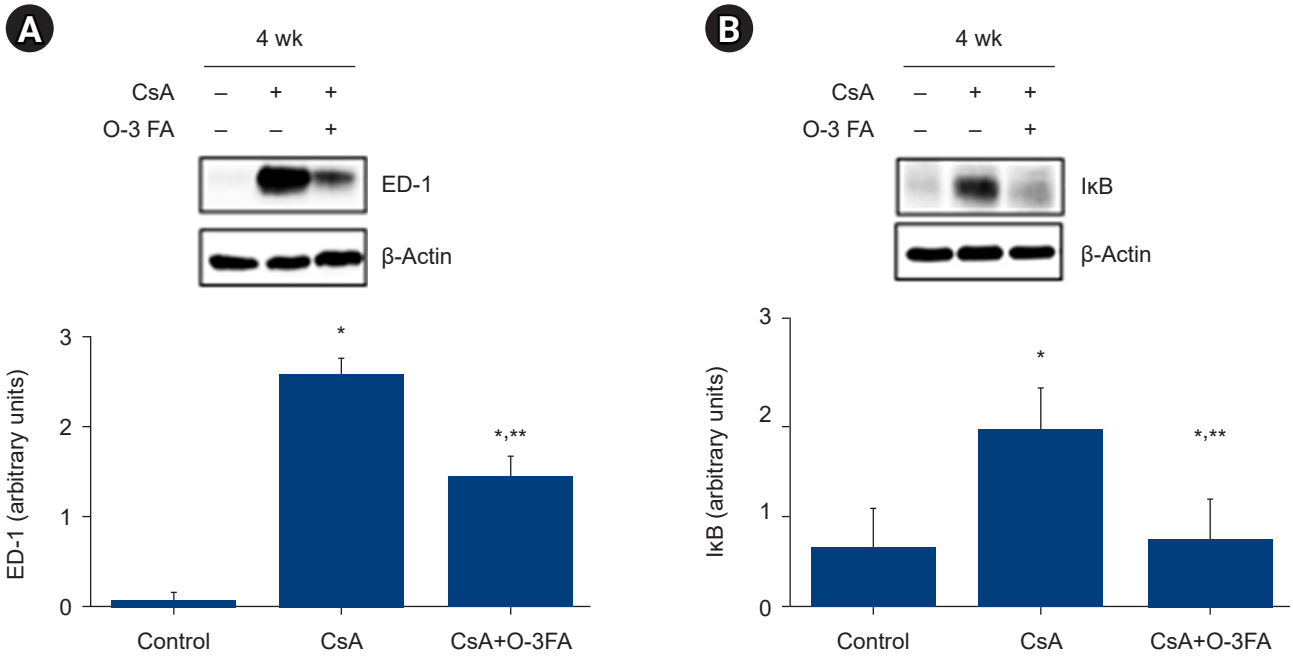

Fig. 4. ED-1 and IκB expression in the kidneys of cyclosporine A (CsA)-induced rats. Compared to the control group, significantly increased expression of ED-1 (A) and IκB (B) was observed in CsA-treated rats, which was ameliorated by omega-3 fatty acid (O-3 FA) treatment. *p<0.05 compared to control, **p<0.05 compared to the CsA group.

expression (Fig. 4). The total Smad2/3 and Smad4 levels were activated in the CsA-treated group and O-3 FA supplementation attenuated the upregulation of these Smads. An important pro-fibrotic molecule, TGF-β1, was activated in the CsA-treated rats and TGF-β1 was attenuated by O-3 FA supplementation (Fig. 5).

5. Nrf2 and immunohistochemical staining of Nrf2

The CsA-treated rats showed significant downregulation of Nrf2. O-3 FA supplementation increased Nrf2 expression compared to CsA treatment (Fig. 6A). Nrf2 expression in the kidney was also confirmed by immunohistochemical staining. Nrf2 expression was mostly manifested in the kidney tubules of the normal rats (Fig. 6B). This was reduced in the CsA-treated rats (Fig. 6C) and restored by O-3 FA supplementation (Fig. 6D).

Discussion

In this study, we found that O-3 FA upregulates Nrf-2 expression and potentiates anti-inflammatory, anti-apoptotic, and anti-fibrotic processes in CsA-induced nephropathy model. Nrf2 acts as a master regulator of anti-inflammatory and antioxidant gene expression [4]. Previous studies have shown that Nrf2-deficiency enhanced the susceptibility to both ischemic and nephrotoxic renal injury. Compared to wild-type mice, the kidney damage and interstitial fibrosis resulting from CsA treatment were relatively higher in Nrf2-knockout mice [18]. Thus, a potential therapeutic target against kidney injury may be increasing Nrf2 expression and O-3 FA may be a new therapeutic agent for the prevention of CsA-induced kidney injury.

Bardoxolone methyl is an Nrf2 activator, the first orally available antioxidant. In a 52-week bardoxolone methyl treatment of patients with type 2 diabetes mellitus and chronic kidney disease, bardoxolone methyl reduced serum creatinine concentrations [23]. However, bardoxolone methyl did not decrease the risk of end-stage renal disease or death from cardiovascular disease. In addition, heart failure, nonlethal myocardial infarction, nonlethal stroke, hospital admission for heart failure, or death from cardiovascular causes was higher in the bardoxolone methyl group than in the placebo group [24]. In contrast, O-3 FA is beneficial for cardiovascular health and is a natural Nrf2 activator. Further human and animal studies are needed to investigate the effects of O-3 FA on Nrf2. In this study, we also demonstrated Nrf2 expression in the tubular cells of the kidney. Therefore, we suspect that Nrf2, rather than podocytes, may be the predominant protection mechanism against tubular cell injury [25].

Bcl-2 family proteins are the regulators of apoptosis, and the overexpression of Bcl-2 improves cell survival by

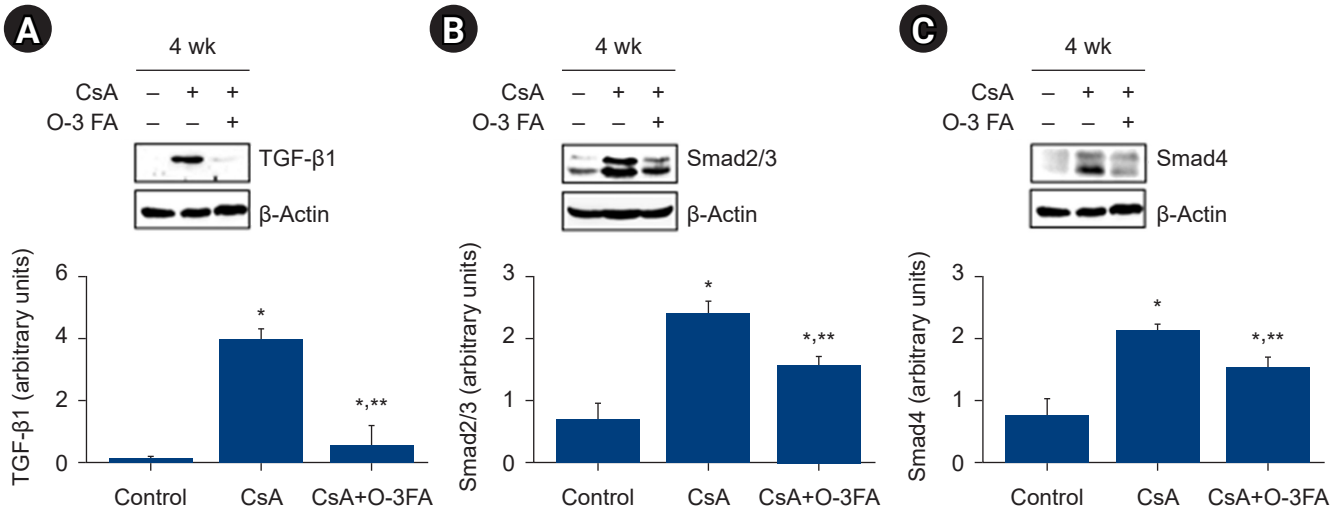

Fig. 5. Smads2/3, Smad4, and transforming growth factor-β1 (TGF-β) expression in the kidneys of cyclosporine A (CsA)-induced rats. Compared to the control group, CsA treatment significantly increased the expression of TGF-β1, Smad2/3, and Smad4. Omega-3 fatty acid (O-3 FA) supplementation significantly prevented the upregulation of TGF-β1, Smad2/3, and Smad4. *p<0.05 compared to control, **p<0.05 compared to the CsA group.

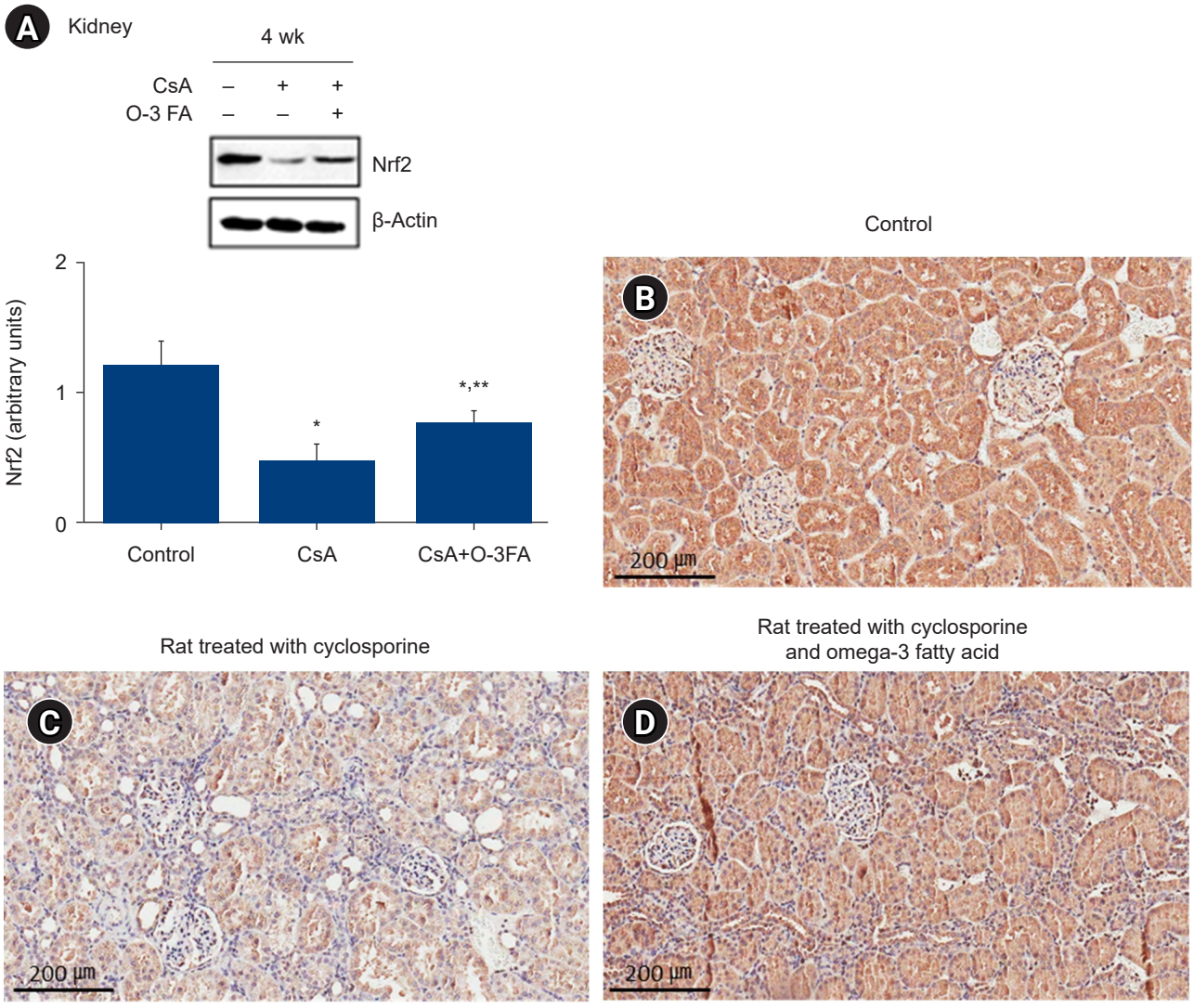

Fig. 6. Nuclear factor erythroid 2-related factor 2 (Nrf2) expression in the kidney of cyclosporine A (CsA) induced rats detected by Western blots. (A) Compared to the control group, Nrf2 expression was downregulated in the kidneys of the CsA group and upregulated by omega-3 fatty acid (O-3 FA) supplementation. (B) Immunohistochemically stained specimens from the control group. Nrf2 was mainly expressed in the tubules of the normal control rats. (C) Nrf2 expression was increased in the kidneys of CsA-induced rats. (D) Nrf2 expression was decreased by O-3 FA supplementation in the kidney of CsA-induced rats. *p<0.05 compared to control, **p<0.05, compared to the CsA group.

inhibiting apoptosis [26]. Bax belongs to a family of proteins that share homology with Bcl-2 and the formation of heterodimers between Bax and Bcl-2 homologs with death repressor function leads to suppression of the death-promoting effects of Bax. The relative ratio of Bax and Bcl-2 has been suggested to determine cell survival after apoptotic stimuli [27]. In this study, the relative ratio of Bax and Bcl-2 was changed by O-3 FA, suggesting that O-3 FA prevented apoptosis in the CsA-treated rat model. We also demonstrated that apoptosis-related effector caspase-3 and caspase-7 were inhibited by O-3 FA supplementation in CsA-induced nephropathy.

Nuclear factor-κB (NF-κB) signaling plays an important role in interstitial inflammation in the kidney [28]. In the unstimulated state, NF-κB is present in the cytoplasm attached to the suppressor protein inhibitor of NF-κB, but certain stimulators generate free NF-κB through the degradation and phosphorylation of IκB. Dissociated NF-κB pro-

teins translocate to the nucleus and promote macrophage infiltration and interstitial inflammation [28]. We also observed increased inflammatory processes by increased IκB and pan-macrophage marker ED-1 expression in CsA nephropathy and O-3 FA supplementation attenuated the inflammatory process. In a rat model of progressive proteinuric renal disease, interstitial macrophages produced TGF-β1 and the levels correlated with interstitial inflammatory infiltration [29]. TGF-β1 is a well-known key mediator, activating its downstream Smad signaling pathway in renal fibrosis [30]. In this study, we found that TGF-β1 and a Smad-dependent pathway were inhibited by O-3 FA supplementation.

In conclusion, O-3 FA attenuates tubulointerstitial injury in a CsA-induced nephropathy rat model by mitigating apoptosis, inflammation, and fibrosis and increasing Nrf2 expression. These observations showed the potential role of O-3FA supplementation as an adjunct in the management of delaying CsA-induced nephropathy.

Article information

Conflicts of interest

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

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Author contributions

Conceptualization: WSA. Data curation: JYL, YKS, MHL, SML, WSA. Methodology: JYL, YKS, MHL, SML, SEK, WSA. Formal analysis: MHL, SML, WSA. Supervision: JYL, YKS, MHL, SML, SEK, WSA. Writing - original draft: JYL, YKS, WSA. Writing - review & editing: JYL, YKS, WSA. Approval of final manuscript: all authors.

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The COVID-19 pandemic's impact on prostate cancer screening and diagnosis in Korea

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Background: The global coronavirus disease 2019 (COVID-19) pandemic, which started in early 2020, has had multiple impacts on cancer care. This study assessed how the COVID-19 pandemic influenced prostate cancer (PCa) screening and diagnosis in South Korea.

