Aims and scope

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Implementation of a care coordination system for chronic diseases

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The number of people with chronic diseases has been increasing steadily but the indicators for the management of chronic diseases have not improved significantly. To improve the existing chronic disease management system, a new policy will be introduced, which includes the establishment of care plans for hypertension and diabetes patients by primary care physicians and the provision of care coordination services based on these plans. Care coordination refers to a series of activities to assist patients and their families and it has been known to be effective in reducing medical costs and avoiding the unnecessary use of the hospital system by individuals. To offer well-coordinated and high-quality care services, it is necessary to develop a service quality assurance plan, track and manage patients, provide patient support, agree on patient referral and transition, and develop an effective information system. Local governance should be established for chronic disease management, and long-term plans and continuous quality improvement are necessary.

Keywords: Chronic disease; Patient care management; Referral and consultation; Transitional care

Introduction

Population aging has led to a steady increase in the number of people with chronic diseases, and the socio-economic costs due to the related complications, disabilities, and premature deaths, as well as direct medical costs, are rising rapidly [1]. Despite this current status, the management of chronic diseases has not improved significantly. For example, the blood pressure control rates of patients with hypertension and the glucose control rates of those with diabetes have remained at approximately 60% and 20%, respectively [2]. To improve the above described problems in the management of chronic diseases, numerous public projects have been implemented in some regions. Although these projects have resulted in some achievements related to developing awareness on diseases and medication adherence [3,4], this approach comprising sporadically implemented individual projects has not shown population-level effects in the South Korean environment, where there are no incentives for healthcare providers to engage in chronic disease management [5].

Recently, the Ministry of Health and Welfare announced the plan for the “Pilot Project for Primary Care Chronic Disease Management,” and it will introduce a system for the stepwise strengthening of chronic disease management based on primary care services. The current pilot project includes the establishment of care plans for patients with hypertension and diabetes by primary care physicians and the provision of care coordination services based on these plans. Care coordination refers to a series of activities that assist patients and their families in self-managing their health conditions and related psychosocial problems more effectively, coordinating their care among multiple health and community providers, bridging gaps in care, and receiving the appropriate level of care. Numerous countries, such as the United Kingdom (UK), the Netherlands, the United States, and Japan, have already introduced care coordination...
systems for the management of chronic diseases, or they have implemented care coordination services within the national healthcare system. Such measures have proven effective in reducing medical costs and avoiding the unnecessary use of the hospital system by individuals [6].

However, it is difficult to apply these foreign systems directly to South Korea. The reasons for the difficulty in introducing a care coordination system could include the nature of medical practice, which is based on a private-dominated supply system; opposition from the medical field against the introduction of the prospective payment system; and lack of experience and resources on chronic disease management. Therefore, it is important to introduce a system by adapting it to our conditions and circumstances. Nevertheless, because the conditions described above have been formed during the historical development of the South Korean healthcare system, these conditions should be considered in terms of overall chronic disease management, including care coordination.

This study aimed to examine the factors of care coordination in the management of patients with chronic diseases, and further investigate the conditions and plans for implementing the policy successfully in South Korea.

### Key elements of care coordination

The Chronic Care Model (CCM) has been used widely as a guideline for effective chronic disease management since its emergence in the 1990s [7,8]. It emphasizes the need for change in the existing health and medical system for dealing with chronic diseases management, presenting the factors that are important for appropriate chronic diseases management, such as linkage with community resources, reflecting on the characteristics of healthcare institutions, self-management support, design of a delivery system, decision-making support, and establishment of a clinical information system. This approach purports that the community and healthcare system should collaborate to improve treatment outcomes by creating “informed and activated patients” and “prepared and proactive practice teams,” and developing a productive relationship between patients and the practice team. Studies have revealed that interventions based on the CCM have led to improvements in treatment outcomes changing the manner in which medical and care services are provided [7]. Therefore, the present study utilized the CCM to examine the elements necessary for care coordination and further discuss practical measures for South Korea.

### Structure and responsibilities of care coordination team

The CCM model emphasizes that chronic diseases management should be organized, coordinated, and provided by the management team. Further, it is recommended to employ a population-based approach to influence treatment outcomes [9]. Such an approach should include a care plan based on complex and comprehensive assessments and rationales on the problems of patients, and it should involve cooperating with patients to identify hurdles, solve their own problems, and achieve service goals. Previous studies reported that the care coordination team is effective in the areas of heart failure, diabetes, treatment of the frail elderly, and mental health services integrated with chronic pain [10-14], further revealing that other healthcare professionals and office workers can play a partial role equivalent with that of physicians, in treating chronic diseases [15,16].

The care coordination team could include the patient and his/her family, the primary care provider, the care coordinator, and the assistant clinician, if necessary. These team members should fulfill their respective treatment responsibilities and further share responsibilities pertaining to intervention and follow-up observation. The first step in chronic disease management is to create a team that provides comprehensive care based on the needs of patients with a sense of responsibility.

However, it is difficult to construct and operate a team that provides care with a comprehensive responsibility for disease management in the current private sector-led healthcare delivery system in South Korea. Although the National Health Insurance Service is a single insurance provider in South Korea, the public sector’s role in financing the healthcare system is low. Further, more than 90% of the beds for patients with acute diseases are owned by the private sector. In South Korea, neither can the government be responsible for all services, as in the case of National Health Service in UK, nor can each insurer assume the responsibilities, as in the United States, the Netherlands, and Japan. Although the labor cost for care coordinators could be financially aided by the National Health Insurance (NHI), the care services need to be coordinated through a network of service providers in each region, and individual institutions responsible for the coordination of care services should modify them based on the local situation.

Currently, among the various institutions that could shoulder the responsibility of coordinating care services, possible candidates could be public organizations, such as public health centers and the NHL, and civil organizations, including local medical associations. Public health centers could be
advantageous for providing comprehensive services because they can be linked easily with community resources. Associations of service providers, such as local medical associations, could be responsible for service quality assurance in clinical terms, because their accessibility could be used to foster cooperation between service providers. Although the NHI can stably employ care coordinators, it is difficult to connect them with local communities. More importantly, there is no consensus on whether care coordinators employed by the NHI could be dispatched owing to the long-lasting conflict with service providers on medical payments. Thus, it is difficult to implement a care coordination system without the cooperation of or at least agreement by providers [17].

A more important issue than that pertaining to the care coordination team is the need to establish local governance based on the involvement of multiple service providers to provide and coordinate comprehensive services from the viewpoint of patients. It is difficult for one institution to have comprehensive responsibility for patients in the South Korean context. Therefore, it is necessary for one institution to lead the coordination of care services and simultaneously encourage the participation of other service providers. Furthermore, the participation of community health workers should be guaranteed to provide comprehensive services and to communicate with service providers [17].

Plan for service quality assurance

In many primary healthcare institutions, care is not coordinated by merely enforcing a system and supporting the manpower and finances. The improvement of the coordination system requires concerted efforts. It is necessary to relocate the personnel and train new roles for care coordination, and further establish networks with numerous service providers and organizations in the local community. Moreover, the timely sharing of information between patients and various service providers should be ensured. These efforts would lead primary care providers to make decisions that improve care coordination.

The next step after establishing a care coordination team is to develop quality management plans and monitor the operations [18]. Planning should begin with clear goals such as receiving a reply on a consultation report each time after specialist referral, or contact with a patient within 3 days after hospital discharge. The quality indicators of chronic disease management projects that have been implemented thus far have directly measured changes in patients, including self-recognition of blood pressure or blood glucose levels, drug intake, health behaviors relating to nutrition and physical activities, and blood pressure and blood glucose control. On the other hand, process indicators that can directly show the status of care coordination, such as patient referral, report reply, and patient information sharing, have not been measured. Though not in the community, several cases of improvement through inter-department cooperation within regional cardiovascular centers have been reported by monitoring several indicators, including the time from hospital arrival to treatment for emergency patients with conditions such as myocardial infarction and stroke [19]. In the medical field, if consensus is reached between participating experts, quality indicators that reflect the clinical viewpoint could be identified within a short period.

The most important components of quality assurance for services over the long term is the content of the services offered by the care coordinator and the training of the workforce. However, it is unrealistic to conduct the educational programs developed in foreign countries in the absence of a care coordination service in South Korea. Indeed, it is logical to manage quality and educate personnel based on services that can be performed effectively owing to the implementation of the system. Several aspects of healthcare services need to be provided by experts, which requires vertical quality control. However, the care coordinator also needs to reflect on the needs of patients and to ensure access to services. The strict assessment and monitoring of the contents of the services offered is therefore essential. Simultaneously, it is important to provide autonomy to service providers to enable them to develop services to suit the needs of patients. Therefore, educational programs for personnel should be developed continually through linking theoretical education and practice of the services provided onsite, rather than through standardized processes. Thus, educational institutions for healthcare personnel should be able to cater to a sizeable number of students to meet the needs of onsite service providers. Considering that the size of the city and county is too small, and it would be appropriate to divide them into several districts.