Methods: Patients who visited the outpatient clinic at a single institution for PCa evaluation were included in this study and divided into a pre-COVID-19 group and a COVID-19 pandemic group, based on the start of the COVID-19 pandemic and social distancing policies on March 1, 2020. The number of prostate-specific antigen (PSA) tests, patients with elevated PSA levels, and prostate biopsy results were analyzed.

Results: In total, 8,926 PSA tests were administered during the COVID-19 pandemic, compared to 15,654 before the pandemic (p<0.05). Of 2,132 patients with high PSA levels, 1,055 (49.5%) received prostate biopsies before the pandemic and 1,077 (50.5%) did so during the COVID-19 pandemic. The COVID-19 pandemic group had a higher detection rate of PCa, and increased rates of Gleason scores (GS) 7 and 9–10, while the rate of GS 6 decreased compared to the pre-COVID-19 group (p<0.05). The rate of clinically significant PCa (csPCa) was also higher during the pandemic (p<0.05). In both magnetic resonance imaging-guided and standard biopsies, the GS 6 rate decreased, and the csPCa rate increased during the COVID-19 pandemic (each, p<0.05).

Conclusions: During the COVID-19 pandemic, the detection rate of prostate biopsies and the rate of csPCa increased significantly. Thus, PCa was diagnosed at a more advanced state in Korea during the COVID-19 pandemic.

Keywords: COVID-19; Prostate-specific antigen; Prostatic neoplasms

Introduction

The number of diseases worldwide has increased dramatically after the coronavirus disease 2019 (COVID-19) appeared in the last month of 2019 in the Chinese province of Wuhan. Crowd control protocols have been implemented worldwide as a response to the outbreak [1]. In Korea, COVID-19 has spread nationwide since the first case occurred on January 20, 2020. On February 29, 2020, the Korean government announced its social distancing policy. Since then, it has continued for 2 years, with several phase changes [2]. The worldwide pandemic influenced cancer screening, diagnosis, and transfer [3,4]. That negatively affected both regular prostate-specific antigen (PSA) screening and the diagnosis of prostate cancer (PCa), as evidenced by research conducted in the United States. The absolute PCa screening shortfall in the U.S. population is 1.6 million [5-7]. Studies evaluating the pandemic of

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COVID-19's effects on PCa detection, diagnosis, and therapy have been published in several nations [8-11]. Several studies conducted in Korea analyzed the influences of the pandemic situation on colorectal and breast cancer. Still, PCa research has not yet been published [1,12]. This study examined if the COVID-19 epidemic in Korea delayed PCa screening and detection, deteriorating clinical status at diagnosis.

Methods

Ethical statements: This study was approved by the Institutional Review Board of Pusan National University Hospital (IRB No. 2303-019-125) and was conducted in accordance with the recent Declaration of Helsinki. Informed consent was waived by the board.

1. Patients

We analyzed the number of PSA tests, cases diagnosed with elevated PSA, and patients with PCa at a single Korean institution during the study period. Additionally, we examined the pathology reports of prostate biopsies at our institution. The patients were divided into a pre-COVID-19 period (from March 2018 to February 2020) and a COVID-19 pandemic period (from March 2020 to February 2022). This division was chosen because the Korean government started its social distancing campaign on March 1, 2020 [2]. This study excluded patients who got a diagnosis of PCa.

2. Prostate biopsy procedure

The inclusion criteria regarding prostate biopsy consisted of a PSA over 4.0 ng/mL, a palpable hard nodule from digital rectal examination, or any lesion exhibiting a Prostate Imaging Reporting and Data System (PI-RADS) score of 3 or higher on magnetic resonance imaging (MRI) [13,14]. All prostate biopsies were performed transrectally using ultrasound-guidance (standard biopsy, 12 cores). Patients with pre-biopsy MRI underwent targeted biopsy using MRI-ultrasound fusion (two cores for each target lesion) plus standard biopsy (MRI-guided biopsy) [15]. We divided all patients into MRI-guided and standard biopsy groups and analyzed the differences in prostate biopsy results preceding and following the COVID-19 pandemic in each group. Patients were not strictly categorized into the MRI-guided and sstandard biopsy groups with specific indications. However, Patients with high cancer risk, such as atypical small acinar proliferation or extensive prostatic intraepithelial neoplasia on previous biopsy, were strongly recommended to undergo MRI. Additionally, MRIs were recommended for men with persistently elevated PSA following a negative biopsy. All patients with PI-RADS ≥3 on MRI underwent MRI-guided biopsy [16].

3. Assessment variables

The following clinical parameters consisted of the analysis: PSA level, age, diagnosis of elevated PSA, the detection rate of PCa, Gleason score (GS), and clinically significant PCa (csPCa; defined as GS of 7 or greater).

4. Statistical analysis

A *p*-value of lower than 0.05 was determined to be statistically significant. The statistical analyses were conducted utilizing IBM SPSS Statistics for Windows, version 22.0 (IBM Corp.).

Results

1. PCa screening number

Throughout the research duration, 24,580 PSA tests were performed, with 8,926 tests completed during the Korean pandemic of COVID-19. This was less than the 15,654 cases conducted prior to the emergence of the COVID-19 virus (63.7% vs. 36.3%). In addition, the number of outpatient visits for elevated PSA (n=1,756 [54.7%] vs. n=1,457 [45.3%], $p<0.05$) and PCa (n=3,084 [63.2%] vs. n=1,795 [36.8%], $p<0.05$) was significantly lower in the pandemic period. The patients' number who had return visits decreased significantly during the pandemic ($p<0.05$) (Table 1).

2. Prostate biopsy results in the pre-COVID-19 and COVID-19 pandemic periods

A total of 2,132 patients diagnosed with elevated PSA levels underwent prostate biopsy. One thousand fifty-five patients (49.5%) and 1,077 patients (50.5%) underwent prostate biopsy during pre-COVID-19 and the COVID-19 pandemic, respectively. The average age (68.1 years vs. 69.0 years) and median PSA (6.88 vs. 7.05) were higher during the pandemic, but the differences were insignificant. The COVID-19 pandemic group had a higher PCa detection rate (53.7% vs. 48.7%, $p<0.05$) (Table 2). The rates of GS 7 (4+3

Table 1. Number of prostate cancer-related cases before and during the COVID-19 pandemic

Variable	Pre-COVID-19	COVID-19 pandemic	Total	*p*-value
No. of PSA tests	15,654 (63.7)	8,926 (36.3)	24,580 (100)	
No. of patients with elevated PSA	1,756 (54.7)	1,457 (45.3)	3,213 (100)	<0.05
First visits	1,216 (69.2)	1,311 (90.0)	2,527 (78.6)	
Return visits	540 (30.8)	146 (10.0)	686 (21.4)	<0.05
No. of patients with prostate cancer	3,084 (63.2)	1,795 (36.8)	4,879 (100)	<0.05
First visits	1,080 (35.0)	1,066 (59.4)	2,146 (44.0)	
Return visits	2,004 (65.0)	729 (40.6)	2,733 (56.0)	<0.05

Values are presented as number (%).
COVID-19, coronavirus disease 2019; PSA, prostate-specific antigen.
p-values were calculated using the chi-square test for categorical variables.

Table 2. Prostate biopsy outcomes before and during the COVID-19 pandemic

Variable	No. (%)			*p*-value
	Pre-COVID-19	COVID-19 pandemic	Total	
No. of patients	1,055 (49.5)	1,077 (50.5)	2,132 (100)	
Average age (yr)	68.1	69.0	68.5	0.89
PSA (ng/mL)				
Median	6.88	7.05	6.96	0.62
Interquartile range	4.86–12.20	4.76–12.45	4.81–12.30	
PCa detection				<0.05
PCa	514 (48.7)	578 (53.7)	1,092	
No tumor	541 (51.3)	499 (46.3)	1,040	
Gleason score (GS)				<0.05
3+3	130 (25.3)	65 (11.2)	195 (17.9)	<0.05
3+4	92 (17.9)	148 (25.6)	240 (22.0)	
4+3	96 (18.7)	150 (25.6)	246 (22.5)	
8	127 (24.7)	111 (19.2)	238 (21.8)	
9-10	69 (13.4)	104 (18.0)	173 (15.8)	<0.05
csPCa (GS≥7)				<0.05
Yes	384 (74.7)	513 (88.8)	897 (82.1)	
No	130 (25.3)	65 (11.2)	195 (17.9)	

COVID-2019, coronavirus disease 2019; PSA, prostate-specific antigen; PCa, prostate cancer; csPCa, clinically significant PCa.

and 3+4: 51.2% vs. 36.6%, $p<0.05$) and 9–10 increased (18.0% vs. 13.4%, $p<0.05$) in the period of the pandemic, while the rate of GS 6 decreased by contrast to the period of pre-COVID-19 (11.2% vs. 25.3%, $p<0.05$). The csPCa was more frequent in the period of the pandemic than in the period of pre-COVID-19 (88.8% vs. 74.7%, $p<0.05$) (Table 2, Fig. 1).

3. Results of MRI-guided biopsy and standard biopsy

Of the 2,132 patients who underwent biopsy, 921 (43.2%) and 1,211 (56.8%) underwent MRI-guided biopsy and standard biopsy, respectively. More MRI-guided biopsies were performed during the pandemic of COVID-19 than during the period of pre-COVID-19 (n=369 [40.1%] vs. n=552 [59.9%], $p<0.05$). We divided the patients into an MRI-guided biopsy group and a standard biopsy group and analyzed the biopsy results (Table 3). Of the 921 men who underwent MRI-guided biopsy, 552 (59.9%) underwent prostate biopsy during the COVID-19 pandemic, and the age and PSA levels were significantly higher in the group that underwent biopsy during this time ($p<0.05$). The PCa detection rate was also higher in the group that underwent MRI-guided biopsy during the pandemic ($p<0.05$). Comparing the GS results of

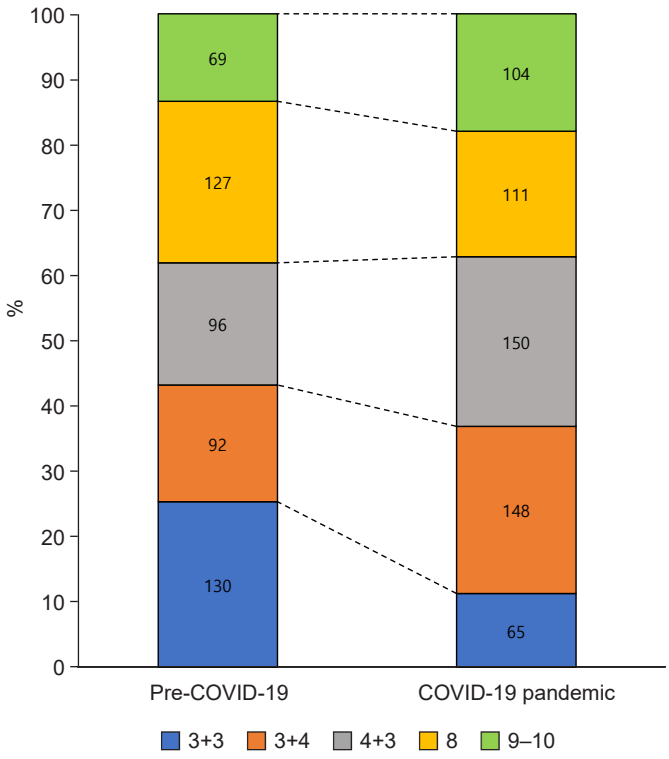

Fig. 1. Differences in the distribution of Gleason scores (GS) in prostate biopsies before and during the coronavirus disease 2019 (COVID-19) pandemic. Each GS is represented by a color, and the number inside the bar represents the number. The y-axis represents the proportion of each GS.

the MRI-guided biopsy, the proportion of GS 6 decreased by 18.7% from 28.7% to 10.0% during the pandemic. The ratio of csPCa with a GS 7 or higher increased from 71.3% to 90.0% (p<0.05) (Table 3, Fig. 2).

We analyzed 1,211 patients with standard biopsies throughout the pre-COVID-19 and COVID-19 pandemic, and the two periods had no significant differences in age or PSA levels. The PCa detection rate did not exhibit a statistically significant difference (n=340 [49.6%] vs. n=268 [51.0%], p=0.61). However, GS6 rates declined significantly from 23.5% to 12.7%, while csPCa rates increased by 10.8% from 76.5% to 87.3% (p<0.05) (Table 3, Fig. 2).

Discussion

Our research indicates a significant reduction in outpatient visits for elevated PSA and PCa following the emergence of the Korean pandemic of COVID-19. As the pandemic progressed, social distancing policies reduced access to hospital care [2]. Patients may also have postponed cancer screening tests or biopsies during outpatient appointments or hospital visits due to anxiety about the risk of COVID-19 infection.

The 2020 Korean Cancer Registry Statistics showed results similar to our study's. The incidence ranking of PCa went up to third place from fourth; however, the incidence rate per 100,000 decreased from 34.5 to 32.7 [17]. This phenomenon is similar to those observed in other countries. The U.S. has an estimated deficit of 1.6 million PCa cases in the entire population. The pattern of decline and recovery in PCa screening varies according to geographic region and socioeconomic status. The Northeast region saw the fastest decline in screenings from March to May 2020, while the West recovered more slowly than the Midwest and southern regions [7].

According to Dutch research, the COVID-19 epidemic considerably reduced the incidence of PCa diagnosis and prostate biopsies. In contrast, the detection rate of biopsies showed a substantial increase in 2020. The researchers explained that patients were reluctant to visit their general practitioners for PSA testing during the lockdown, reduction of PSA testing among asymptomatic individuals. Urologists also conducted fewer biopsies and encouraged low-risk patients to postpone biopsies [9]. These results are consistent with our findings.

In contrast, researchers from Australia reported that PSA screening, prostate MRI, and prostate biopsy showed no significant differences in 2020 owing to the pandemic of COVID-19, except in Victoria. Compared to other nations, the pandemic had little influence, and the effectiveness of Australia's medical care system is credited to researchers. For example, while the COVID-19 outbreak, Australia had less restrictive social policies than other countries, resulting in a lower pandemic influence [10]. These findings indicate that, with some regional variations, PCa screenings and diagnoses reduced globally throughout the COVID-19 pandemic. Differences in basic health systems, government policies on infectious diseases, and sociocultural factors can explain disparities in how various nations have been affected by the COVID-19 pandemic.

The biopsy counts conducted before and after the pandemic did not significantly differ according to this study.

Table 3. MRI-guided and standard prostate biopsy outcomes before and during the COVID-19 pandemic

Variable	No. (%)			*p*-value
	Pre-COVID-19	COVID-19 pandemic	Total	
Biopsy protocol				<0.05
MRI-guided biopsy (target+standard)	369 (40.1)	552 (59.9)	921 (43.2)	
Standard biopsies	686 (56.6)	525 (43.4)	1,211 (56.8)	
Total biopsies	1,055 (49.5)	1,077 (50.5)	2,132 (100)	
MRI-guided biopsies				
No. of patients	369 (40.1)	552 (59.9)	921 (100)	
Average age (yr)	66.2	68.5	67.6	<0.05
Median PSA (ng/mL)	6.82	6.91	6.89	<0.05
PCa detection				<0.05
PCa	174 (47.2)	310 (56.2)	484 (52.6)	
No tumor	195 (52.8)	242 (43.8)	437 (47.4)	
Gleason score (GS)				<0.05
3+3	50 (28.7)	31 (10.0)	81 (16.7)	
3+4	29 (16.7)	81 (26.1)	110 (22.7)	
4+3	38 (21.8)	87 (28.1)	125 (25.8)	
8	42 (24.1)	57 (18.4)	99 (20.5)	
9–10	15 (8.7)	54 (17.4)	69 (14.3)	
csPCa (GS≥7)				<0.05
Yes	124 (71.3)	279 (90.0)	403 (83.3)	
No	50 (28.7)	31 (10.0)	81 (16.7)	
Standard biopsies				
No. of patients	686 (56.6)	525 (43.4)	1,211 (100)	
Average age (yr)	69.1	69.5	69.3	0.34
Median PSA (ng/mL)	6.83	7.03	7.02	0.38
PCa detection				0.61
PCa	340 (49.6)	268 (51.0)	608 (50.2)	
No tumor	346 (50.4)	257 (49.0)	603 (49.8)	
GS				<0.05
3+3	80 (23.5)	34 (12.7)	114 (18.8)	
3+4	63 (18.5)	67 (25.0)	130 (21.4)	
4+3	58 (17.1)	63 (23.5)	121 (19.9)	
8	85 (25.0)	54 (20.1)	139 (22.9)	
9–10	54 (15.9)	50 (18.7)	104 (17.0)	
csPCa (GS≥7)				<0.05
Yes	260 (76.5)	234 (87.3)	494 (81.2)	
No	80 (23.5)	34 (12.7)	114 (18.8)	

MRI, magnetic resonance imaging; COVID-2019, coronavirus disease 2019; PSA, prostate-specific antigen; PCa, prostate cancer; csPCa, clinically significant PCa.

However, during the pandemic, the detection rate of PCa from prostate biopsy increased significantly, and PCa was diagnosed with a higher GS. According to the European Association of Urology risk stratification classification, the rate of csPCa increased accordingly, and the proportion of the low-risk group decreased significantly [18]. Therefore, we confirmed that more advanced cancers were diagnosed during this period. This finding suggests that biopsies were performed later during the pandemic, and PCa patients were diagnosed at more advanced stage than pre-COVID-19.

It is interpreted that this trend is partly reflected in the

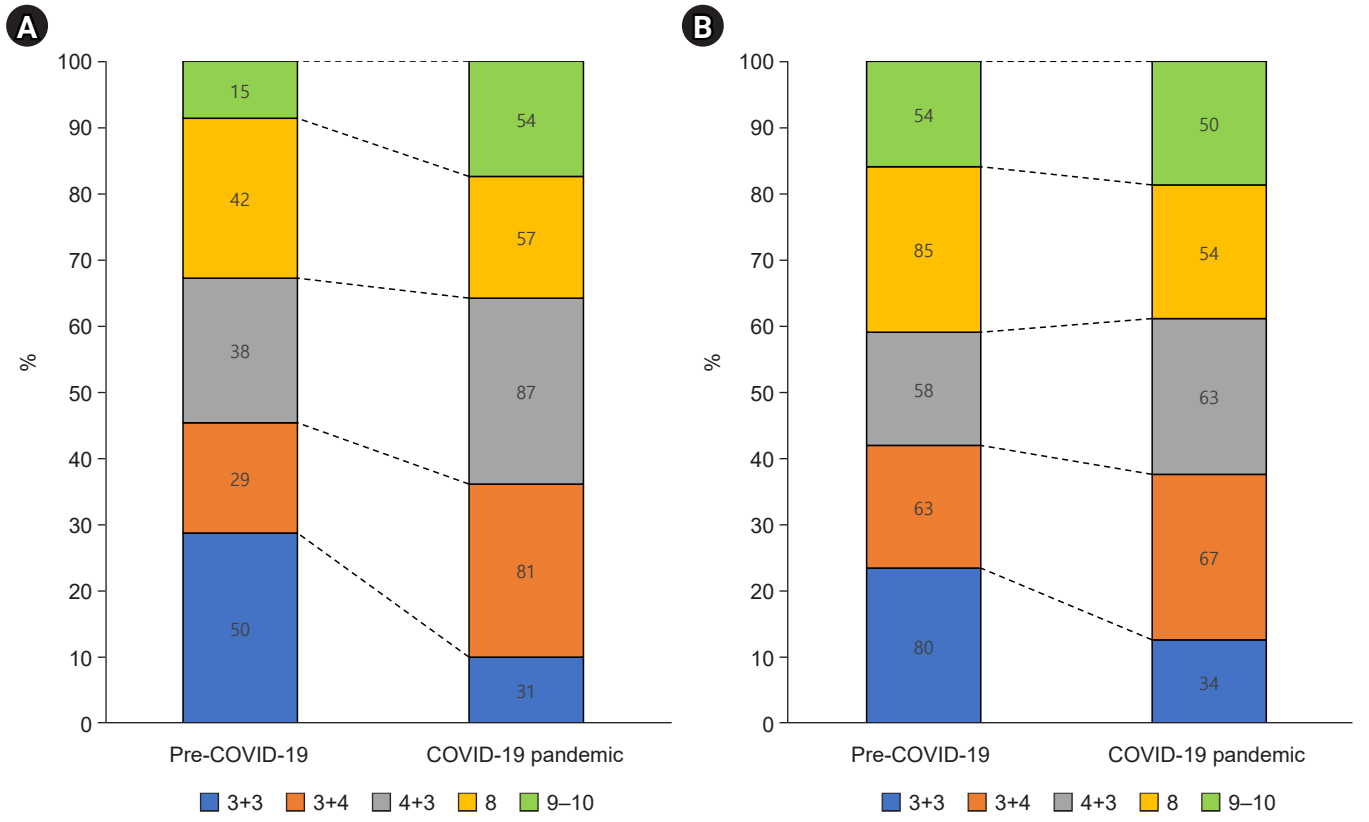

Fig. 2. Differences in the Gleason score (GS) distribution of magnetic resonance imaging (MRI)-guided and standard biopsies before and during the coronavirus disease 2019 (COVID-19) pandemic. Each GS is represented by a color, and the number inside the bar represents the number. The y-axis represents the proportion of each GS. (A) The MRI-guided biopsies comprised MRI-ultrasound fusion targeted target biopsies (two cores per target lesion) and standard biopsies. The group with these biopsies was compared before and during the COVID-19 pandemic. (B) The standard biopsy group was compared before and during the COVID-19 pandemic.

significant increase in age and PSA after the COVID-19 pandemic in the MRI-guided biopsy group. In the MRI-guided biopsy group, a higher proportion of patients were diagnosed with atypical small acinar proliferation and prostatic intraepithelial neoplasia after a biopsy was performed at the other institution and had a higher risk of cancer, but the diagnosis was delayed. These patients are typically older and have higher PSA than prostate biopsy naïve patients. It is inferred that the screening and biopsy of these patients have been delayed at a particularly high rate since the COVID-19 pandemic, which may explain the significant increase in age and PSA in the MRI-guided biopsy group.

While it is debatable whether the postponed detection and management of PCa due to the pandemic has impacted patient oncologic outcomes [19], several countries have published similar analyses. Nyk et al. [8] found that adverse pathological outcomes after robot-assisted radical prostatectomy were associated with the pandemic. They indicated that the pandemic in Poland had resulted in lockdowns or limitations, which had a negative impact and increased the chance of the disease worsening without prompt, effective treatment. They also explained that the pandemic might have made screening tools less available, leading to incorrect preoperative risk assessments [8]. The Netherlands study noted a 1.5% increase in patients with metastatic disease and a 2% drop in the proportion of low-risk groups during the wave of COVID-19 [9].

The diagnosis and management of PCa were influenced by social concerns and regulations in addition to the COVID-19 pandemic. One such example is the restrictions on PSA screening in the United States. In 2012, the U.S. Preventive Services Task Force advised avoiding PSA

testing in light of grade D evidence, citing concerns that PCa may be overdiagnosed and overtreated [20]. A grade D recommendation means that the task force recommends against a particular screening or intervention, as the potential harm outweighs the potential benefits. After these policies were implemented, PSA screening declined, and PCa-specific mortality plateaued or increased [21]. Butler et al. [22] found that from 2012 to 2015, the occurrence of localized disease declined, whereas distant metastatic disease continued to increase. In Korea, PSA screening tests are not included in national cancer screening workups. The pandemic may have further increased the rate of advanced PCa at diagnosis, especially in Korean circumstances. We can explain that this effect is reflected in our results.

The 2020 cancer statistics report for Korea states that the relative survival rate for PCa patients from 2016 to 2020 reached 102.6% in localized PCa and 99.9% in locally advanced PCa but decreased significantly to 45.9% in distant metastatic PCa [17]. Hence, it is probable that a more significant number of individuals got diagnoses at more aggravated states linked with the COVID-19 pandemic, thereby carrying the potential to exert a substantial adverse influence on oncological outcomes, including cancer-specific survival in PCa. Long-term follow-up is needed to determine how government policies and sociological responses to pandemic have affected the oncological outcomes.

Various constraints limited the present research. The present study was conducted retrospectively at a single institution. Thus, the possibility of bias in selection could not be entirely eradicated. The results of this study from a single tertiary referral center are limited in understanding the screening and diagnostic patterns of patients with PCa in Korea. Therefore, further multicenter studies or studies using a Common Data Model for the screening and diagnosing PCa in the course of the Korean pandemic. Second, this study analyzed the COVID-19 pandemic's influence using only outpatient visits, screening tests, and biopsy results related to PCa. Additional information on the results of imaging screening tests, such as MRI, bone scans, and post-prostatectomy pathologic reports, would provide a more accurate PCa patients' clinical presentation. Third, this study did not examine the long-term outcomes of PCa patients. In the future, it will be necessary to establish a multicenter database to evaluate recurrence-free or cancer-specific survival after treating patients diagnosed at that time.

In conclusion, during the COVID-19 pandemic, outpatient visits and screenings for PCa decreased. Simultaneously, the diagnostic rate of PCa with biopsies increased during this period, and patients with PCa tended to be diagnosed at a more aggravated state. Additional research is required to examine the ongoing influence of the COVID-19 pandemic on the oncological outcomes of PCa in Korea.

Article information

Conflicts of interest

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

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Author contributions

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Post-percutaneous core needle biopsy sputum cytology: diagnostic value and factors for positive prediction in diagnosing malignancy

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Background: This study evaluated the diagnostic yield and positive predictive factors of post-percutaneous core needle biopsy (PCNB) sputum cytology in diagnosing malignancy.

Methods: This retrospective study included patients who underwent PCNB at a single center from January 2014 to March 2022. Patient demographics, lung lesion characteristics on computed tomography, underlying lung disease, post-PCNB complications, histopathologic results of PCNB, post-PCNB sputum specimens, and final diagnoses were reviewed. The diagnostic yields and related factors were analyzed.

Results: Overall, 177 consecutive patients with sputum specimens obtained after PCNB for intrapulmonary lesions were enrolled. Among them, 152 patients had a final diagnosis of malignancy. Diagnostic sputum specimens with atypical or malignant cells were obtained in 12 patients. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of sputum cytology were 7.89%, 100%, 20.90%, 100%, and 15.15%, respectively. Lesion size, air-bronchogram, lesion multiplicity, and the cell type of squamous cell and adenocarcinoma differed significantly between the groups with diagnostic versus non-diagnostic sputum (p<0.05). The lesion size (odds ratio [OR], 1.035; 95% confidence interval [CI], 1.008–1.064; p=0.013), presence of air- bronchogram (OR, 23.485; 95% CI, 2.532–217.316; p=0.005), and squamous cell carcinoma (OR, 7.397; 95% CI, 1.773–30.865; p=0.006) were significantly associated with a diagnostic sputum specimen post-PCNB.

Conclusions: Although post-PCNB sputum cytology had low sensitivity in diagnosing lung cancer, it showed diagnostic results in some peripheral lung cancer patients who have squamous cell types, relatively large tumors, and air-bronchograms in the lesions.

Keywords: Core needle biopsy; Cytology; Lung neoplasms; Sputum

Introduction

Worldwide, lung cancer is the second most common cancer and the leading cause of cancer mortality. In 2020, 2.21 million people were newly diagnosed with lung cancer, and 1.8 million died from lung cancer [1]. The crude incidence rate of lung cancer in Korea in 2019 was 58.4 per 100,000 people, making it the second highest of all cancers, and the 5-year relative survival rate of lung cancer patients was only 34.7%. Characteristically, lung cancer had the highest proportion of patients diagnosed at a distant metastatic stage, approximately 50%, with a corresponding 5-year survival

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rate of 7.6% [2].

To reduce the mortality rate of lung cancer, early diagnosis is essential. In the 1970s and 1980s, large-scale trials using chest X-rays and sputum cytology for lung cancer screening failed to significantly reduce lung cancer mortality [3-5]. Since the 1990s, efforts have been made to diagnose lung cancer early using low-dose chest computed tomography (LDCT). In 2011, the National Lung Screening Trial reported a 20% reduction in lung cancer mortality from screening with LDCT [6]. However, using LDCT for lung cancer screening has low specificity, i.e., its ability to differentiate between benign and malignant lesions is poor [6,7]. Invasive pathological confirmation is imperative to diagnose malignancy in many cases.

Sputum cytology is an examination widely used for the respiratory system because of its cost-effectiveness and non-invasiveness. The detection of atypical cells in sputum can also be helpful in diagnosing cancer. Furthermore, biomolecular analyses of sputum cytology show potential for risk assessment and the early detection of lung cancer [8]. However, sputum cytology plays a limited role because of its low sensitivity [9]. The many factors that influence the sensitivity of sputum cytology include the location, stage, and cytologic subtype of the tumor; the number of sputum specimens; the specimen-collection method; and the specimen preparation technique [10].

Percutaneous core needle biopsy (PCNB) is a minimally invasive procedure for the pathological confirmation of peripheral lung lesions; it has high sensitivity and specificity of around 90% [11]. PCNB is a technique widely used for diagnosing lung cancer, although its value is limited in some cases of central lung cancer.

Our purpose in this study was to evaluate the diagnostic value of sputum cytology after PCNB for lung cancer diagnosis and to investigate which factors influence the results.

Methods

Ethical statements: This study was approved by the Institutional Review Board (IRB) of Kosin University Gospel Hospital (IRB No: KUGH 2023-02-004). The requirement for patient consent was waived due to the retrospective nature of this study.

1. Study population

Under approval of institutional review board, we retrospectively reviewed the medical records of 987 consecutive patients who underwent PCNB of the thorax in a single center from January 2014 to March 2022. Patients who underwent PCNB for extrapulmonary lesions, including pleura and chest wall lesions (n=19); those from whom no sputum could be obtained after PCNB (n=778); and those without a pathologically or clinically confirmed diagnosis (n=13) were excluded from enrollment. Therefore, 177 consecutive patients from whom sputum specimens were obtained after PCNB for the diagnosis of intrapulmonary lesions were included. We reviewed their medical records to collect demographic information (age, sex, and history of smoking), the results of pulmonary function testing, operator's procedure record, results of post-PCNB sputum specimens, results of PCNB, and final diagnoses.