Tracking and management of patients

The primary goals of care coordination are to manage the quality of care services provided at the time of patient referral and transition. Additionally it should enable service providers and related organizations, as well as patients, to have the information and resources they need for providing appropriate care. These tasks the core tasks of the care coordination team. The primary care provider can refer the patient to a specialist or other provider
for services that he/she needs. Therefore, the primary care provider becomes primarily responsible for patient referrals and care coordination. On the other hand, with reference to inter-hospital transitions of patients, the primary service provider at the institution from which the patient is discharged, or the institution itself, is responsible for the transition. However, the direction of transition could be reversed, such as transition from a primary care facility to a hospital. When various service providers are involved in the treatment of patients, they are solely responsible for the individual services they provide, while it is often unclear who is responsible for coordinating the various services provided to the patient. Indeed, the care coordination team should assume this responsibility [18].

Care coordination requires the consideration of patient activities outside the institution to which the provider belongs. Thus, the recognition of these activities is essential in care coordination. The tracking of extra-organizational activities by patients begins with recording basic information on the referral and transition documentation. This task develops into strategies to evaluate and record the implementation of major steps, including scheduling consultation with a specialist, delivery of information for consultation, implementation of the referred consultation, and handing over a report after the referral and transition [18].

Because incidents occur suddenly, such as hospitalization and emergency room visits, it is difficult to acquire the necessary information in a timely manner if no information delivery system is established in advance. Patients could lack information on their illness or the doctor in charge of the treatment may not be available when the patients visit the hospital suddenly. An electronic record system can help in such cases, and a card that patients can carry in his/her wallet could provide all the important information to the medical staff. Furthermore, when the care coordinator intends to acquire the information on the entry and exit of patients, the care coordinator could rely on the hospital physician and the emergency room physician. However, it is the best to have a daily entry/discharge report from the hospital [18]. In the case of infected patients, an infection manager in the hospital can easily access and manage the information on such patients. However, currently, hospitals do not have a system for managing the information on the entry and discharge of patients with chronic diseases. Although the insurance claim data could be helpful, the acquired information would not be timely. Therefore, it is necessary to establish a system for tracking and managing patients in cooperation with local hospitals.

Patient support

Care coordination can be divided into multiple steps, such as logistic support in service delivery, clinical monitoring, and support for self-management or drug use. Particularly, case management and care coordination should be distinguished clearly. Nurses and other care managers in primary care clinics have been provided clinical support regarding clinical evaluation, follow-up observation, self-management support, and medication management. Case management primarily provides some care coordination functions for high-risk patients, while the care coordinator strives to complete patient referrals by handling logistic or financial hurdles. Moreover, the care coordinator allows patients to receive timely referred treatment, and further resolves problems faced by patients by delivering clinical information and tracking the referral process. Most benefits from patient support arise mainly from handling logistic steps or information on patient support related to patient referral or transition [18].

Numerous diseases, such as diabetes, require continuous and comprehensive management. The more severe the patient’s condition is, the more likely he/she is to require services from other specialists and health and welfare services in addition to those offered in primary care clinics [20]. It is therefore important to develop linkages with community resources to meet the comprehensive needs of patients with chronic diseases, and to ensure the continuity and completeness of treatment [17]. Although the introduction of a care coordination system would help improve the community linkage, the first challenge in achieving the same is the conflict between the public health agencies and the private hospitals and clinics. Private clinics are commonly annoyed by patients’ use of services at public health centers. However, it is difficult to link clinics with the community if they do not accept such practices. Indeed, minimal acceptance is essential [17].

The easiest way to strengthen the linkage of service providers to a local community is to conduct conversations on common issues. Private clinics commonly lack the information on services provided by public health centers. This is also observed between departments within a public health center. Therefore, it is necessary for individual institutions or organizations to share information on both private and public services provided in the local community, and to conduct subsequent meetings on case management. Currently, some meetings on case management are being conducted occasionally in public health clinics and public health centers, particularly regarding the visiting healthcare services project. In this case, the challenge lies in the participation
of doctors. Even within the same institution, the participation of healthcare providers is insufficient. Although physicians could participate by creating a care plan for patients, the provision of the information related to the care, the mechanism to participate in the decision-making process, and the provision of incentives, if necessary, should be considered to improve the linkage and cooperation among healthcare providers.

**Agreement on patient referral and transition**

It is important for service providers to agree on the purpose and importance of referral, and their respective roles in patient referral and transition. The agreement begins by focusing on the service providers and institutions that are frequently referred to, and further building relationships with the major service providers in the community. Continuing conversations could lead to consensus to improve the referral system [21]. Further, relationships need to be established with providers of services such as social services, health behavior support, and peer support, as well as with major specialists, hospitals, and emergency medical institutions [18]. The inclusion of some of the service providers from the community in the care coordinator team could help facilitate communication between providers [17].

Even if service providers agree with the standardized format and method of patient referral or transition, the practice needs to be managed continuously. If a hospital or clinic is open or closed and new public services are created, the newcomers should be asked to agree on the existing methods or new methods need to be agreed upon. Although the agreed expectations could be formalized in writing, dialogue between the parties as well as the individual relationships formed accordingly are crucial factors. Official regulations or guidelines are important, but it is more important to build a network of service providers. Although guidelines agreed in writing are important, there could be some unsettled issues, and informal consent about the details is also important. In this case, human relationships play an important role. The relationship between providers has weakened in comparison to that in the past, which has become an obstacle to service linkage among providers [22]. The same is applicable to South Korea. Another manner in which agreed expectations can be systemized is the use of an electronic referral system [23], especially if appropriate consent is obtained, unnecessary referrals are reduced, duplicate surveys are avoided, and optimal treatment is provided after referral and discharge [24,25].

**Information system**

A crucial factor in successful patient referrals and transition is to have the information that service providers require for providing their services. Hospitals, clinics, and public health centers used different electronic medical record systems, and it is difficult to standardize medical information, which in turn is a hurdle in establishing an electronic information system [23]. If the practice team is organized and consensus is established among service providers, information that is essential for the referral and transition of patients could be standardized [24]. The electronic referral system can help ensure that this information is delivered in a timely manner, incorporating the agreed guidelines for the referral and transition of patients to reduce unnecessary transfer and transitions of patients. Furthermore, it could be a trusted source of information for primary care providers and specialists [23]. However, the delivery of this information could be performed through a pen-and-paper-based medium by structuring and standardizing referral requests and medical notes.

Although the amount and accessibility of the delivered information is important, ongoing management of the information system is more vital. In addition to the organizations participating at the beginning, as local networks expand, more providers could participate. Different regions have different conditions and additional needs may be added over time. Accordingly, to increase the usability of the information system, it is necessary to be able to respond immediately to such changing conditions. It is necessary to set up a system which allows various stakeholders to access the information system and develop various applications (apps). With the recent increase in the use of cloud services or open-source programs, computer non-specialists are developing their own apps. If health professionals can participate directly in app development, they will be able to respond to a variety of needs and changes in the field.

Service providers require the information system to obtain any information they need. Moreover, patients should have access to the information system to enable them to provide information on their diseases during self-management, including that on care plans, or unexpected hospitalizations or emergency room visits. Continued feedback from patients would further help improve the utility and quality of the information system.

**Conclusion**

This study examined the major factors of care coordination based on the CCM, and further examined the difficulties and practical measures required for introducing the system in South Korea.
The first consideration in introducing care coordination in South Korea is to form a network among the community and medical service providers. Although there have been numerous attempts to provide comprehensive services for the management of patients with chronic diseases, such efforts have not been successful. In addition to methodological problems and financial difficulties, these attempts could not overcome the problems related to the segmentation of the healthcare delivery system. Financial support is important for securing consent from healthcare providers, but it is more essential to continuously develop the network that is organized in the community, by including a participation mechanism in the decision-making process.

In addition to the lack of experience regarding the management of chronic diseases, there is absolutely no manpower to provide care coordination. Even if a foreign curriculum is introduced in South Korea, it needs to be adapted to the contextual realities of the field. Eventually, the curriculum could be improved as the system is implemented and related experiences are accumulated. However, it is important to note that success of the system is ultimately determined by the quality of the services provided. Therefore, continuous quality control and improvement should be ensured for the services provided in the care coordination system.

It would take time for the care coordination system to settle. All the factors for care coordination described in this study require continuous management and improvement after the actual implementation of the system, including planning for service quality assurance, tracking and managing patients, providing patient support, maintaining consent and agreement on patient referral and transition, and developing and maintaining an electronic information system. Furthermore, most indicators on chronic diseases cannot be improved immediately after the implementation of the system. Therefore, a long-term plan should be established and continuous improvement of the system should be emphasized.

Conflicts of interest

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

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Pathological interpretation of connective tissue disease-associated lung diseases

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Connective tissue diseases (CTDs) can affect all compartments of the lungs, including airways, alveoli, interstitium, vessels, and pleura. CTD-associated lung diseases (CTD-LDs) may present as diffuse lung disease or as focal lesions, and there is significant heterogeneity between the individual CTDs in their clinical and pathological manifestations. CTD-LDs may presage the clinical diagnosis a primary CTD, or it may develop in the context of an established CTD diagnosis. CTD-LDs reveal acute, chronic or mixed pattern of lung and pleural manifestations. Histopathological findings of diverse morphological changes can be present in CTD-LDs airway lesions (chronic bronchitis/bronchiolitis, follicular bronchiolitis, etc.), interstitial lung diseases (nonspecific interstitial pneumonia/fibrosis, usual interstitial pneumonia, lymphocytic interstitial pneumonia, diffuse alveolar damage, and organizing pneumonia), pleural changes (acute fibrinous or chronic fibrous pleuritis), and vascular changes (vasculitis, capillaritis, pulmonary hemorrhage, etc.). CTD patients can be exposed to various infectious diseases when taking immunosuppressive drugs. Histopathological patterns of CTD-LDs are generally nonspecific, and other diseases that can cause similar lesions in the lungs must be considered before the diagnosis of CTD-LDs. A multidisciplinary team involving pathologists, clinicians, and radiologists can adequately make a proper diagnosis of CTD-LDs.

Keywords: Airway diseases; Connective tissue diseases; Histopathology; Interstitial lung diseases; Pleural diseases

Introduction

In patients with connective tissue disease-associated lung diseases (CTD-LDs), lung biopsies show fibro-inflammatory changes in various compartments of the lung, including the airways, alveolar walls, alveolar spaces, pleura, and vascular structure. CTD-LDs often reveal acute, subacute, and chronic fibro-inflammatory lesions within the same biopsy specimen, indicating an ongoing pathological process [1,2]. Most CTD–associated interstitial lung diseases (CTD-ILDs) show nonspecific microscopic patterns that are indiscernible from idiopathic interstitial pneumonias of usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), lymphocytic interstitial pneumonia (LIP), diffuse alveolar damage (DAD) or organizing pneumonia (OP) [1]. It is important to confirm the patient’s underlying systemic disease before pathological interpretation and diagnosis of CTD-LDs [3,4]. CTD-LDs reveal certain histological patterns of ILD, and it is possible in many cases to make a diagnosis of CTD-ILD and give important information about patient management through surgical lung biopsy including video-assisted thoracoscopic biopsy [1].

Some histopathological changes in CTD-LDs are not direct manifestations induced by CTD on the lung, but can be induced by drug therapy or other systemic complications in patients with
CTD. It is difficult to discriminate which findings are related to the patient’s CTD or secondary effects in the lungs on microscopic examination [3,5]. The case of CTD-LDs should be reviewed by a team of specialists, including pathologists, rheumatologists, pulmonologists, and radiologists, for accurate diagnosis and confirmation of underlying etiology. This article will cover the pathological interpretation and diagnosis of the pulmonary changes seen in rheumatoid arthritis (RA), systemic sclerosis (SyS), systemic lupus erythematosus (SLE), polymyositis and dermatomyositis (PM-DM), Sjögren syndrome (SjS), and mixed connective tissue disease (MCTD).

**Histopathological pulmonary patterns in rheumatoid arthritis**

RA associated with lung diseases can affect all anatomical areas of the lungs, including airways, alveoli, interstitium, vessels, and pleura [1]. Common pleural manifestations of RA are pleural effusion, acute fibrinous pleuritis, chronic fibrous pleuritis, and pleural adhesions [1,6,7] (Fig. 1). RA reveals single or multiple nodular lesions in the pleura, interlobular or alveolar septa, and the rheumatoid nodules are considered the most diagnostic finding of RA-ILD. Pathologically, rheumatoid nodules measure up to 2 to 3 cm and consist of a central portion of eosinophilic necrosis, occasionally neutrophils, that is surrounded by appearance of epithelioid histiocytes and scattered giant cells [1,6,8]. The most common histological pattern of RA-ILD is NSIP (30–67%), followed by UIP (13–57%) [1-3,9,10].

The microscopic features of RA-NSIP or RA-UIP mimic those seen in idiopathic NSIP and UIP. More prominent interstitial lymphocyte aggregates and/or occasional germinal centers can be seen in the alveolar walls or peribronchiolar areas in RA-UIP [1,8] (Fig. 2). In contrast, fibroblast foci are more characteristic findings in idiopathic UIP rather than RA-UIP [3,11]. Histological patterns of inflammatory airway diseases, such as bronchiectasis, chronic bronchitis, or follicular bronchiolitis can be present in the RA-NSIP or RA-UIP [10,12]. Acute and subacute inflammatory lung lesions including DAD and OP are often seen in RA-ILD with an acute exacerbation, or initial pulmonary lesions of RA [6,13,14]. Acute vascular manifestations including vasculitis, capillaritis or pulmonary hemorrhage, and chronic vascular manifestations are rare in RA-ILD [15].

![Fig. 1. Chronic fibrous pleuritis in a patient with rheumatoid arthritis. Pleural adhesion (black arrows), fibrotic interstitial pneumonitis (asterisks) and focally lymphoid aggregates (white arrow) are present (hematoxylin and eosin stain, x40).](https://doi.org/10.12701/yujm.2019.00101)

**Histopathological pulmonary patterns in systemic sclerosis**

ILD occurs in up to 80% of SyS patients, and ILD is the most characteristic finding in SyS than in any other CTD [1]. The major histological features of SyS-ILD are collagenous interstitial fibrosis and hypertensive vascular changes [16]. The characteristic findings of SyS-ILD are fibrotic NSIP with paucicellular and collagen-rich interstitial fibrosis, maintaining the lung architecture and often preserving the subpleural area [1,17] (Fig. 3).

When lung lesions progress, the fibrotic changes becomes confluent and this leads to honeycombing or end-stage lung [1]. The hypertensive vascular changes reveal fibromyxoid

![Fig. 2. Usual interstitial pneumonia pattern with lymphoid hyperplasia in a patient with rheumatoid arthritis. Microscopic findings of honeycombing (black arrows) and lymphoid follicle (white arrow) are seen. Fibrous pleural thickening is noted (arrow heads) (hematoxylin and eosin stain, x40).](https://doi.org/10.12701/yujm.2019.00101)

![Fig. 3. Usual interstitial pneumonia pattern with lymphoid hyperplasia in a patient with rheumatoid arthritis. Microscopic findings of honeycombing (black arrows) and lymphoid follicle (white arrow) are seen. Fibrous pleural thickening is noted (arrow heads) (hematoxylin and eosin stain, x40).](https://doi.org/10.12701/yujm.2019.00101)
intimal thickening and mild medial hypertrophy, which leads to thickening and narrowing of pulmonary arterioles [16]. True vasculitis and pulmonary hemorrhage are infrequent features of SyS [18]. Patients with SyS occasionally complain of esophageal motility disorder with gastrointestinal reflux; which leads to superimposed aspiration pneumonia [1,19].

Histopathological pulmonary patterns in systemic lupus erythematosus

Pleuritis is the most common pleuropulmonary change of SLE, and microscopic features such as nonspecific pleural inflammation with fibrin deposition and fibrosis can be observed [20]. Cytological findings of the pleural fluid in SLE patients reveal characteristic lupus erythematosus cells consisting of neutrophils or macrophages with cytoplasmic degenerated nuclei [1]. Histologically, SLE reveals acute lupus pneumonitis with nonspecific acute and organizing lung injury patterns [21,22]. The most common microscopic features of SLE-LDs are acute and organizing DAD with fibrinoid microthrombi, hemorrhage, and focal hyaline membranes [1,21] (Fig. 4A). OP also can be a common finding in SLE [23] (Fig. 4B). Diffuse alveolar hemorrhage (DAH) can be a fatal acute complication of SLE with capillary immune complex deposition. DAH reveals fresh hemorrhage or hemosiderin pigments in the alveolar spaces and alveolar septa, associated with or without capillaritis [1,24] (Fig. 5). Histological features of chronic lung disease are also observed in SLE patients.

Mononuclear or lymphoplasmacytic cells are infiltrated in the interstitial and peribronchiolar areas, with variable cellularity of NSIP pattern but less prominent fibrosis [1,20,22]. Pulmonary vascular disease can occur in SLE by chronic thromboembolic disease or repeated vasculitis with healing [1]. The features of vascular disease show active vasculitis or chronic fibrous intimal thickening to plexiform lesions [1,2]. UIP, diffuse interstitial fibrosis with NSIP pattern, LIP, or amyloidosis are occasionally reported in the literature [3,5,25].