2. CT acquisition and assessment

Computed tomography (CT) scans were acquired using a multidetector CT system (Somatom Sensation 64 or dual-source Somatom Definition Flash 128 or dual-source Somatom Force 192 multidetector CT system; Siemens Medical Solutions) with or without intravenous administration of contrast medium. Scanning parameters were 80–120 kVp, 90–150 mA, 0.5 seconds tube rotation time, and 1.2 pitch. The image data were reformatted with 1- to 2-mm slice thickness for transverse images and a 2.0-mm slice thickness for coronal and sagittal images. Two radiologists with 2 and 15 years of experience in chest CT interpretation retrospectively reviewed preprocedural CT images by consensus. Images were displayed with a lung window setting of level –500 Hounsfield units (HU) and width 200 HU and mediastinal window of level 25 HU and width 40 HU. One-dimensional size measurements were performed on the maximum diameter of the lesion on axial images. We divided the location of lung lesions into central and peripheral lesions. Central lung lesions were limited to the trachea, bronchi, or segmental bronchi, and peripheral lesions were found farther to the periphery than the subsegmental bronchi. The distance from the pleura to the lesion on the CT scan was also measured. We evaluated lesion solidity according to the Fleischner 2017 guideline. Solid nodules had homogenous soft-tissue attenuation, part-solid nodules had both ground-glass and solid soft-tissue attenua-

tion components, and pure ground glass nodules had only ground glass attenuation [12]. Other characteristics (the presence or absence of necrosis, cavity, air-bronchogram, mediastinal lymphadenopathy, multiplicity of lung lesions, and underlying lung disease of emphysema or fibrosis) were also reviewed. Necrosis was defined as low-attenuated, non-enhancing regions within lung lesions. Cavity was defined as a gas-filled space, seen as lucency or a low-attenuation area, within lung lesions. Air-bronchogram was defined as a pattern of air-filled bronchi on a background of a high-attenuation airless lesion. Lymphadenopathy was defined as enlarged lymph nodes greater than 1cm in the short axis diameter in the mediastinum. We regarded pulmonary emphysema as focal areas or regions of low attenuation, usually without visible walls, in whole lung parenchyma [13]. Reticular and ground glass opacities seen in a subpleural or peribronchial area with or without honeycombing were classified as underlying pulmonary fibrosis, irrespective of a specific diagnosis of fibrosis [14].

3. Percutaneous core needle biopsy

PCNBs using CT (n=143) or fluoroscopy (n=34) were performed by a chest radiologist with 15 years of experience in thoracic biopsy. Postprocedural complications, including pneumothorax and hemorrhage, were evaluated by immediate follow-up CT or fluoroscopy. We divided hemorrhages into minor and major. A minor hemorrhage was defined as a newly developed ground glass opacity around the lung lesion after biopsy. A major hemorrhage was defined as a hemorrhage with hemoptysis, as assessed using the operator's procedural records or patient's medical charts, regardless of the amount of blood [15].

4. Acquisition of sputum specimens

Patients who underwent PCNB expectorated sputum within 7 days after PCNB. The obtained sputum was fixed in 95 % ethanol and prepared using the pick and smear technique. After the prepared slides were stained with Papanicolaou methods, pathologists examined them. Pathologic diagnoses were reported as "non-diagnostic," "negative for malignant cells" or "positive for malignant cells," sometimes referring to a specific histologic type. In cases where a definite diagnosis could not be made, they were reported as "atypical cells" or "suspicious for malignancy" followed by a descriptive diagnosis.

5. Final diagnosis

Final diagnoses of the biopsied lesions were confirmed by PCNB (n=103), independent surgical pathology (n=49), another biopsy such as endobronchial ultrasound-guided transbronchial needle aspiration (n=4), or clinical follow-up (n=21). Clinical proof of benign lesions was accepted if no evidence of malignancy was confirmed by biopsy and any of the following conditions were satisfied: (1) spontaneous resolution, (2) resolution after appropriate treatment such as antibiotics or corticosteroid treatment, and (3) no significant change of morphology on the serial follow-up CT for at least 1 year [16].

6. Statistical analysis

We calculated the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of post-PCNB sputum cytology. The characteristics of patients and pulmonary lesions, presence of underlying pulmonary disease, and post-PCNB complications were compared between the diagnostic and non-diagnostic sputum specimen groups. Comparisons used the Mann-Whitney *U* test for continuous variables and the Fisher exact test for categorical variables. A logistic regression analysis was used to identify factors related to the diagnostic sputum specimen group. All statistical analyses used SPSS software (SPSS 28.0; IBM Corp.)

Results

This study included 177 patients (124 males and 53 females, mean age 68.9 years, range 33–94 years). Overall patient demographics and lesion characteristics are shown in Table 1. One hundred ten patients (62.1%) had a history of smoking, with a mean±standard deviation of 41±23.8 pack years. The mean forced vital capacity (% predicted value of FVC) was 81.75%±13.37%, and the mean FEV1/FVC was 0.703±0.091.

One hundred fifty-two patients (85.8%) had a final diagnosis of primary or metastatic lung cancer. The other 25 patients were diagnosed with benign lesions: infection by bacteria (n=12), mycobacterium (n=7), fungus (n=1), or parasite (n=2); an inflammatory pseudotumor (n=1); non-specific granuloma (n=1); and pneumoconiosis (n=1).

Diagnostic sputum specimens with atypical or malignant cells were obtained from only 12 of the 152 patients who were diagnosed with a malignancy. The individual charac-

Table 1. Overall patient demographics and lesion characteristics

Characteristic	Value (n=177)
Sex	
Male	124
Female	53
Age (yr), mean (range)	68.9 (33–94)
No. of smokers	110 (62.1)
History of smoking (pack-years), mean±SD	41.0±23.8
Pulmonary function testing, mean±SD	
FVC, % predicted	81.7±13.4
FEV1/FVC	0.703±0.091
Lesion size (mm), mean (range)	37.3 (4.1–123.0)
Solidity of nodule	
Solid nodule	149
Part-solid nodule	26
Pure ground glass nodule	2
Final diagnosis	
Squamous cell carcinoma	36
Adenocarcinoma	93
Small cell lung cancer	7
Pulmonary sarcoma	2
Carcinoid tumor	1
Diffuse large B-cell lymphoma	1
Extranodal marginal zone B-cell lymphoma	1
Leiomyosarcoma	1
Pulmonary metastasis	10
Benign pulmonary lesion	25

FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second.

teristics of the patients with diagnostic sputum specimens are given in Table 2. Non-diagnostic sputum specimens, which were found to be negative for malignancy, were obtained from the remaining 140 patients with cancer. The mean time between the PCNB and the collection of sputum specimens was 2 days (range, 0–7 days).

Using sputum cytology after PCNB to diagnose malignancy, the overall sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were 7.89%, 100%, 20.90%, 100%, and 15.15%. When we compared the diagnostic and non-diagnostic sputum specimen groups, there were no significant differences in age, sex, history of smoking, or pulmonary function testing results. There were significant differences between the groups in lesion size, air-bronchogram in the lesion, multiplicity of lesions, and cell type of squamous cell carcinoma and adenocarcinoma. The lesion size was significantly larger (p=0.001), and the proportion of air-bronchogram (p<0.001) and multiplicity of lesions (p=0.027) was significantly higher in the diagnostic specimen group. The proportion of squamous cell carcinoma (p=0.008) was higher in the diagnostic specimen group, and that of adenocarcinoma was higher in the non-diagnostic specimen group (p=0.012) (Fig. 1). The other lung lesion characteristics (location, solidity, depth from pleura to lesion, presence or absence of necrosis, cavity, mediastinal lymphadenopathy, underlying pulmonary disease of emphysema or fibrosis) and post-PCNB complications such as hemorrhage or pneumothorax did not differ significantly between the groups (Table 3).

In the multivariate logistic regression analysis, lesion size (odds ratio [OR], 1.035; 95% confidence interval [CI], 1.008–1.064; p=0.013), air-bronchogram in the lesion (OR, 23.485; 95% CI, 2.532–217.316; p=0.005), and cell type of squamous cell carcinoma (OR, 7.397; 95% CI, 1.773–30.865; p=0.006) were significant factors related to the diagnostic sputum specimen after PCNB.

Discussion

Cytological or pathological confirmation is essential for diagnosing lung cancer. Several methods for the pathologic evaluation of pulmonary lesions can be used, including CT- or fluoroscopic-guided percutaneous transthoracic lung biopsy, transbronchial biopsy using bronchoscopy with or without endobronchial ultrasound, and surgical resection of lung tissue. However, tissue obtained through a lung biopsy can be non-diagnostic or insufficient, and even in non-diagnostic cases, lung cancer was reported as the final diagnosis in about 40% of cases [17]. Sputum cytology is a simple, noninvasive, and adjuvant method for some patients with large, centrally located tumors. Neumann et al. [18] reported that nearly 50% of all patients with lung cancer who produced adequate sputum specimens were sputum-positive for cancer cells and that the detection of premalignant cells in sputum enabled the early diagnosis of lung cancer.

The sensitivity and specificity of sputum cytology for detecting lung cancer were 7.89% and 100%, respectively, in this study. Previous studies reported that the overall sensitivity and specificity of sputum cytology were 66% and 99%, respectively, which is a large difference in sensitivity from this study [9]. This difference in sensitivity might reflect the

Table 2. Patients with diagnostic sputum specimens

No.	Age (yr)	Sex	Smoking (pack-years)	FVC (% predicted)	FEV1/FVC	Results of PCNB	Final diagnosis	Location	Size (mm)	Solidity	Air-bronchogram	Multiplicity of lesions
1	63	M	Former smoker (90)	70	0.56	SQCC	SQCC	Peripheral	66	Solid	Presence	Presence
2	76	M	Never smoker	46	0.88	ADC	ADC	Peripheral	100	Solid	Presence	Presence
3	81	M	Former smoker (20)	69	0.71	SQCC	SQCC	Peripheral	73	Solid	Presence	Absence
4	63	M	Never smoker	91	0.70	SQCC	SQCC	Peripheral	42	Solid	Presence	Absence
5	78	M	Current smoker (13)	81	0.79	SQCC	SQCC	Peripheral	15	Solid	Presence	Absence
6	81	M	Former smoker (45)	54	0.60	SQCC	SQCC	Peripheral	46	Solid	Presence	Absence
7	83	F	Never smoker	81	0.80	Carcinoma with sarcomatous differentiation	NSCLC	Peripheral	68	Solid	Presence	Absence
8	64	M	Current smoker (48)	60	0.62	ADC	ADC	Peripheral	43	Solid	Absence	Presence
9	74	M	Current smoker (50)	93	0.62	SQCC	SQCC	Central	53	Solid	Presence	Presence
10	66	M	Current smoker (47)	96	0.67	Only necrotic tissue	SQCC	Central	80	Solid	Presence	Presence
11	62	M	Former smoker (30)	78	0.74	Only necrotic tissue	ADC	Peripheral	56	Solid	Presence	Presence
12	64	M	Current smoker (40)	70	0.70	Carcinoma	SCC	Central	43	Solid	Presence	Absence

FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; PCNB, percutaneous core needle biopsy; M, male; F, female; SQCC, squamous cell carcinoma; ADC, adenocarcinoma; NSCLC, non-small cell lung cancer; SCC, small cell carcinoma.

characteristics of the patient groups who underwent PCNB and the process of collecting sputum from those patients. Most patients in this study who underwent PCNB had peripheral lung lesions; few patients with centrally located tumors were included. Also, the sensitivity of sputum cytology in the previous study increased with the number of satisfactory sputum samples tested, starting at 45% for one satisfactory sample, increasing to 55% for two, and rising to 60% for three [19]. In our study, the instruction was to collect only spontaneous sputum without forceful coughing to prevent bleeding after PCNB, which probably affected the sensitivity of the results.

The lesion size, presence of air-bronchogram, and cell type of lung cancer differed significantly between the non-diagnostic and diagnostic sputum specimen groups.

A larger lesion size and cell type of squamous cell carcinoma are consistent with the results of previous studies [9,19,20]. Compared with other subtypes of carcinoma, squamous cell carcinoma is mainly located in the airway, grows into the bronchi, and causes atypical changes over a wide area of the respiratory mucosa. Although most lesions in our study were located in the periphery, this characteristic of squamous cell carcinoma might have affected the positive results from sputum specimens.

Lung cancer with an air-bronchogram was significantly associated with diagnostic sputum in our study. Although the correlation between air-bronchogram and sputum cytology has not been reported in previous studies, we suggest that the presence of a patented bronchus to the lesion affects the sputum cytology results. Atypical cells might emerge into the sputum specimen through the patent open bronchus or airways damaged after PCNB.

A multiplicity of lung lesions was significantly associated with diagnostic sputum specimens in our study. This result has not been reported previously. By the time the diameter of a lung cancer reaches 1cm, the tumor has more than 10^9 cells and might have already invaded the bronchial epithelium and vascular epithelium [21]. In cases with multiple lesions or advanced lesions with lung to lung metastases, a multiplicity of lung lesions might be distributed over a larger surface area than a solitary nodule or primary lesion, have high cellularity, and be aggressive.

Among the commonly known factors, cancer in a central location did not yield significant results in this study. Most of the patients who underwent PCNB in this study had peripheral lung cancers, with few patients with central lung cancer included, which is probably why statistically significant results were not obtained.

This study had several limitations. First, it was a single-center, retrospective study. Second, only a few patients

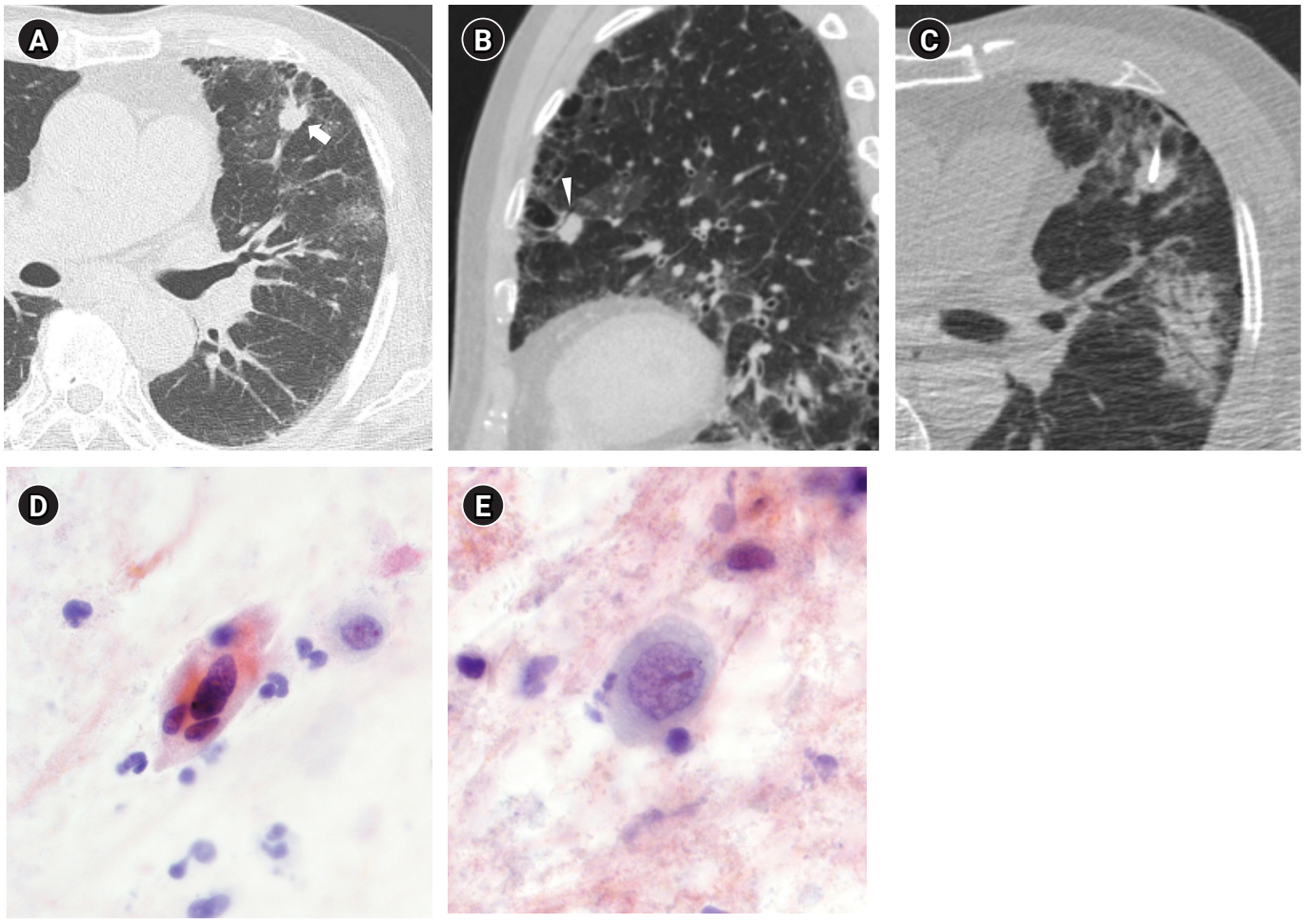

Fig. 1. An 83-year-old man with a diagnostic sputum specimen taken the day after percutaneous core needle biopsy (PCNB). (A) Axial computed tomography (CT) image shows an approximately 1.5-cm solid nodule with a spiculated margin in the left lingular division (arrow). (B) A sagittal CT image shows an air-bronchogram in the peripheral portion of the nodule (arrowhead). (C) CT image obtained during PCNB shows the needle tip targeting the nodule. There are peribronchial and subpleural ground glass opacities and reticulation in both lungs, suggestive of pulmonary fibrosis. (D, E) High-magnification microscopic images of the sputum specimen show the keratinizing squamous cell with hyperchromatic nuclei and non-keratinizing squamous cell with sharp "cookie-cutter" edge of dysplastic nuclei (Papanicolaou stain, ×1,000).

with diagnostic sputum specimens were included. Among 987 patients, only 177 patients met our inclusion criteria of pathologic confirmation, follow-up, and sputum samples after PCNB for intrapulmonary lesions, and only 12 of those patients produced diagnostic sputum specimens. Despite the challenges in obtaining these specimens, we were trying to analyze factors related to the results in the sputum cytology. The factors not included in the diagnostic sample group, such as part-solid and pure ground glass nodules, might have shown statistical insignificance even if they were actually significant. Third, most of our patients who underwent PCNB were peripheral lung cancer patients. Thus, it is difficult to apply our results to the entire population of lung cancer patients. Fourth, sputum specimens were not obtained through an optimal process. As described above, to prevent bleeding after PCNB, patients were instructed to collect only spontaneous sputum that did not require deep coughing, so the method and number of specimens obtained could not satisfy the optimal environment. In addition, because patients were on bed rest, it is unknown whether the obtained specimens were immediately received in the laboratory. Fifth, we did not conduct

Table 3. Comparisons between diagnostic and non-diagnostic specimen groups

Factor	Diagnostic specimen (n=12)	Non-diagnostic specimen (n=140)	*p*-value
Mean age (yr)	71.25	70.05	0.756
Sex			0.108
Male	11 (91.7)	95 (67.8)	
Female	1 (8.3)	45 (32.2)	
Smokers	9 (75.0)	85 (60.7)	0.375
Mean history of smoking (pack-years)	42.56	42.00	0.887
Pulmonary function test			
Mean FVC (% of predicted)	74.08	81.80	0.083
Mean FEV1/FVC (%)	69.92	69.69	0.771
Mean lesion size (mm)	57.1	35.7	0.001
Location			0.099
Central	3 (25.0)	12 (8.6)	
Peripheral	9 (75.0)	128 (91.4)	
Mean distance from pleura (mm)	6.3	5.2	0.643
Multiple lesions	6 (50.0)	28 (20.0)	0.027
Underlying lung disease			
Emphysema	5 (41.7)	40 (28.6)	0.340
Fibrosis	3 (25.0)	13 (9.3)	0.117
Post-PCNB complications			
Minor hemorrhage	4 (33.3)	70 (50.0)	0.370
Major hemorrhage	2 (16.7)	18 (12.9)	0.660
Pneumothorax	0	36 (25.7)	0.070
Solidity of nodules			
Solid nodule	12 (100)	116 (82.9)	0.215
Part-solid nodule	0	22 (15.7)	0.216
Pure ground glass nodule	0	2 (1.4)	0.999
CT characteristics			
Cavity	3 (25.0)	28 (20.0)	0.711
Necrosis	9 (75.0)	65 (46.4)	0.073
Air-bronchogram	11 (91.7)	55 (39.3)	<0.001
Mediastinal lymph node	4 (33.3)	31 (22.1)	0.473
Final diagnoses			
Squamous cell carcinoma	7 (58.3)	29 (20.7)	0.008
Adenocarcinoma	3 (25.0)	90 (64.3)	0.012
Small cell lung cancer	1 (8.3)	6 (4.3)	0.444
Pulmonary sarcoma	1 (8.3)	1 (0.7)	0.152
Carcinoid tumor	0	1 (0.7)	0.999
Diffuse large B-cell lymphoma	0	1 (0.7)	0.999
Extranodal marginal zone B-cell lymphoma	0	1 (0.7)	0.999
Leiomyosarcoma	0	1 (0.7)	0.999
Metastatic lung cancer	0	10 (7.1)	0.999

Values are presented as number (%) unless indicated otherwise.
FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; PCNB, percutaneous core needle biopsy; CT, computed tomography.

a comparison of diagnostic yield between pre- and post-PCNB sputum cytology. Because sputum samples were not routinely obtained before PCNB, it was not possible to compare sputum samples before and after PCNB, so we cannot suggest what effect the PCNB procedure itself had on sputum cytology.

In conclusion, although post-PCNB sputum cytology has low sensitivity in diagnosing lung cancer, it has shown diagnostic results in some peripheral lung cancer patients who have squamous cell types, relatively large tumors, and air-bronchograms in the lesions. Although this study has many limitations, that is the first study to show the results of post-PCNB sputum analysis and provides insights into the diagnostic yields of post-PCNB sputum cytology in diagnosing lung cancer.

Article information

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

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Case report

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Scrub typhus with complications of acute myocarditis and cardiac tamponade in metropolitan areas: two case reports

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Scrub typhus is known as one of the most common seasonal infections in endemic rural areas, but life-threatening cardiac complications in cases of scrub typhus are very infrequent. In addition, scrub typhus infection has been rarely reported among workers assembling pallets using manufactured wood in metropolitan areas. Herein, we present two cases involving myocarditis and cardiac tamponade as complications of scrub typhus. One patient died and the other patient survived. These cases indicate that scrub typhus infection could be an environmental hazard in metropolitan areas, especially in locations with poor hygiene, and highlight the need for timely diagnosis and proper management of severe scrub typhus infections. Therefore, we present these two informative fatal cases of scrub typhus infection presenting with myocarditis and cardiac tamponade as an environmental hazard in metropolitan areas.

Keywords: Cardiac tamponade; Case reports; Public health; Scrub typhus

Introduction

Mite-borne scrub typhus is geographically distributed in the rural area and a median incidence of 4.6/100,000, a mortality rate of 6.0% among those untreated, and 1.4% among those treated has been reported [1]. Based on the pathophysiology, scrub typhus invades blood vessels in the host's body, causing generalized vasculitis in major organs which results in various complications [2-4]; pneumonitis, hepatitis, and meningoencephalitis are relatively common, whereas cardiac complication including myocarditis or cardiac tamponade is unusual [5-7]. Environment assembling pallets using manufactured wood in the metropolitan show two unusual complications of fatal myocarditis and cardiac tamponade, who is the first documented carpentry-related environmental hazard due to scrub typhus infection.

Cases

Ethical statements: Informed consent was waived from the patients to participate in the study (EMC IRB 2017-10-006) and case report (EMC IRB 2023-01-010).

Case 1

A 56-year-old man visited the emergency unit on November 2, 2015. His heart rate and body temperature were 130 beats/min and 39.1 °C, respectively. The physical examination revealed a 1×1 cm necrotic skin lesion with an erythematous rim in the right axilla, and several erythematous maculopapular lesions on his trunk, right side of the neck, and on both arms. An electrocardiogram (ECG) showed sinus tachycardia (heart rate, 124 beats/min) with left ventricular hypertrophy and biochemical results included

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aspartate transaminase (AST) level of 228 IU/L, alanine aminotransferase (ALT) level of 130 IU/L, creatine kinase level of 1,204 IU/L, C-reactive protein (CRP) level of 6.34 mg/dL. An immunochromatographic assay for scrub typhus was positive, and immunofluorescent antibody IgG titer against *Orientia tsutsugamushi* was 1:1,280. On the 5th day of treatment with intravenous azithromycin, he complained of aggravated dyspnea. Vital signs were stable, but the chest X-ray revealed haziness in the lower lobes, bilaterally. An echocardiogram revealed severe left ventricular systolic dysfunction (ejection fraction: 13% with diffuse global hypokinesia). A chest computed tomography confirmed bilateral pleural effusions, and cardiac magnetic resonance imaging revealed dilated cardiomyopathy. Coronary computed tomography angiography showed no evidence of significant luminal narrowing. The patient was diagnosed with scrub typhus-associated myocarditis according to the diagnostic criteria for clinical myocarditis from the consensus and guidelines [8]. The patient presented unstable vital signs showed a blood pressure 80/60 mmHg, heart rate of 132 beats/ min, respiratory rate of 31 breaths/min, and body temperature of 37 °C. Arterial blood gas analysis revealed severe metabolic acidosis; blood pH was 7.18, $PaCO_2$ 13.0 mmHg, PaO_2 114.0 mmHg, HCO_3^- 4.9 mEq/L, and base excess –20.7 mEq/L. Furthermore, N terminal pro-brain natriuretic peptide and D-dimer levels were elevated to 27,157 pg/mL and 1.62 μg/dL, respectively. The white blood cell count was 1,290/μL and CRP was 15.43 mg/dL and liver enzymes were elevated with AST 188 IU/L, ALT 169 IU/L. Cardiogenic shock was clinically suspected and urgent extracorporeal membrane oxygenation with inotropic agents was applied in the intensive care unit, however, the patient expired due to cardiac death the next day.

On the medical records, an insect bite was reported at his workplace on October 26, 2015. No outdoor activities such as gardening/farming, landscaping or traveling to a rural area were reported in the preceding couple of months. He had been assembling pallets using processed wood for 2 years and wild logs were piled up adjacent to his workplace (Fig. 1A). In addition, poor hygiene as well as insect vectors and rats were present in the metropolitan (Fig. 1B).

Case 2

A 39-year-old woman was referred with an intermittent fever that became progressively worse. She periodically handled the wild log in the poor hygiene workplace of metropolitan as a carpenter for few months. Her vital signs were as follows: blood pressure 90/50 mmHg, body temperature 38.4 °C, pulse 132 beats/min, respiratory rate 20 breaths/min, and clear consciousness. On physical examination, she had no skin rash but, dark red crust presenting suspicious eschar was detected on the left lower abdominal wall. On the biochemical test, white blood cells 20,350/μL (segment 71%, lymphocyte 19.4%), AST 82 U/L, ALT 78 U/L, and CRP 3.16 mg/dL were elevated. Scrub typhus infection was clinically suspicious by the eschar with infectious sign and doxycycline was immediately started. On

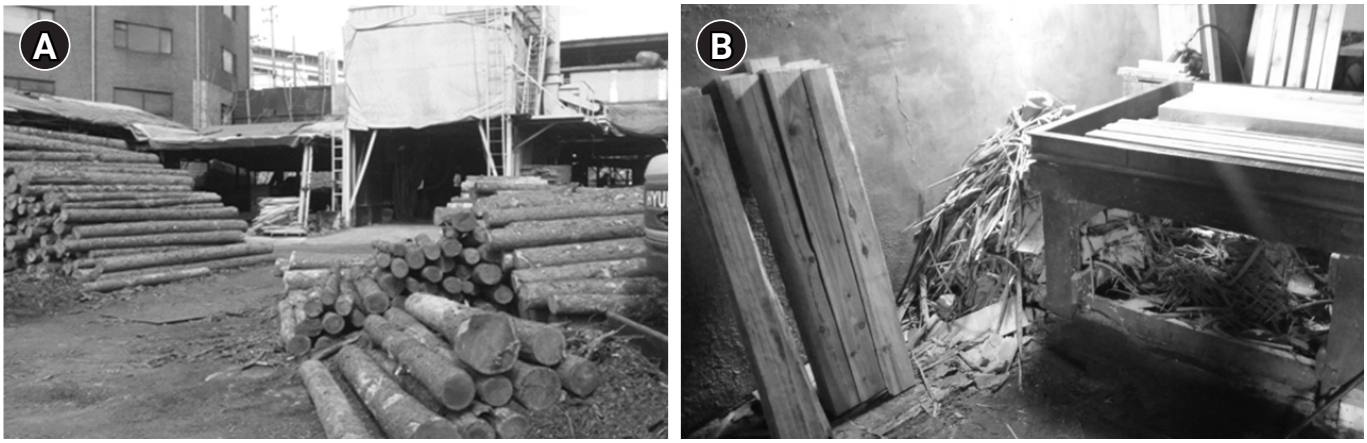

Fig. 1. The photo of public workplace in the infection. (A) The pallet manufacturing plant in a metropolitan area. Piles of logs in a neighboring carpentry workplace. (B) Pictures of the plant and surroundings show poor hygiene.

day 3 of the patient's hospital stay, the antibody titer to *O. tsutsugamushi* was elevated to 1:1,280 according to the indirect immunofluorescent antibody test, which confirmed the diagnosis of scrub typhus, but the resting dyspnea with generalized edema worsened slightly. A drowsy mentality with unstable vital sign was suddenly noted in the patient and urgent ECG revealed an overall low voltage QRS with a nonspecific ST-T change (Fig. 2A). Emergent chest computed tomography and echocardiogram revealed a marked cardiac tamponade with a collapse of the right atrium and right ventricle (Fig. 3). Subsequently, an emergent pericardiocentesis was performed to alleviate the symptoms and hemodynamic status and pericardial fluid in amount of 485 mL was drained in the catheterization laboratory and