Histopathological pulmonary patterns in polymyositis and dermatomyositis

Chronic lung changes in PM-DM reveal diffuse interstitial pneumonias with cellular, mixed cellular and fibrotic or pure fibrotic NSIP patterns [1,2,26,27] (Fig. 6A). The OP pattern can
Fig. 4. Lung parenchymal changes in a patient with systemic lupus erythematosus. Histological patterns of organizing diffuse alveolar damage (A) and organizing pneumonia (arrows) (B) are present (hematoxylin and eosin stain, x40 [A] and [B]).

Fig. 5. Pulmonary parenchymal hemorrhage in a patient with systemic lupus erythematosus. (A) Diffuse parenchymal hemorrhage associated with lymphoid aggregates (arrow) and vascular congestion in the fibrotic interstitium. (B) Diffuse and fresh alveolar hemorrhage (arrows) with no distinct capillaritis (hematoxylin and eosin stain, x40 [A] and x100 [B]).
be seen in PM-DM patients treated with steroid treatments [28]. PM-DM shows UIP pattern, and the lesion contains abundant lymphoplasmacytic or mononuclear cell aggregates [2,11,28] (Fig. 6B). Patients with PM-DM occasionally show features related to DAD, which suggests an acute exacerbation of the underlying chronic lung disease and poor prognosis [1,27-29]. Occasionally patients with PM-DM reveal follicular bronchiolitis, LIP, DAH, pleuritis, airway involvement, and vasculitis [30].

**Histopathological pulmonary patterns in Sjögren syndrome**

Sjögren syndrome associated ILDs (SjS-ILD) reveals various features of cellular NSIP pattern, follicular bronchiolitis, lymphoid interstitial hyperplasia, and LIP [1,31]. Common histological patterns of SjS-ILD are NSIP with cellular, fibrotic, or mixed patterns [32]. SjS-LIP shows florid peribronchiolar and interstitial lymphoplasmacytic infiltrates with expansion to the alveolar septa (Fig. 7). The LIP lesion also reveals germinal centers, and occasionally non-necrotizing granulomas [1,33]. LIP is most frequently associated with SjS among CTD-LDs, and can be a precursor lesion of malignant lymphoma. About 25% of LIP patients have SjS [1,31,33]. SjS frequently shows involvement of the large and small airways, and the small airway reveals chronic bronchiolitis and follicular bronchiolitis with bronchiolocentric nodular lymphocytic infiltrates and reactive germinal centers [1,31,33]. CTD-ILDs and pleuritis are rare in SjS. Acute and subacute DAD patterns are seen in acute SjS-ILD exacerbation cases [31].

**Histopathological pulmonary patterns in mixed connective tissue disease**

Histologically, MCTD patients show one or more specific CTD patterns [1,34]. MCTD patients frequently show acute fibrinous or chronic organizing pleuritis [34]. MCTD patients with acute exacerbations of underlying pulmonary disease reveal findings of OP or DAD [1,23]. MCTD patients with fibrotic NSIP or UIP patterns are rarely reported in the literature [35]. MCTD-LD frequently shows pulmonary vascular disease, and the vascular

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Fig. 6. Fibrotic nonspecific interstitial pneumonia pattern in a patient with polymyositis (A). Usual interstitial pneumonia pattern is present in another lesion of the same patient (B). Diffuse interstitial fibrotic thickening associated with honeycombing (asterisk) and scattered lymphoid aggregates (arrows) are present (hematoxylin and eosin stain, ×10 [A] and ×100 [B]).
change is related to fatal pulmonary hypertension. Microscopic examination shows endothelial hyperplasia, intimal fibromyxoid hyperplasia, and medial muscular hypertrophy in the vascular structures of MCTD-LD. Advanced cases of MCTD-LD occasionally show plexiform arteriopathy. Vascular changes are an isolated lung lesion or are associated with interstitial fibrosis.

**Differential diagnosis of connective tissue disease-associated lung diseases**

Histological findings of CTD-ILDs can overlap with pulmonary manifestations of CTDs and various drug treatments which induce histological changes in the underlying lung diseases. Differential diagnosis of CTD-ILDs includes UIP, NSIP, LIP, and OP patterns. Several CTD-ILDs showing these histological patterns should be considered as a differential diagnosis of non CTD-ILDs. Viral infections can show cellular NSIP patterns, resolving phase of bacterial pneumonia can show an OP pattern, and fungal or mycobacterial infections reveal necrotizing granulomas morphologically similar to polyangitis with granulomatosis. Pulmonary toxicity induced by immunosuppressive drugs for CTD can cause lung injury patterns such as OP, cellular NSIP, DAD, and vasculitis. Lung injury caused by inhalation or aspiration of toxic materials can cause bronchiolitis and bronchiectasis. Chronic hypersensitivity pneumonia sometimes requires a differential diagnosis with UIP and NSIP. Lymphoproliferative disorders in SjS-ILD are similar to cellular NSIP and LIP, and should be ruled out if the inflammatory infiltrate is florid. Fibroblastic foci are diagnostic histological findings in idiopathic UIP, but it is absent or rarely seen in CTD-ILDs. On the other hand, histological findings of prominent lymphoplasmacytic infiltrates associated with lymphoid follicles are rare in idiopathic UIP or NSIP.

**Conclusion**

ILDs, large and small airway diseases, pleural and pulmonary vascular changes are common in CTD patients. Histological patterns of UIP, cellular and/or fibrotic NSIP, LIP, DAD, and OP can be seen in the lungs of patients with CTDs (Table 1). Pulmonary changes of CTDs can be caused by the direct involvement of the CTD in the lung by drug reactions from the patient’s medications, or by infections caused by autoimmune...
diseases and their related treatments. Clinical information and laboratory findings of patients with CTDs are important for the interpretation and accurate diagnosis of CTD-LDs in lung biopsy, and a multidisciplinary team with the participation of pathologists, clinicians and radiologists can do an accurate diagnosis of CTD-LDs.

**Conflicts of interest**

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

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Comparison of three different endoscopic approaches in the treatment of bladder calculi

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Background: This study compared the following three endoscopic techniques used to treat bladder stones: transurethral cystoscope used with a pneumatic lithoclast or nephroscope used with a pneumatic lithoclast and nephroscope used with an ultrasonic lithoclast.

Methods: Between January 2013 and May 2016, 107 patients with bladder stones underwent endoscopic treatment. Patients were classified into three groups based on the endoscopic techniques and energy modalities used in each group as: group 1 (transurethral stone removal using a cystoscope with pneumatic lithoclast), group 2 (transurethral stone removal using a nephroscope with pneumatic lithoclast), and group 3 (transurethral stone removal using a nephroscope with ultrasonic lithoclast). Baseline and perioperative data were retrospectively compared between three groups.

Results: No statistically significant intergroup differences were observed in age, sex ratio, and stone size. A statistically significant intergroup difference was observed in the operation time—group 1, 71.3 ± 46.6 min; group 2, 33.0 ± 13.7 min; and group 3, 24.6 ± 8.0 min. All patients showed complete stone clearance. The number of urethral entries was higher in group 1 than in the other groups. Significant complications did not occur in any patient.

Conclusion: Nephroscopy scores over cystoscopy for the removal of bladder stones with respect to operation time. Ultrasonic lithoclast is a safe and efficacious modality that scores over a pneumatic lithoclast with respect to the operation time.

Keywords: Bladder stone; Cystoscopy; Nephroscope

Introduction

Bladder stones are the most common type of lower urinary tract stones, accounting for approximately 5% of all urinary lithiasis [1]. They are classified as primary or secondary varieties. Primary stones are common in children, particularly in those receiving low-protein, low-phosphorous diets (in endemic regions). They are usually solitary and rarely recur after treatment. However, secondary stones are commonly detected in men aged >60 years and are usually associated with urinary stasis secondary to bladder outlet obstruction and a neurogenic bladder among other such etiologies [2].

There are several procedures for the surgical treatment of bladder stones, including open or transurethral surgery, percutaneous procedures, and extracorporeal shock wave lithotripsy (ESWL). The choice of treatment is determined by stone size and composition, stone location, the patient's surgical history, comorbidities, costs, and the availability of equipment [3].

The transurethral approach is commonly used in endoscopic surgery, and various advanced surgical modalities are being used following the development of newer endoscopic and crushing equipment. Although a cystoscope is commonly used,
it has recently been replaced by other endoscopic instruments, including resectoscopes and nephroscopes. Transurethral stone disintegration can be performed using a pneumatic, electrolydraulic, or ultrasonic lithotripter [4-6].

A variety of devices are available, and urologists can choose the optimal modality based on an individualized treatment plan. However, whether a few devices are better than others in terms of operation and recovery time and the rate of complications, among other such considerations remains controversial.