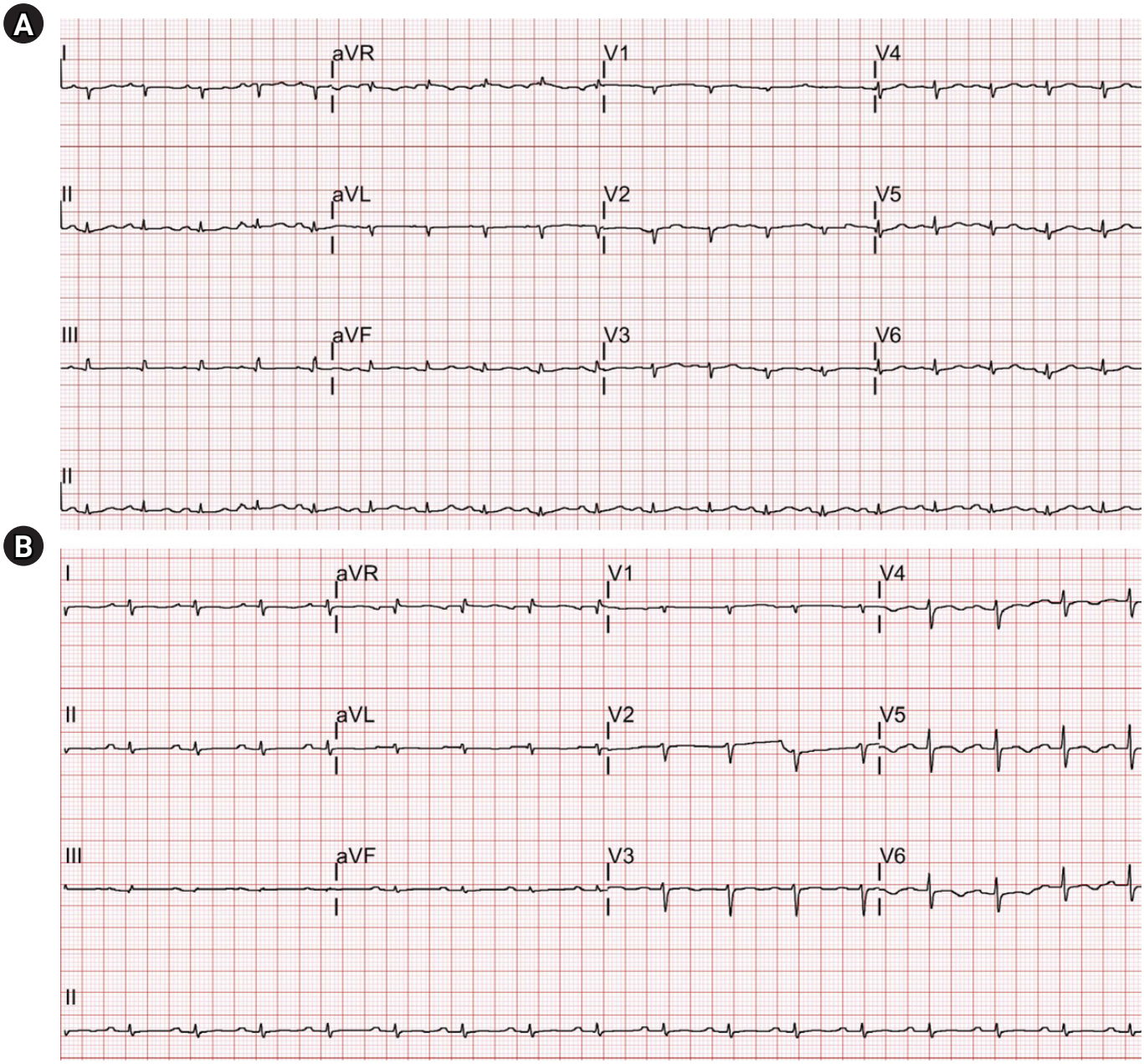

Fig. 2. The change of QRS voltage in the serial electrocardiogram (ECG) during the cardiac complication. (A) Initial ECG shows a low voltage of QRS amplitude in the intensive care unit. (B) Post-pericardiocentesis ECG shows improvement in the low voltage of QRS amplitude at discharge.

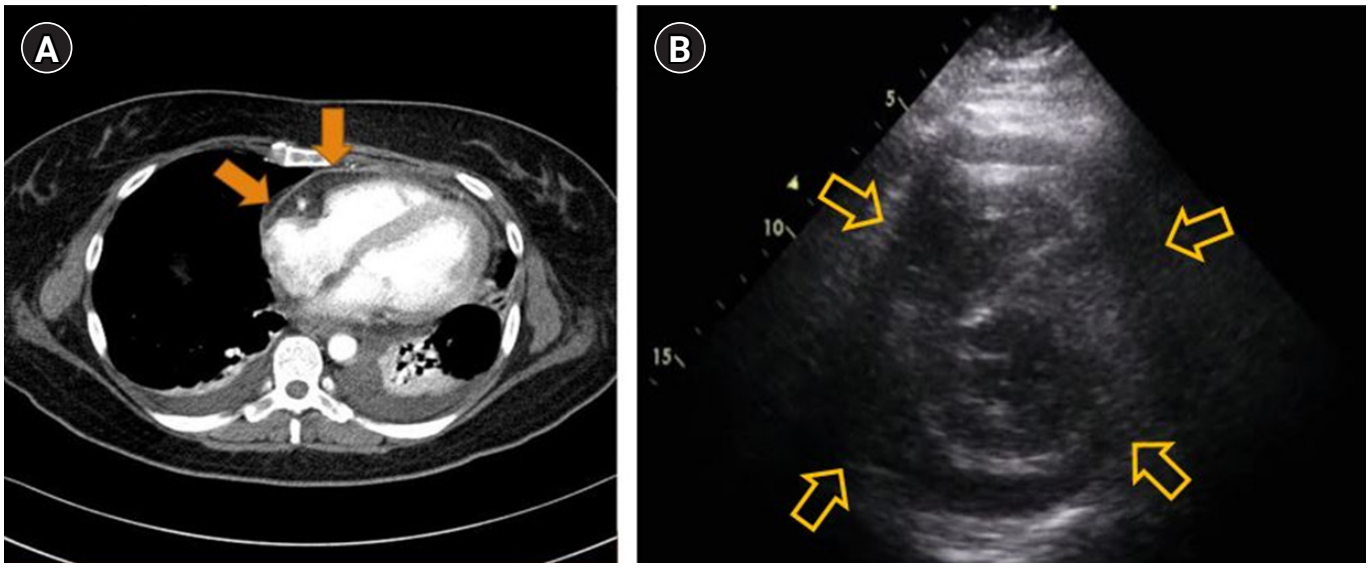

Fig. 3. The image of cardiac complications. (A) Chest computed tomography shows pericardial effusion with pericardial enhancement (filled arrows). (B) Echocardiography shows marked pericardial effusion (hollow arrows).

additional 618 mL of fluid was drained from the following day. The patient's overall clinical symptoms had suddenly improved (post-pericardiocentesis ECG) (Fig. 2B), and we finished doxycycline administration and removed the drain tube on hospital day 8.

Discussion

The incidence of scrub typhus is positively correlated with the physical environment (temperature, precipitation, and climate change) and human activities in the rural area [9,10]. Sepsis was the most prevalent serious complication followed by pneumonitis, hepatitis, and acute respiratory distress syndrome in the scrub typhus infection [4]. However, fatal acute myocarditis, arrhythmia and coronary artery disease complicated by scrub typhus and environmental hazard in the metropolitan have been recently reported in Korea [5,6,11]. The most common cause of occupation-related infection was scrub typhus, followed by tuberculosis, viral hepatitis, and viral influenza and unskilled laborers were the most vulnerable to scrub typhus, followed by health care professionals [12]. In addition, cardiac complications including myocarditis, myocardial infarction, and pericarditis have been recently reported in South Korea [6,11]. The incidence rate of cardiac complication might have been underestimated because a scrub typhus infection, which generally presents with mild chest pain, dyspnea, and edema, might remain nonspecific and under-detectable. But, early detection of cardiac complications prior to hemodynamic instability is crucial because the clinical outcome depends on adequate hemodynamic support and proper intervention. Especially, ECG screening can be useful in the timely diagnosis of cardiac complications in the infection status [11].

Considering that scrub typhus infection could steadily increase in the public park and workplace-related environments in the metropolitan [9,10], hygiene protection in the workplace should be re-emphasized to reduce environmental hazards from scrub typhus in endemic geographies. In particular, these cases also serve as a warning for the risk of fatal complication and timely surveillance in severe scrub typhus infection.

Article information

Conflicts of interest

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

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Author contributions

Conceptualization: KWK. Data curation: KWK. Formal analysis: KWK. Methodology: KWK. Project administration: KWK. Visualization: KWK. Writing - original draft: WK, KWK. Writing- review and editing: KWK.

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Case report

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Dermoscopic features of an unusual case of targetoid hemosiderotic nevus

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Targetoid hemosiderotic nevus (THN) is a rare variant of melanocytic nevus, characterized by a sudden development of a targetoid ecchymotic halo around a pre-existing nevus. THN clinically raises concern for malignant transformation due to its abrupt change in color and size. THN should be distinguished from other diseases showing a peripheral halo, including targetoid hemosiderotic hemangioma, halo nevus, and Meyerson nevus. Dermoscopy can help clinicians to differentiate THN from these diseases. The typical dermoscopic features of THN are known to be divided into two distinctive areas: the central melanocytic area and the peripheral ecchymotic area. In our case, dermoscopy revealed a novel bull's eye pattern composed of a central area with characteristic features of benign melanocytic nevus, an intermediated white circular ring, and a peripheral milky red area. When a sudden change occurs in a pre-existing nodule showing targetoid features, dermoscopy should be considered before conducting a biopsy or surgical intervention.

Keywords: Case reports; Dermoscopy; Targetoid hemosiderotic nevus; Traumatized nevus

Introduction

Targetoid hemosiderotic nevus (THN), first described in 2005 by Tomasini et al. [1], is a variant of traumatized melanocytic nevus mimicking malignant melanoma. It needs to be evaluated carefully by a dermatologist to exclude the possibility of malignant transformation. Dermoscopy is a noninvasive method of diagnosis which allows the visualization of pigmented and vascular structures. It has been reported that dermoscopy reveals typical patterns of a benign melanocytic nevus on the central nodule in THN [1-3]. To date, there are only a few reports of dermoscopic features of THN. Herein, we report a case of THN with characteristic dermoscopic features.

Case

Ethical statements: This report was approved by the Institutional Review Board of Kosin University Gospel Hospital (IRB No. #2022-06-034). The patient in this manuscript has given written informed consent to the publication of his case details.

A 66-year-old man presented with a solitary, round, 2 cm in diameter, blackish nodule surrounded by an ecchymotic halo on the abdomen (Fig. 1). The lesion at first was a brownish nodule, which existed since birth. It had developed a sudden change in size and color within 3 days. The patient denied any type of overt trauma. He was not taking anticoagulant drugs at the time of the development of ec-

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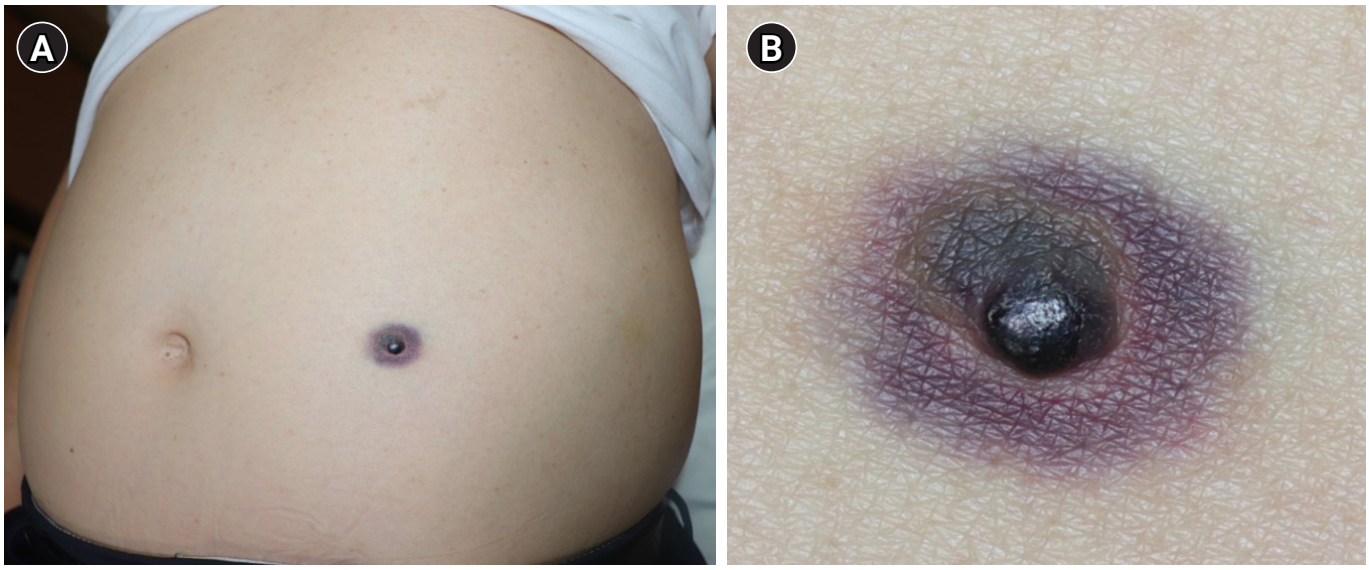

Fig. 1. (A, B) Blackish nodule on the abdomen surrounded by an ecchymotic targetoid halo.

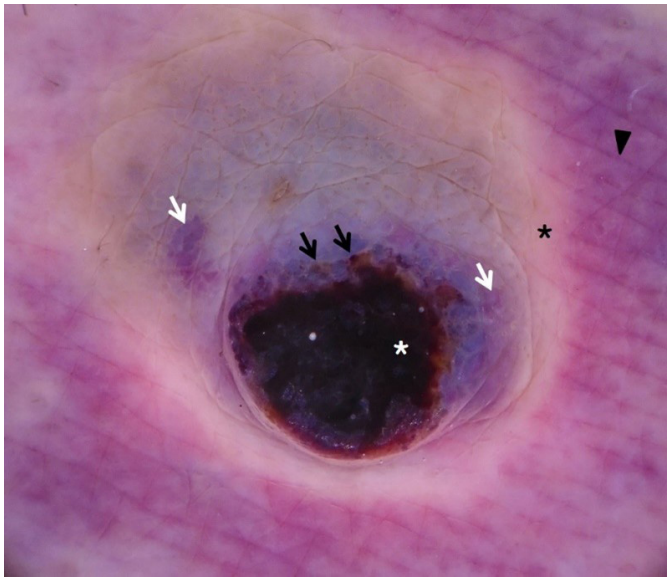

Fig. 2. Dermoscopy revealed brownish globules (black arrows) and violaceus globules (white arrows) intermingled with a structureless jet-black area (white asterisk) on the center. On the periphery, homogeneous milky red areas (black arrowhead) with an intermediate whitish circular ring (black asterisk) surrounding the central nodule were seen (×10).

chymotic halo and did not complain any symptoms. Dermoscopic findings of the central nodule revealed brownish to violaceus globules intermingled with a structureless jet-black area. Dermoscopic findings of the peripheral ecchymotic halo revealed an intermediate white circular ring surrounded by a homogeneous milky red area (Fig. 2). Skin biopsy was performed with a suspicion of hemangioma or other melanocytic lesions, including melanoma. Histology of the completely excised nodule showed dome-shaped architecture, a well-circumscribed proliferation of melanocytes arranged in nests in the dermis, and extravasation of red blood cells throughout the lesion. Neither atypical cells nor mitoses were observed (Fig. 3). Based on clinicopathological findings, a diagnosis of THN was made. After 10 months of monitoring, there was no recurrence.

Discussion

THN is a distinctive variant of traumatized melanocytic nevus. The pathomechanism of THN remains unclear, but it is presumed to be related to mild and repeated trauma. It has a predilection for the chest, abdomen, and shoulder, which are convex areas, and appears slightly elevated or exophytic, making it prone to receive chronic mechanical irritation. The peripheral ecchymotic halo usually appears abruptly without a history of overt trauma and regresses spontaneously within 4 weeks after the reabsorption of the extravasated red blood cells and hemosiderin with no recurrence [1,3]. Due to its benign nature, close observation using dermoscopy with short-term follow-up could be enough to manage it.