This study compared the following three endoscopic techniques used to treat bladder stones: transurethral cystoscope used with a pneumatic lithoclast or a nephroscope used with a pneumatic lithoclast and nephroscope used with an ultrasonic lithoclast.

Materials and methods

This study included 107 patients who underwent endoscopic surgical treatment for bladder stones at our medical center between January 2013 and May 2016. Patients were randomly classified into three groups based on the endoscopic technique and energy modalities used for treatment.

In group 1 (n=65), all surgeries were performed using a 22-Fr cystoscope with a pneumatic lithoclast. In group 2 (n=21), all surgeries were performed using a 24-Fr nephroscope with a pneumatic lithoclast. In group 3 (n=21), all surgeries were performed using a 24-Fr nephroscope with transurethral placement of an ultrasonic lithoclast. Routine hematological laboratory tests, urinalysis, and radiological imaging including abdominal radiography and computed tomography were performed in all patients for the preoperative diagnosis of bladder stones. The stone burden was estimated by integrating maximum diameters of calculi. All patients received prophylactic antibiotics 24 hours prior to surgery. All surgeries were performed by a single experienced surgeon under general or spinal anesthesia with all patients placed in the lithotomy position.

Intraoperatively, following adequate fragmentation, the stone fragments were removed using an Ellik evacuator. Removal of fragments failed in cases where the size of the stone fragments was larger than the diameter of the endoscopic sheath. In such cases, the stone crushing forceps were introduced through the endoscopic sheath for stone removal. A 16-Fr Foley catheter was inserted after completion of the procedure. Postoperatively, a blood test was performed, and patients were closely monitored for hematuria. The catheter was removed after cessation of hematuria, and patients were discharged thereafter.

Baseline and perioperative data were retrospectively compared between the three groups. Statistical analyses were performed using the Mann–Whitney U test. The SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all analysis. A p-value ≤0.05 was considered statistically significant.

Results

The mean age of patients was 66.7±13.5, 67.9±13.3, and 63.5±13.7 years in groups 1, 2, and 3, respectively (p≤0.622). The mean stone burden in patients was 5,238.2±4,818.4, 4,656.8±2,252.1, and 6,350.7±5,534.1 in groups 1, 2, and 3, respectively (p≤0.472).

Stone fragments were removed completely in all patients. The mean operation time was 71.3±46.6, 33.0±13.7, and 24.6±8.0 min in groups 1, 2, and 3, respectively. A statistically significant intergroup difference was observed in the mean operation time (p<0.001). The mean urethral entries were 2.1±1.7, 1.03±0.12, and 1.05±0.08 in groups 1, 2, and 3, respectively (p≤0.081).

The length of hospitalization (days) was 2.2±0.9, 2.3±0.4, and 2.1±0.3 in groups 1, 2, and 3, respectively (p≤0.594) (Table 1).

No statistically significant intergroup differences were observed in age, sex ratio, and the stone burden. However, a statistically significant intergroup difference was observed in the operation time. All patients showed complete stone clearance. Significant complications did not occur in any patient.

Table 1. Comparison of all the three groups for various variables

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>65</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Male:female</td>
<td>57:8</td>
<td>19:2</td>
<td>18:3</td>
<td>0.726</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66.7±13.5</td>
<td>67.9±13.3</td>
<td>63.5±13.7</td>
<td>0.622</td>
</tr>
<tr>
<td>Stone burden (mm³)</td>
<td>5,238.2±4,818.4</td>
<td>4,656.8±2,252.1</td>
<td>6,350.7±5,534.1</td>
<td>0.472</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>71.3±46.6</td>
<td>33.0±13.7</td>
<td>24.6±8.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean urethral entries</td>
<td>2.1±1.7</td>
<td>1.03±0.12</td>
<td>1.05±0.08</td>
<td>0.081</td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>2.2±0.9</td>
<td>2.3±0.4</td>
<td>2.1±0.3</td>
<td>0.594</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation.
Discussion

The treatment options for bladder stones are diverse and include open and percutaneous surgery, transurethral cystolitholapaxy, lithotripsy, and ESWL [3]. The transurethral approach shows high efficacy and is associated with minimal morbidity and is therefore the most commonly used method [4]. Several modalities for stone fragmentation including mechanical/ballistic, ultrasonic, electrohydraulic, and pneumatic lithotripsy, as well as the holmium laser are used via the transurethral approach [7]. Additionally, various types of endoscopes, including a cystoscope, nephroscope, and resectoscope, among other such devices are used transurethrally. All these modalities are intended to achieve complete stone clearance, a short operation time, short length of hospitalization, and minimal complications [6]. A combination of various fragmenting devices and endoscopes can effectively remove most bladder stones [8]. The choice of treatment method is determined by the size and the number of stones [9]. Several studies have been performed to assess lithotripsy devices. Oktay et al. concluded that pneumatic lithotripsy is an easy, reliable, and cost-effective endoscopic lithotripsy modality. Pneumatic lithotripsy was performed in 92 patients with 98 lower or mid-ureteral stones and in eight patients with bladder stones. Successful stone fragmentation was achieved in 96 patients [10]. Pneumatic lithotripsy is useful for rapid fragmentation of large and hard stones [11] and can be safely performed without injuring the urothelial mucosa [12,13]. Electrohydraulic lithotripsy is useful to treat bladder stones; however, it is associated with a relatively high incidence of bladder injury and mucosal damage to the urinary tract. Bülow and Frohmüller reported 305 consecutive cases of bladder stones treated using electrohydraulic lithotripsy and observed that bladder perforations occurred in five patients [14]. Ultrasonic lithotripsy is an effective and safe modality that offers the advantage of fragmentation with simultaneous evacuation. It shortens the operation time and minimizes urethral mucosal injury [15]. Holmium laser lithotripsy is useful for successful fragmentation of large bladder stones (>4 cm). Additionally, it is associated with a low complication rate with proven efficacy in recent years [16].

The diameter of the endoscopic sheath used during the operation is a topic of interest [7]. Sathaye performed transurethral pneumatic lithotripsy using a nephroscope and a 25-Fr cystoscope sheath in four patients to treat bladder stones measuring >10 cm. All patients showed complete stone clearance without any complications [17]. Ener et al. compared two different techniques (a 24-Fr nephroscope and a 22-Fr cystoscope) used to treat large bladder stones and concluded that removal of large bladder stones is achieved more rapidly and effectively with a transurethrally inserted nephroscope than with a cystoscope [6]. Kawahara et al. reported the usefulness of a 30-Fr Amplatz sheath with holmium laser in three women who underwent cystolithotripsy. All three women were successfully treated without postoperative complications [18].

In this study, we compared three different combinations of endoscopic modalities: group 1 (cystoscope/pneumatic lithoclast), group 2 (nephroscope/pneumatic lithoclast), and group 3 (nephroscope/ultrasonic lithoclast). Removal of fragmented stones is easier with a nephroscope than with a cystoscope because of its larger diameter. Cystoscopic removal of large fragmented stones involves drawing out the cystoscope with the stone via the urethral meatus. This procedure and the subsequent reinsertion of the cystoscope may cause urethral mucosal injury. Additionally, the operation time is longer. Ultrasonic lithotripsy enables simultaneous aspiration of fragmented stones. It offers a better endoscopic view, which shortens the time required for evacuation of the stone fragments. We observed that combined modality therapy using a nephroscope/ultrasonic lithoclast is a rapid and more effective intervention than other modalities. Limitations of this study include the retrospective data assessment and lack of follow-up data regarding postoperative complications such as the development of urethral strictures.

A previous study has compared different endoscopic modalities similar to our analysis. A study reported by Singh and Kaur compared a transurethral approach using a 24-Fr nephroscope and a 22-Fr cystoscope and a percutaneous approach using a 24-Fr nephroscope. The nephroscope offered a better view and additionally facilitated emptying of the overdistended bladder through the inlet port. The percutaneous approach offered a better view, and prolonged instrumentation of the urethra was avoided. However, placement of the suprapubic catheter prolonged the length of postoperative hospitalization [19].

Studies reporting newer techniques are reported in recent years. Ali et al. performed transurethral pneumatic cystolithotripsy with a semirigid ureteroscope in 53 patients with bladder stones. Complete stone clearance was achieved in all patients without any surgical complications. Patients underwent follow-up for 18 months postoperatively, without urethral stricture reported in any patient. Thus, the authors concluded that this technique is safe and effective [20].

Transurethral removal of bladder stones using a nephroscope is an effective procedure that additionally shortens the operation time compared with a cystoscopy. Ultrasonic lithoclast is a safe and efficacious energy modality that shortens the operation time.
compared with a pneumatic lithoclast.

**Conflicts of interest**

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

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Awareness of occupational hazards and personal protective equipment use among dental hygienists

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Introduction

Dental clinics and hospitals are exposed to various harmful substances depending on the nature of treatment. Many medical materials used in dental offices can cause abnormal and hypersensitivity reactions [1]. The risk of damage to hearing and vision is high, owing to harmful physical factors such as loud noises, radiation, and ultraviolet light [1].