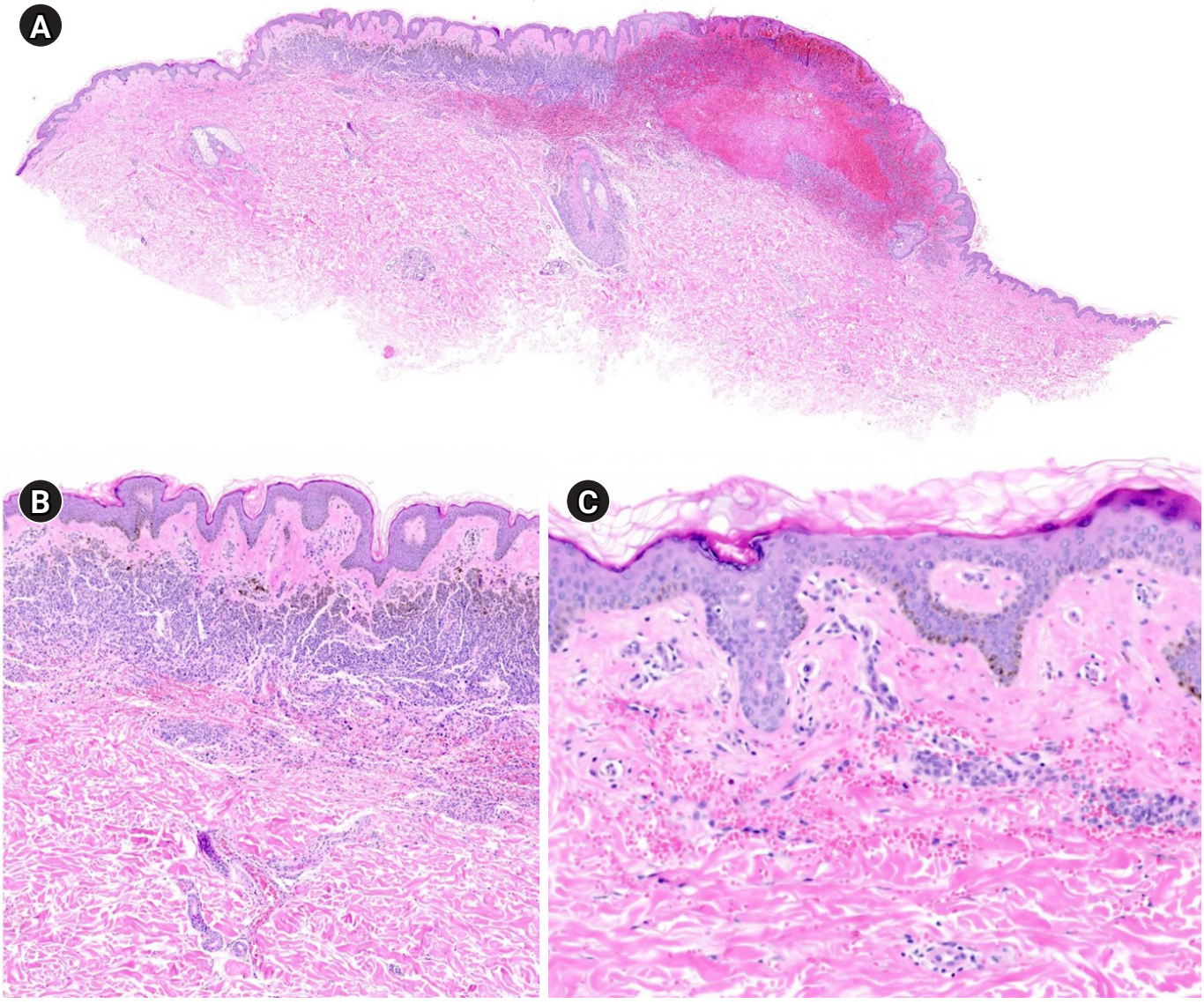

Fig. 3. Histological findings. (A) Dome-shaped architecture was seen along with the extravasation of red blood cells throughout the lesion (hematoxylin-eosin [H&E] stain, ×20). (B) Well-circumscribed proliferation of dermal melanocytes arranged in a nest and vessel dilatation were noted (H&E stain, ×200). (C) On the periphery, only extravasation of red blood cells was seen (H&E stain, ×400).

To date, it has been reported that dermoscopic findings of THN show two distinctive areas; benign melanocytic area on the center and homogeneous ecchymotic area on the periphery. Dermoscopy for the center shows a brownish globular pattern in addition to an irregular jet-black area and comma-shaped vessels. On the periphery, a homogeneous milky red area surrounding the entire lesion has been described [1-3]. Our case was novel in that an intermediate white circular ring was found between the central brown to black area and the peripheral homogenous milky red area, forming a bull's eye pattern composed of three distinct zones.

THN should be distinguished from other diseases showing peripheral halo, including targetoid hemosiderotic hemangioma (THH), halo nevus, and Meyerson nevus. Dermoscopy can help clinicians to differentiate THN from these diseases.

The main differential diagnosis of THN is THH. On the naked eye examination, it is difficult to differentiate THH from THN because of their morphologic similarity; reddish

to violaceous central nodule with the peripheral ecchymotic halo. On dermoscopy, THH presents as central red and/or dark lacunae characterized by multiple well-demarcated structures with a round to oval shape on the central nodule. An intermediate skin-colored, yellow, or whitish circular area surrounded by peripheral homogenous milky red area can also be found [4]. Our case of THN showed violaceus globules and an intermediate whitish circular area mimicking THH. However, the presence of brownish globules and structureless jet-black area, which are characteristic features of benign melanocytic nevus, made it possible to distinguish it from THH.

Halo nevus is characterized by a hypopigmented border around a pigmented melanocytic nevus. On dermoscopy, halo nevus has a peripheral rim of a whitish depigmented area rather than the peripheral reddish area found in THN [5]. Meyerson nevus is a benign melanocytic nevus with an eczematous halo. It is usually pruritic and presents with an erythematous scaly border. Meyerson nevus is dermoscopically differentiated from THN by the presence of yellow crusts, white scales, and dotted vessels [6].

In conclusion, dermoscopy considerably aids in the diagnosis of THN. In THN, dermoscopy reveals the bull's eye pattern composed of three distinct zones, the central area with characteristic features of benign melanocytic nevus, the intermediated white circular ring, and the outermost homogenous milky red area. When a sudden change occurs in a pre-existing nodule showing targetoid features, dermoscopy should be considered first before conducting a biopsy or surgical intervention.

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Case report

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Central diabetes insipidus following COVID-19 mRNA vaccination: a case report

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The coronavirus disease 2019 (COVID-19) has been a major public health emergency worldwide. Vaccines were rapidly developed and approved to prevent the spread of viral infection. However, various side effects of the COVID-19 messenger RNA (mRNA) vaccines have been reported after their commercialization. A 24-year-old man visited our emergency department with polyuria and polydipsia that occurred after he received a COVID-19 mRNA vaccine 10 days beforehand. The initial laboratory findings showed very low urine osmolality with hyperosmolar hypernatremia. Based on these findings, diabetes insipidus was suspected, and sella magnetic resonance imaging showed an enlarged pituitary gland and the absence of posterior pituitary higher intensity. After 12 hours of using oral desmopressin acetate, urine volume decreased, and after 5 days of administration, serum electrolyte and serum osmolality improved. This case report of diabetes insipidus occurring after vaccination with the BNT162b2 mRNA COVID-19 vaccine is presented as a reminder that close monitoring is necessary for patients with polyuria and polydipsia after vaccination.

Keywords: Case reports; Diabetes insipidus; Hypernatremia; mRNA vaccine; Polyuria

Introduction

The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a major worldwide public health emergency. To prevent infection, many vaccines rapidly tested in small groups and emergency use authorization was given [1].

Phase 3 clinical trials of the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer-BioNTech) indicated efficacy against COVID-19 infection, and showed that the vaccine had an acceptable safety profile [2]. However, this clinical trial included only 43,548 participants; thus, it was difficult to identify less common adverse events. After post-marketing of the COVID-19 mRNA vaccine, side effects were reported including myocarditis [3,4], cerebral venous sinus thrombosis [5], immune thrombocytopenic purpura [6], and new-onset autoimmune diseases [7].

Here, we report the case of diabetes insipidus after administration of the BNT162b2 mRNA vaccine in a 24-year-

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old male patient.

Case

Ethical statements: This report was exempted from review by the Institutional Review Board (IRB) of Gyeongsang National University Hospital (IRB No. 2023-01-001). Written informed consent was obtained from the patients to participate in the study.

A 24-year-old man visited the emergency department of our hospital with polyuria and an electrolyte abnormality. He had no medical history, and was otherwise in good health (September 2021). Symptoms of polyuria and polydipsia developed 10 days after he received the COVID-19 mRNA vaccine. At that time, blood analysis was performed at a local hospital and no abnormalities, including in glucose and electrolyte levels, were detected. However, the symptoms were persistent. He visited another hospital and was referred to our institution for further evaluation of the electrolyte imbalance. He had an increased urine output of more than 6–8 L/day at that time (November 2021). A COVID-19 test was negative.

Physical examination on admission revealed a blood pressure of 140/80 mmHg, heart rate of 110/min, body temperature of 37.8 °C and alert mental status.

The laboratory findings at admission were as follows: hemoglobin, 17.8 g/dL; white blood cell count, 8.56×10^9/L (segmented neutrophils, 47%; lymphocytes, 40%); platelets, 239×10^9/L; protein, 7.1 g/dL; albumin, 4.7 g/dL; alkaline phosphatase, 72 U/L; aspartate aminotransferase, 88 U/L; alanine aminotransferase, 98 U/L; blood urea nitrogen, 12.8 mg/dL; and creatinine, 1.06 mg/dL. His serum electrolytes and blood gas parameters were as follows: sodium, 175.6 mM/L; potassium, 3.9 mM/L; chloride, 140 mM/L; osmolality, 357 mOs/kg; calcium, 9.4 mg/dL; phosphorus, 2.5 mg/dL; pH, 7.38; pCO_2, 46 mmHg; pO_2, 81 mmHg; and bicarbonate, 27 mM/L. Finally, his urinalysis results were as follows: osmolality, 112 mOs/kg (normal range, 300–900 mOs/kg); and density, 1.002.

Diabetes insipidus was suspected based on the test results. Further hormonal testing was performed, and the results were as follows: thyroid-stimulating hormone, 1.58 (0.27–4.2) μIU/mL; T3, 152 (80–200) ng/dL; free T4, 1.4 (0.93–1.7) ng/dL; adrenocorticotropic hormone, 72 pg/mL; cortisol, 22.2 μg/dL; follicle-stimulating hormone, 0.87 UI/L; luteinizing hormone, 2.57 UI/L; insulin-like growth factor-1, 243 (88–209) ng/mL; prolactin, 34.7 (4.0–15.2) μg/L; and arginine vasopressin, 3.0 pg/mL.

Magnetic resonance imaging (MRI) of the pituitary gland revealed an enlarged pituitary gland and stalk, with no posterior pituitary hyperintensity (Fig. 1).

Diabetes insipidus was diagnosed based on these findings. The patient was started on oral 0.2 mg desmopressin acetate (three times per day). Although no pituitary biopsy was conducted, other plausible causes of central diabetes

Fig. 1. Magnetic resonance imaging of the pituitary gland. (A) Sagittal pre-contrast T1 image showing an enlarged pituitary gland and stalk, with no posterior pituitary hyperintensity. (B) Sagittal and (C) coronal post-contrast T1 images showing an enlarged pituitary gland and stalk, with heterogeneous enhancement.

insipidus (CDI) were ruled out, including IgG4-related disease and autoimmune diseases.

After 12 hours of using oral desmopressin acetate, the urine volume decreased and the urine osmolality increased rapidly. The serum electrolytes and serum osmolality were improved after 5 days of administration (sodium, 139.3 mM/L; potassium, 4.4 mM/L; chloride, 98 mM/L; osmolality, 290 mOs/kg; 24-hour urine volume, 2,300 mL; urine osmolality, 437 [300–900] mOs/kg; urine density, 1.011) (Fig. 2).

At the last visit in May 2022, 7 months after the first visit, the patient was taking 0.2 mg desmopressin acetate three times per day and had no polydipsia or polyuria. MRI of the pituitary gland 3 months later showed that the pituitary stalk was still enlarged.

Discussion

We report a case of diabetes insipidus developing after BNT162b2 COVID-19 mRNA vaccination, suggesting that it is necessary to monitor patients after mRNA vaccinations. CDI is characterized by hypotonic polyuria due to impaired AVP secretion from the posterior pituitary [8]. First, we confirmed the presence of hypotonic polyuria. The most commonly used test for diagnosing diabetes insipidus is the water deprivation test. However, we did not conduct this test because the sodium and osmolality levels of our patient were very high. Instead, we diagnosed diabetes insipidus after a therapeutic trial of desmopressin, which eliminated the polyuria and lowered the plasma osmolarity/sodium levels. We also measured the plasma

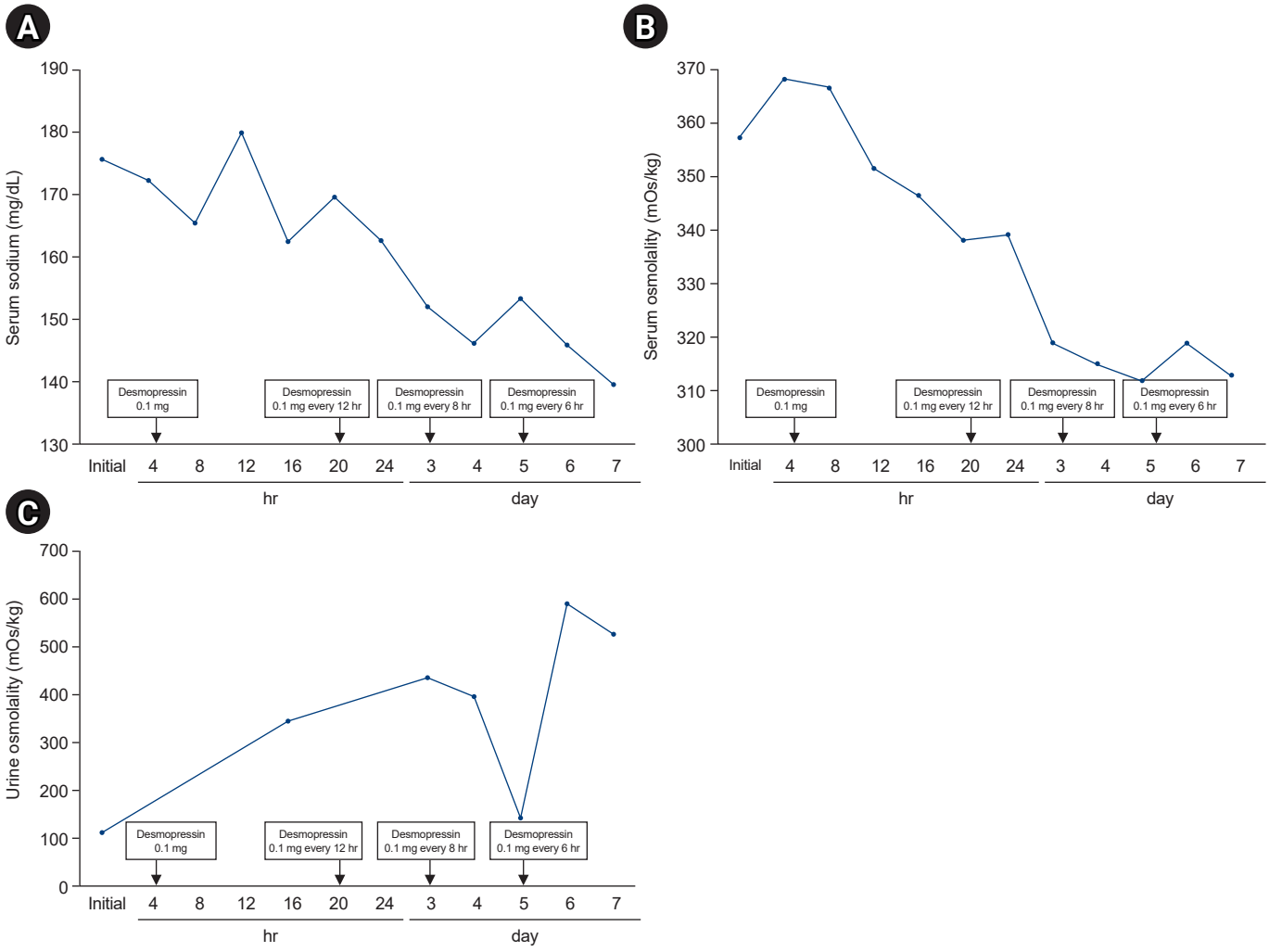

Fig. 2. Clinical course of (A) serum sodium concentration, (B) osmolality, and (C) urine osmolality.