Dental hygienists (DHs) in dental clinics and hospitals are exposed to various harmful physical and chemical elements during conservation and reinforcement work, such as calculus removal, diagnostic mouth radiography, impression acquisition, and production of temporary crowns. Hazardous exposures can lead to musculoskeletal disorders, eye damage, vibration-induced neuropathy, hearing loss, and psychological stress [2-4]. Specifically, there are risks of eye damage due to direct blue light irradiation during resin preparation [5]. Harm may arise when chemicals are inhaled during prosthetic dentistry [6]. In addition, methyl methacrylate, which is used in dental cement, can induce mucous irritation, allergic reactions, hypersensitivity, asthma reactions, nerve symptoms, and skin diseases, among others [7]. Jang [8] reported that 46.5% of respondents complained of coughing due to alginate or stone dust inhalation and 61.1% of experienced respondents complained of coughing. Lee et al. [9]
reported that noises from ultrasonic scalers, high- and low-speed handpieces, and dental suction affect the hearing of DHs.

Most existing studies have focused their investigations on either exposure to infectious diseases and its awareness, use of protective gear to prevent infection [10-12], or symptoms of musculoskeletal diseases [13-17].

Despite the known physical and chemical hazards of dental care environments, available research on DHs’ awareness about, management of, and protection against these harmful environments is insufficient. DHs’ work, like other hazardous tasks, requires personal protective equipment but the level of awareness of the hazardous work environment in dental offices is still low [18].

This study was conducted to identify DHs’ awareness of the physically and chemically hazardous working conditions associated with dental care and to understand the need for appropriate protective equipment.

Materials and methods

The study participants were DHs working in 6 dental hospitals and 35 dental clinics in Daegu and Gyeongsangbuk-do provinces. Those who performed reception duty or pregnant, were excluded. The dental hospitals and clinics were selected through convenient sampling. We obtained consent after providing an explanation of the study to the DHs and the corresponding dental hospitals and clinics.

Data collection was conducted between April 1, 2017 and April 20, 2017 in the form of self-administered questionnaires. A total of 280 questionnaires were collected. After excluding 9 owing to unreliable responses, 271 were included in the final dataset. This study was approved by the institutional review board of the institution to which the researcher is affiliated (YU 2017-02-007-003).

Regarding general characteristics, demographic factors such as gender, age, and educational level, and work-related factors such as the type of working institution, as well as total duration of employment (in years), were investigated.

Work environment-related survey items were constructed based on a study by Choi [19], which adapted the Air Quality and Work Environment Symptom Survey developed by the National Institute for Occupational Safety and Health, to fit the Korean context. Work environment is an item that examines the frequency of work in dental hospitals and clinics that can prove harmful, including tasks that involve dust, volatile substances, noises, and light-curing units.

In order to identify the management characteristics of hazardous substances during dental work, items from a previous study by Kim and Choi [18] were used. Survey content included whether instructions are read before using dental materials, recognition of Material Safety Data Sheet (MSDS), DHs’ knowledge about the potential harmfulness of dental materials, and awareness regarding the need for education.

The collected data were analyzed using IBM SPSS version 22.0 (IBM Co., Armonk, NY, USA) to compute frequencies and percentages of participants’ general characteristics, awareness of health hazards in dental work, and the use of personal protective equipment.

Results

The most common age group was under 25 with 133 participants (49.1%), followed by 26-30 with 76 participants (28.0%) and 31 or older with 62 participants (22.9%). There were 227 college graduates (83.8%) and 44 university graduates or above (16.2%).

A total of 144 participants (53.1%) were from dental hospitals and 127 (46.9%) were from dental clinics. Total career duration was 5 years or less for 168 participants (62.0%), followed by 6 to 10 years for 55 participants (20.3%), and more than 11 years for 48 participants (17.7%).

Regarding the average number of patients seen per day, 94 participants (34.7%) saw more than 51 patients, 89 (32.8%) saw fewer than 30, and 88 (32.5%) saw between 31 and 50. The use of eyewear among participants was considered: 137 participants (50.6%) wore neither, 62 (22.9%) wore contact lenses, 38 (14.0%) wore glasses, and 34 (12.5%) wore both (Table 1).

Among those involved in physically or chemically harmful work, 255 participants (94.1%) performed work involving dust, 233 (86.0%) volatile substances, 263 (97.0%) noises, and 262 (96.7%) light-curing units.

For work involving dust, 234 participants (91.8%) were engaged in alginate mixing, 202 (79.2%) in prosthodontics polishing and restoration, 202 (79.2%) in tooth preparation, and 164 (64.3%) in plaster mixing.

For the types of volatile substances used, 194 participants (83.2%) used disinfectants, 166 (71.2%) resin monomers, 120 (51.5%) formocresol, and 111 (47.6%) zinc oxide eugenol.

For the types of noises, 256 participants (97.3%) used suction and air, 248 (94.3%) operated with dental hand pieces, 119 (45.2%) were exposed to the cries of young patients during treatments, and 62 (23.6%) performed plaster trimming (Table 2).

Based on multiple responses, participants considered the following as occupational hazards: inhalation of volatile substances at work (140; 51.7%); work involving dust (115;
Participants (31.0%) responded that they wear protective goggles. Reasons for not wearing protective goggles included: "being busy" (62; 41.2%), "no protective equipment" (54; 36.0%), "not necessary" (18; 12.0%), and "no protective equipment use guidelines" (14; 10.0%) (Table 4).

One hundred and thirty-eight participants (50.9%) read the instructions before using dental material. The dental MSDS was recognized by 62 (22.9%). Fifty-five participants (20.3%) had some experience of education regarding occupational hazards. Of those who had not received any education on occupational hazards, 178 participants (82.4%) intended to gain this knowledge if given the opportunity (Table 5).

Values are based on multiple responses.

Table 2. Frequency of hazardous dental works the subjects involved (n=271)

<table>
<thead>
<tr>
<th>Hazardous dental work</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work making dust</td>
<td>255 (94.1)</td>
</tr>
<tr>
<td>Type of work making dust</td>
<td></td>
</tr>
<tr>
<td>Alginate mixing</td>
<td>234 (91.8)</td>
</tr>
<tr>
<td>Prosthodontics polishing and restoration</td>
<td>202 (79.2)</td>
</tr>
<tr>
<td>Tooth preparation</td>
<td>202 (79.2)</td>
</tr>
<tr>
<td>Plaster mixing</td>
<td>164 (64.3)</td>
</tr>
<tr>
<td>Others</td>
<td>17 (6.6)</td>
</tr>
<tr>
<td>Work using volatile substance</td>
<td>233 (86.0)</td>
</tr>
<tr>
<td>Type of volatile substance used</td>
<td></td>
</tr>
<tr>
<td>Disinfectant</td>
<td>194 (83.2)</td>
</tr>
<tr>
<td>Resin monomer</td>
<td>166 (71.2)</td>
</tr>
<tr>
<td>Formocresol</td>
<td>120 (51.5)</td>
</tr>
<tr>
<td>Zinc oxide eugenol</td>
<td>111 (47.6)</td>
</tr>
<tr>
<td>Others</td>
<td>21 (9.0)</td>
</tr>
<tr>
<td>Work making noise</td>
<td>263 (97.0)</td>
</tr>
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<td>Type of noise made</td>
<td></td>
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</tr>
<tr>
<td>Plaster trimming</td>
<td>62 (23.6)</td>
</tr>
<tr>
<td>Others</td>
<td>6 (2.3)</td>
</tr>
<tr>
<td>Work using light curing unit</td>
<td>262 (96.7)</td>
</tr>
</tbody>
</table>

Table 3. Dental works perceived to be hazardous by the subjects (n=271)

<table>
<thead>
<tr>
<th>Dental work</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work making dust</td>
<td>115 (42.4)</td>
</tr>
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<td>Work using volatile substance</td>
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<td>25 (9.2)</td>
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<tr>
<td>Work using light curing unit</td>
<td>85 (31.4)</td>
</tr>
</tbody>
</table>

None of the participants used ear plugs, which should be worn while performing work involving noises. Reasons for not wearing ear plugs included: "no protective equipment" (122; 56.3%), "not necessary" (38; 17.9%), "being busy" (37; 17.0%), and "no protective equipment use guidelines" (15; 6.9%).

When performing work involving light-curing units, 62 participants (31.0%) responded that they wear protective goggles. Reasons for not wearing protective goggles included: “being busy” (62; 41.2%), “no protective equipment” (54; 36.0%), “not necessary” (18; 12.0%), and “no protective equipment use guidelines” (14; 10.0%) (Table 4).

One hundred and thirty-eight participants (50.9%) read the instructions before using dental material. The dental MSDS was recognized by 62 (22.9%). Fifty-five participants (20.3%) had some experience of education regarding occupational hazards. Of those who had not received any education on occupational hazards, 178 participants (82.4%) intended to gain this knowledge if given the opportunity (Table 5).

Table 1. General characteristic of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
</tr>
<tr>
<td>≤25</td>
<td>133 (49.1)</td>
</tr>
<tr>
<td>26–30</td>
<td>76 (28.0)</td>
</tr>
<tr>
<td>31 s</td>
<td>62 (22.9)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
</tr>
<tr>
<td>College graduate</td>
<td>227 (83.8)</td>
</tr>
<tr>
<td>University or above</td>
<td>44 (16.2)</td>
</tr>
<tr>
<td>Type of working institution</td>
<td></td>
</tr>
<tr>
<td>Dental clinic</td>
<td>127 (46.9)</td>
</tr>
<tr>
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<td>144 (53.1)</td>
</tr>
<tr>
<td>Total job carrier (yr)</td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>168 (62.0)</td>
</tr>
<tr>
<td>6–10</td>
<td>55 (20.3)</td>
</tr>
<tr>
<td>11 s</td>
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</tr>
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</tr>
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</tr>
<tr>
<td>Glasses or contact lens use</td>
<td></td>
</tr>
<tr>
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<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

Table 2. Frequency of hazardous dental works the subjects involved (n=271)

<table>
<thead>
<tr>
<th>Hazardous dental work</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work making dust</td>
<td>255 (94.1)</td>
</tr>
<tr>
<td>Type of work making dust</td>
<td></td>
</tr>
<tr>
<td>Alginate mixing</td>
<td>234 (91.8)</td>
</tr>
<tr>
<td>Prosthodontics polishing and restoration</td>
<td>202 (79.2)</td>
</tr>
<tr>
<td>Tooth preparation</td>
<td>202 (79.2)</td>
</tr>
<tr>
<td>Plaster mixing</td>
<td>164 (64.3)</td>
</tr>
<tr>
<td>Others</td>
<td>17 (6.6)</td>
</tr>
<tr>
<td>Work using volatile substance</td>
<td>233 (86.0)</td>
</tr>
<tr>
<td>Type of volatile substance used</td>
<td></td>
</tr>
<tr>
<td>Disinfectant</td>
<td>194 (83.2)</td>
</tr>
<tr>
<td>Resin monomer</td>
<td>166 (71.2)</td>
</tr>
<tr>
<td>Formocresol</td>
<td>120 (51.5)</td>
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</tbody>
</table>

Values are based on multiple responses.

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</tr>
<tr>
<td>Total</td>
<td>271 (100.0)</td>
</tr>
</tbody>
</table>

42.4%); working with specialized equipment such as light-curing units (85; 31.4%); and work involving noises (25; 9.2%) (Table 3).

Four participants (1.1%) responded that they wear dust-proof masks when performing work involving dust. Reasons for not wearing dust-proof masks included “being busy” (47; 52.3%), “not necessary” (20; 22.3%), “no protective equipment use guidelines” (13; 14.4%), and “no protective equipment available” (4; 4.4%).

Two participants (0.7%) responded that they use gas-proof masks and 84 (31.2%) responded that they use gloves when performing work using volatile substances. Reasons for not wearing protective equipment when working with volatile substances included: “being busy” (31; 42.5%), “not necessary” (17; 23.3%), and “no protective equipment use guidelines” (16; 21.9%).

None of the participants used ear plugs, which should be worn while performing work involving noises. Reasons for not wearing ear plugs included: “no protective equipment” (122; 56.3%), “not necessary” (38; 17.9%), “being busy” (37; 17.0%), and “no protective equipment use guidelines” (15; 6.9%).

When performing work involving light-curing units, 62 participants (31.0%) responded that they wear protective goggles. Reasons for not wearing protective goggles included: “being busy” (62; 41.2%), “no protective equipment” (54; 36.0%), “not necessary” (18; 12.0%), and “no protective equipment use guidelines” (14; 10.0%) (Table 4).

One hundred and thirty-eight participants (50.9%) read the instructions before using dental material. The dental MSDS was recognized by 62 (22.9%). Fifty-five participants (20.3%) had some experience of education regarding occupational hazards. Of those who had not received any education on occupational hazards, 178 participants (82.4%) intended to gain this knowledge if given the opportunity (Table 5).
Discussion

Owing to the diversification and specialization of dental care, DHs must perform various tasks in prosthetics, preservation, corrective care, and preventive care [20]. In the course of their work, DHs are consistently exposed to physically and chemically harmful environments [18]. This study was conducted to identify DHs’ exposure to hazardous work environments and to examine their use of personal protective equipment.

The frequencies of physically and chemically harmful work involving dust, volatile substances, noises, and light-curing units were 94.1%, 86.0%, 97.0%, and 96.7%, respectively. In contrast, the degree of perceived harm were in the following order: work involving volatile substances that induce inhalation (51.7%); work involving dust (42.4%); work using specialized equipment such as light-curing units (31.4%); and work involving noise (9.2%). Hazard perception was lower than the hazardous work factors to which DHs are exposed to in the dental setting. In particular, the recognition of noise hazards was very low.

Among the participants, 1.1% responded that they wore dust-proof masks while doing work involving dust. While 0.7% and 31.2% responded that they wore gas-proof masks and protective gloves, respectively, while doing work with volatile substances. None of the participants wore ear plugs while doing work involving noises. Moreover, only 31.0% wore protective goggles while doing work using light-curing units. Although “being busy” and “no protective gear” were common reasons for not using appropriate personal protective equipment, the fundamental reason relates to low awareness of work-related hazards.

None of the DHs wore earplugs. This can be attributed to low awareness of the dangers of noises in dental hospitals and clinics. In terms of workplace noises, the working environment standard for DHs is much lower than the 8-hour average of 90 dB prescribed by the Industrial Safety and Health Act. However, Kim et al. [21] reported that noises produced in dental offices may lead to noise-induced deafness. Park and Kim [22] also reported that noises in dental settings affect the hearing and job performance of DHs.

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Our study showed that only 22.9% of the participants were familiar with the MSDS, and only 20.3% were educated about the harmfulness of dental materials. These findings suggest that there is a lack of awareness about occupational hazards in the dental work environment. Contrarily, Jeong et al. [23] reported that while dental workers know about the need for basic protection, the compliance rate is low because of reasons such as increased

Table 4. Frequency of the subjects use protective equipment according to hazardous dental works and reasons for not use

<table>
<thead>
<tr>
<th>Item</th>
<th>Work making dust</th>
<th>Work using volatile substance</th>
<th>Work making noise</th>
<th>Work using light curing unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of protective equipment use&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mask (gas/dust proof)</td>
<td>4 (1.1)</td>
<td>2 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Goggles</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>62 (31.0)</td>
</tr>
<tr>
<td>Ear plug</td>
<td>-</td>
<td>-</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Gloves</td>
<td>-</td>
<td>84 (31.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reason for not use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Being busy</td>
<td>47 (52.3)</td>
<td>31 (42.5)</td>
<td>37 (17.0)</td>
<td>62 (41.2)</td>
</tr>
<tr>
<td>Not necessary</td>
<td>20 (22.3)</td>
<td>17 (23.3)</td>
<td>38 (17.9)</td>
<td>18 (12.0)</td>
</tr>
<tr>
<td>No protective equipment</td>
<td>4 (4.4)</td>
<td>5 (6.8)</td>
<td>122 (56.3)</td>
<td>54 (36.0)</td>
</tr>
<tr>
<td>No protective equipment use guideline</td>
<td>13 (14.4)</td>
<td>16 (21.9)</td>
<td>15 (6.9)</td>
<td>14 (10.0)</td>
</tr>
<tr>
<td>Others</td>
<td>2 (2.2)</td>
<td>4 (5.5)</td>
<td>4 (1.9)</td>
<td>1 (0.7)</td>
</tr>
</tbody>
</table>

Table 5. Characteristics related to management of hazardous dental work condition

<table>
<thead>
<tr>
<th>Item</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read the instruction before using dental material</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>138 (50.9)</td>
</tr>
<tr>
<td>No</td>
<td>133 (49.1)</td>
</tr>
<tr>
<td>Recognition of MSDS</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62 (22.9)</td>
</tr>
<tr>
<td>No</td>
<td>209 (77.1)</td>
</tr>
<tr>
<td>Education experience on harmfulness of dental material</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>55 (20.3)</td>
</tr>
<tr>
<td>No</td>
<td>216 (79.7)</td>
</tr>
<tr>
<td>Intention to get education on harmfulness of dental material&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>178 (82.4)</td>
</tr>
<tr>
<td>No</td>
<td>38 (17.6)</td>
</tr>
</tbody>
</table>

MSDS, material safety data sheet.
<sup>a</sup>The respondents are 216 subjects who answered not having education experience.
cost of care, cumbersomeness during procedures, and low awareness about cross-contamination risks.