AVP level, which was 3.0 pg/mL; the serum osmolality was 357 mOs/kg.

A several cases of side effects related to the pituitary gland after vaccination with SARS-CoV-2 were reported, and the types of vaccines received were different [9-11]. In this case, there was one case of CDI after BNT162b2 COVID-19 mRNA vaccine in a healthy person, and the case was reported where symptoms occurred 2 days after vaccination and persisted for more than 3 months [11]. Unlike previously reported case, this case is a young male who presented with a relatively rapid and severe electrolyte imbalance.

Hypophysitis is a heterogeneous condition that leads to inflammation of the sella and/or suprasellar region, potentially resulting in hormonal deficiencies and/or mass effects [12]. The etiology of primary hypophysitis is not clearly known, but majority of hypophysitis cases have an autoimmune etiology [13]. Secondary hypophysitis is caused by a variety of causes, including not only systemic inflammatory diseases but also tumors or cysts in pituitary lesions, infections, and various drugs. Various hypotheses have been proposed for the pathophysiological mechanism of post-vaccination hypophysitis, including autoimmune and inflammatory syndromes induced by vaccine adjuvants, hyper-stimulation of the immune system against vaccine components, and systemic inflammatory response [14]. However, since a pathological analysis of the pituitary gland must be performed for a comprehensive pathophysiological understanding, it is considered very difficult to directly prove the pituitary gland biopsy because of the risks.

Chen et al. [7] showed that new-onset autoimmune diseases are being increasingly reported after COVID-19 vaccination. In our case, there were no specific laboratory findings suggesting other causes of CDI, nor was there a history of drug use. Therefore, hypothalamic damage and inflammatory response occurred after vaccination, and CDI caused by primary hypophysitis was most suspected.

CDI is a rare side effect of mRNA vaccination; aside from our case, there have been only several previous reports. Physicians should be aware of the potential for adverse events in relation to mRNA vaccination.

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Case report

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Scrub typhus infection in a kidney transplant recipient: a case report

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Scrub typhus is a febrile disease that is endemic to Asia and the Pacific region. Its clinical manifestations include fever, myalgia, lymphadenopathy, and a characteristic eschar. The main manifestations of this disease are difficult to differentiate from those of other febrile illnesses; thus, a careful clinical examination and a high index of suspicion are crucial for an early diagnosis. Our case involved a 55-year-old female renal transplant recipient who presented with fever and sore throat in November. Her clinical symptoms did not improve after oral amoxicillin/clavulanate administration for 7 days, after which proteinuria and acute kidney injury were identified. After hospitalization, an eschar was found and immunoglobulin M antibodies against *Orientia tsutsugamushi* were detected by indirect immunofluorescence. She received oral doxycycline for 7 days and showed improvement in renal function and proteinuria. This is the first case report of scrub typhus infection in a kidney transplant patient in Korea. It is meaningful to report that the renal abnormalities associated with scrub typhus improved in a renal transplant patient through treatment of the disease. This case highlights the importance of examining the social history and symptoms of patients suspected of having scrub typhus in endemic areas. Early diagnosis and treatment are crucial in kidney transplant patients to preserve graft function and prevent fatal complications.

Keywords: Acute kidney injury; Case reports; Kidney transplantation; Proteinuria; Scrub typhus

Introduction

Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi*. It is transmitted by the bites of larval *Leptotrombidium* mites with *O. tsutsugamushi* [1]. Scrub typhus is endemic to rural areas of Asia and the Pacific region. It is also the most common rickettsial disease in autumn in Korea and occurs nationwide [2]. Clinical findings include fever, myalgia, skin lesions (especially typical eschar), and lymphadenopathy. The disease is usually mild and self-resolving but can occasionally result in severe complications such as interstitial pneumonia, acute kidney injury, multi-organ failure, and death [3]. Renal involvement in scrub typhus ranges from isolated urinary abnormalities to fatal acute kidney injury [4]. As the main manifestations of this disease are difficult to distinguish from other febrile diseases, it is difficult to make an early diagnosis. Eschar is the crucial clue for the diagnosis of scrub typhus, and its overall prevalence ranges from 32.8% to 78.7%. The distribution of eschar is highest in the inguinal area, followed by the anterior area and lower extremities [5]. Because eschar is small and located in moist areas where the skin overlaps, it can be detected through close physical examination. We report the case of a renal trans-

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plant recipient with scrub typhus infection who received a delayed diagnosis.

Case

Ethical statements: This study was exempt from review by the Institutional Review Board of Asan Medical Center (IRB No. 2023-0172). Written informed consent was obtained from the patient to participate in the study.

A 55-year-old woman, a renal transplant recipient, visited the outpatient clinic of a tertiary hospital and was referred to the emergency department due to fever and sore throat in November. She had undergone a living donor kidney transplant 2 years prior, did not have any rejection, and was on triple immunosuppressant therapy (tacrolimus 6 mg/day, mycophenolate mofetil 1,000 mg/day, and methylprednisolone 4 mg/day). Two weeks prior, she visited the outpatient clinic on a regular schedule and complained of general weakness and breast pain near the axillary area without fever. One week prior, she visited the emergency room of our hospital because of sore throat and was prescribed oral amoxicillin/clavulanate for 7 days. On the day of admission to the outpatient clinic, despite taking oral antibiotics, fever and sore throat persisted, and laboratory tests showed decreased renal function and proteinuria. She was admitted to our hospital for further examination, mycophenolate mofetil was discontinued, and the dose of methylprednisolone was increased to 10 mg/day. At the time of admission, her vital signs were as follows: blood pressure, 121/80 mmHg; pulse rate, 105 beats/min; body temperature, 37.7 °C; and respiratory rate, 18 breaths/min. The patient was alert. Physical examination revealed tonsillar hypertrophy and redness. An eschar, characteristic of scrub typhus, was observed in the right breast near the axillary area (Fig. 1). Examination of the cardiovascular, respiratory, and abdominal systems revealed normal results. Complete blood cell counts were as follows: white blood cell count, 9,300/mm^3 (neutrophil-dominant, 77.9%); hemoglobin, 12.1 g/dL; and platelet count, 170,000/mm^3. The biochemistry results were as follows: C-reactive protein level, 10.87 mg/dL; blood urea nitrogen level, 19 mg/dL; creatinine level, 1.25 mg/dL; total protein level, 7.0 g/dL; albumin level, 2.7 g/dL; aspartate transaminase level, 74 IU/L; alanine aminotransferase level, 49 IU/L; uric acid level, 4.2 mg/dL; and lactate dehydrogenase level, 570 IU/L. Microscopic urinalysis showed albumin 1+, occult blood traces, and red blood cells 0-2/high-power field. The urinary creatinine to protein ratio was 1,345.6 mg/g. The results of the anti-human immunodeficiency virus antibody, hepatitis B virus surface antigen, anti-hepatitis B virus surface antibody, and anti-hepatitis C virus antibody tests were negative. The patient lived in a suburban area and had a history of working in gardens. Based on the social history, seasonal factors (November), fever, elevated liver enzyme levels, and typical eschar, serological tests were performed to discriminate between scrub typhus and other febrile diseases. While waiting for the serological test results, rapid diagnostic tests for leptospirosis, scrub typhus, and hemorrhagic fever with renal syndrome were performed. Immunoglobulin (Ig)M antibodies against *O. tsutsugamushi* were positive; IgG antibodies were weakly positive; and all other tests were negative. Based on these findings, oral doxycycline was immediately administered. As decreased renal function and proteinuria were observed in a renal transplant recipient, renal ultrasonography, cytomegalovirus, BK virus polymerase chain reaction (PCR) tests, and donor-specific antibody (DSA) tests were performed. To discriminate between other viral infections, multiple viral PCR tests were performed for adenovirus, parainfluenza virus, rhinovirus, influenza virus, respiratory syncytial virus, metapneumovirus, enterovirus, and severe

Fig. 1. Clinical photograph showing an eschar on the patient's anterior chest.

acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Fever and inflammatory marker levels improved within 48 hours, and renal function and proteinuria improved within 72 hours (Fig. 2). On the third day of hospitalization, the results of an indirect immunofluorescent antibody (IFA) assay were positive for antibodies for *O. tsutsugamushi*. DSA, cytomegalovirus, and BK PCR results were negative, and renal ultrasonography showed no structural abnormalities. The results of multiple viral PCR tests, including SARS-CoV-2 PCR, were negative. The patient was discharged after 6 days, and her renal function and proteinuria improved to the normal range in an outpatient examination performed 10 days after discharge. The patient maintained normal renal graft function.

Discussion

The clinical features of scrub typhus are similar to those of febrile diseases. Thus, it is important to identify eschar as a pathognomonic sign of scrub typhus. At the site of a tick bite, eschar is formed with a diameter of 5 to 20 mm, covered with a black scab in the middle [2,3]. Although eschar is normally painless, patients often complain of pain due to enlarged lymph nodes around the eschar [2]. Therefore, lymphadenopathy may help locate the eschar if it is small or if it is located in skin overlaps. Our patient presented with breast pain near the axilla, and an eschar was detected on the anterior chest. She complained of breast pain at the outpatient clinic; however, we did not find an eschar at that time. This case highlights the importance of careful physical examinations in outpatient clinical settings.

According to the data from the Korea Disease Control and Prevention Agency, the fatality rate of scrub typhus is as high as 0.2% [6]. Therefore, early diagnosis and appropriate treatment are critical during the epidemic season. Among serological diagnostic tests, the IFA test with high sensitivity and specificity is the gold standard for diagnosing scrub typhus [2]. However, because the IFA test requires some time for diagnosis, a rapid diagnostic test can be helpful for quick diagnosis [7]. In our case, we used a rapid diagnostic test for the early detection of scrub typhus infection. Our patient received antibiotics immediately after the

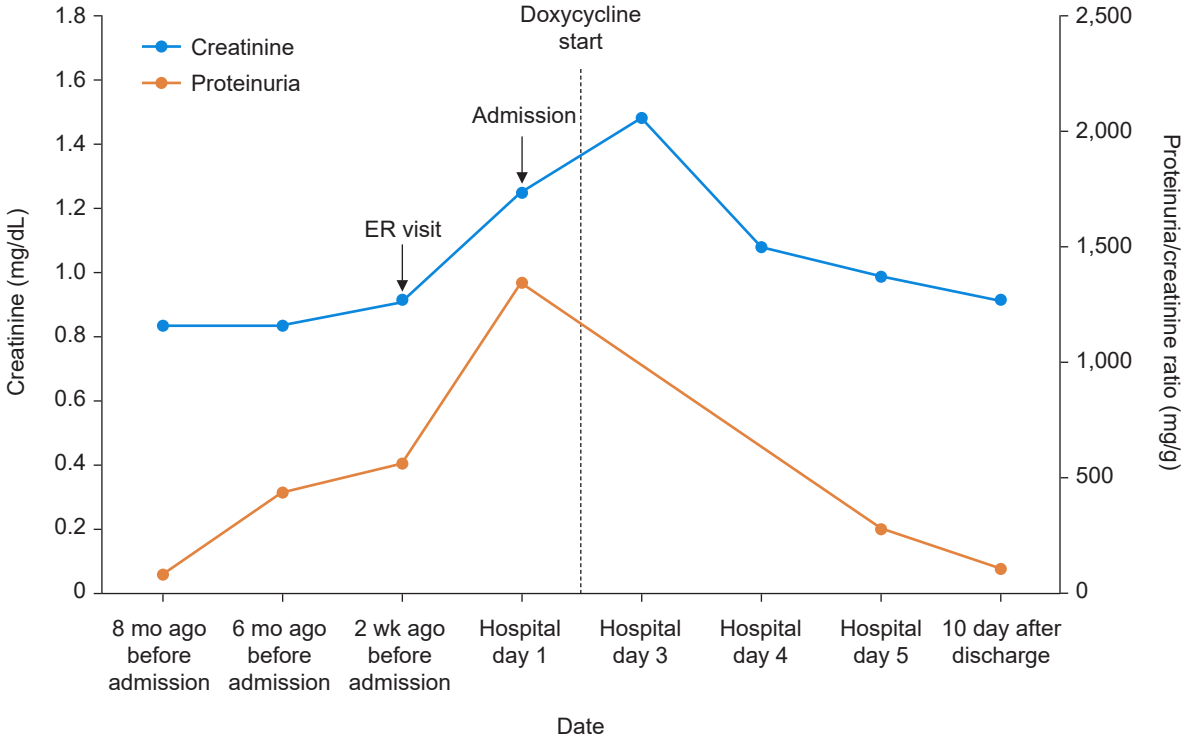

Fig. 2. Change in serum creatinine levels and proteinuria. ER, emergency room.

rapid diagnostic test, and there were no sequelae.

The patient presented with proteinuria and decreased renal function upon admission. Proteinuria and hematuria may occur in 10% to 20% of patients with scrub typhus, and acute renal failure has been reported in 8% to 40% of those patients [8-10]. End-stage renal disease is rarely caused by scrub typhus and requires maintenance hemodialysis, and only one case has been reported in Korea [11]. In most patients, acute renal failure due to scrub typhus is reversible with appropriate antibiotic therapy. Our patient was a renal transplant recipient who received triple immunosuppressant therapy, and her highest creatinine level was 1.48 mg/dL during hospitalization. However, after treatment for scrub typhus, renal function and proteinuria recovered to the normal range in the general population.

Reports of scrub typhus infections in renal transplant recipients are rare; however, there has been a case of scrub typhus meningitis in a kidney transplant recipient in India. The patient presented with fever, severe headache, acute renal failure, and proteinuria. An eschar was found on the patient's back, and IgM antibodies against *O. tsutsugamushi* were positive. She was administered antibiotics and discharged with normal graft function [12]. No case of scrub typhus in a renal transplant patient has been reported in Korea yet; however, in actual clinical practice, it is thought that such cases may have occurred. In India, there has been a recent report of co-infection with coronavirus disease 2019 (COVID-19) and scrub typhus [13]. Considering the ongoing COVID-19 pandemic, it is necessary to differentiate febrile illnesses based on clinical symptoms and epidemiological factors.

Because kidney transplant recipients are immunocompromised, appropriate tests should be performed for general and opportunistic infections if signs of infection are revealed. It is important to assess the social history and symptoms of scrub typhus during the epidemic season. Early diagnosis and proper management are essential in kidney transplant patients to preserve renal graft function and prevent fatal complications.

Article information

Conflicts of interest

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

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Instructions to authors

KMJ
KOSIN MEDICAL JOURNAL

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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.

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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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