The Industrial Safety and Health Act of Korea [24] stipulates the responsibility of the government and accountability of the employer to maintain and promote workers' safety and health by preventing industrial accidents and creating a pleasant working environment. This minimum requirement aims to prevent occupational health injuries. Such measures are important and should be thoroughly complied with. In addition, efforts to establish and implement more stringent standards should be supported.

The best way to prevent occupational health injuries is to transform the working environment into one that causes no harm. However, changing the duties or hazardous factors involved in DHs’ work is difficult. Therefore, it is important to use personal protective equipment while working. Ryu [25] reported that security goggles and facial protection pads should be used for eye and face protection against various physical and chemical hazards in the process of providing care. Also, it is recommended that DHs wear safety goggles because ultraviolet rays from the use of light-curing units in the examination room and dental laboratory can affect the eyes [19]. However, a study by Hwang [26] found that although the rate of using protective gloves and masks was high, the rate of using safety goggles was inadequate. Wearing personal protective gear is important as the dental setting increases aerosol risks to the membranes of the eyes, nose, or lips. Aerosols are difficult to identify with the naked eye, and there is a possibility of cross-contamination between patients and workers through invasive treatment. Supplementing policies relating to the use of personal protective equipment and continued education are needed. It was reported that DHs are aware of the need for noise reduction in dentistry but do not receive noise prevention training [22].

A limitation of this study is that it is difficult to generalize the results as it considered DHs only in Daegu and Gyeongsangbuk-do provinces. Moreover, the study is also limited in that relationships between harmful factors and the induced symptoms have not been considered. In terms of hazardous factors, the perception of the study participants may differ from the actual working environment. Owing to the low rate of protective equipment use, the relationship between work characteristics of the subjects and the perceived degree of harmfulness could not be analyzed. Since all the subjects were female, gender differences in the level of safety and health awareness also could not be analyzed. Additionally, differences in safety and health awareness and participants’ work hours or the time of exposure to hazardous factors were not examined.

The results of the study showed a lack of awareness of the intricacies of working with hazardous factors and a low rate of protective equipment utilization among participants. Training DHs about work-related hazardous risks and continued management of workplace health and safety are necessary.

**Conflicts of interest**

No potential conflicts of interest relevant to this article were reported.

**ORCID**

Hyun-Ju Choi, https://orcid.org/0000-0001-5770-367X
Tae-Yoon Hwang, https://orcid.org/0000-0001-9397-5314
Man-Joong Jeon, https://orcid.org/0000-0001-8255-6202

**References**

Telmisartan increases hepatic glucose production via protein kinase C ζ-dependent insulin receptor substrate-1 phosphorylation in HepG2 cells and mouse liver

Kae Won Cho1, Du-Hyong Cho2

1Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan, Korea
2Department of Pharmacology, Yeungnam University College of Medicine, Daegu, Korea

Background: Dysregulation of hepatic glucose production (HGP) contributes to the development of type 2 diabetes mellitus. Telmisartan, an angiotensin II type 1 receptor blocker (ARB), has various ancillary effects in addition to common blood pressure-lowering effects. The effects and mechanism of telmisartan on HGP have not been fully elucidated and, therefore, we investigated these phenomena in hyperglycemic HepG2 cells and high-fat diet (HFD)-fed mice.

Methods: Glucose production and glucose uptake were measured in HepG2 cells. Expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase α (G6Pase-α), and phosphorylation levels of insulin receptor substrate-1 (IRS-1) and protein kinase C ζ (PKCζ) were assessed by western blot analysis. Animal studies were performed using HFD-fed mice.

Results: Telmisartan dose-dependently increased HGP, and PEPCK expression was minimally increased at a 40 μM concentration without a change in G6Pase-α expression. In contrast, telmisartan increased phosphorylation of IRS-1 at Ser302 (p-IRS-1-Ser302) and decreased p-IRS-1-Tyr632 dose-dependently. Telmisartan dose-dependently increased p-PKCζ-Thr410 which is known to reduce insulin action by inducing IRS-1 serine phosphorylation. Ectopic expression of dominant-negative PKCζ significantly attenuated telmisartan-induced HGP and p-IRS-1-Tyr632. Among ARBs, including losartan and fimasartan, only telmisartan changed IRS-1 phosphorylation and pretreatment with GW9662, a specific and irreversible peroxisome proliferator-activated receptor γ (PPARγ) antagonist, did not alter this effect. Finally, in the livers from HFD-fed mice, telmisartan increased p-IRS-1-Ser302 and decreased p-IRS-1-Tyr632, which was accompanied by an increase in p-PKCζ-Thr410.

Conclusion: These results suggest that telmisartan increases HGP by inducing p-PKCζ-Thr410 that increases p-IRS-1-Ser302 and decreases p-IRS-1-Tyr632 in a PPARγ-independent manner.

Keywords: Hepatic glucose production; IRS-1; Phosphorylation; PKCζ; Telmisartan

Introduction

The anabolic hormone insulin promotes not only glucose uptake and utilization in skeletal muscles and adipose tissues but also represses glycogenolysis and gluconeogenesis in the liver that is a central organ for gluconeogenesis in the body (approximately 90% of endogenous glucose production) [1,2]. Thus, hepatic dysregulation of gluconeogenesis is considered to contribute to the development of insulin resistance and type 2 diabetes mellitus [2,3].

Once insulin binds to the insulin receptor (IR), the IR is autophosphorylated and activated, which mediates tyrosine
phosphorylation of various downstream molecules including the insulin receptor substrate (IRS) [3,4]. Although at least six IRS isoforms (IRS-1 to -6) have been identified [4], IRS-1 has been revealed to play an important role in mediating insulin action [5,6]. In addition to its regulation by gene expression, IRS-1 activity is largely regulated by its extensive phosphorylation [4,5].

Tyrosine phosphorylation of IRS-1 is generally stimulatory (i.e., Tyr632 and Tyr896), whereas serine phosphorylation is mainly inhibitory (i.e., Ser302, Ser307, and Ser1101) [4,5]. Numerous kinases such as phosphatidylinositol 3-kinase (PI3K), Akt, protein kinase C (PKC), mammalian target of rapamycin, p60 S6 kinase, and glycogen synthase kinase-3β (GSK3β) have been identified as responsible kinases for IRS-1 serine phosphorylation [5,7]. Of these, PKC-mediated serine phosphorylation of IRS-1 is widely reported to negatively regulate insulin signaling, which consequently contributes to the development of insulin resistance [7].

Telmisartan belongs to the class of angiotensin II type 1 receptor blockers (ARBs) and is widely used for the treatment of patients with hypertension concomitant diabetes mellitus [8]. Unlike other ARBs, telmisartan exhibits various ancillary effects including improvement of insulin resistance and cardiometabolic profile [9-11]. Telmisartan is reported to improve insulin resistance in skeletal muscles via peroxisome proliferated-activated receptor δ (PPARδ) [12], and adipose tissues by elevating adiponectin and reducing pro-inflammatory cytokines [13]. However, the effects of telmisartan on hepatic glucose production (HGP) and its underlying mechanism have not been fully elucidated. In this study, we investigated these effects, including molecularly, using hyperglycemic HepG2 cells and high-fat diet (HFD)-fed mice, and demonstrated that telmisartan increased HGP by inducing p-PKCζ-Thr410, which increased p-IRS-1-Ser302 and decreased p-IRS-1-Tyr632 in a PPARγ-independent manner and, consequently, impaired insulin action in HepG2 cells and the mouse liver.

Materials and methods

Telmisartan and losartan were purchased from Cayman Chemical (Ann Arbor, MI, USA). Fimasartan was a kind gift from Boryung Pharmaceutical (Seoul, Korea). D-Glucose, GW9662, sodium lactate, HEPES, dimethyl sulfoxide (DMSO), and bovine insulin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against Akt, p-Akt-Ser473, GSK3β, p-IRS-1-Ser302, p-IRS-Ser307, p-IRS-Ser311, p-IRS-Tyr632, p-IRS-Tyr632, IRS-1, and actin were purchased from Cell Signaling Technology (Boston, MA, USA). Antibodies against glucose-6-phosphatase a (G6Pase-a), phosphoenolpyruvate carboxykinase (PEPCK), and PKCζ, p-PKCζ-Thr410 were purchased from Santa Cruz Biotechnology (La Jolla, CA, USA). Antibodies against p-GSK3β-Ser9 and hemagglutinin (HA) were obtained from BD Biosciences (San Jose, CA, USA) and Covance Inc. (Emeryville, CA, USA), respectively. Low or high glucose Dulbecco’s modified Eagle’s medium (DMEM) and Dulbecco’s phosphate-buffered saline were purchased from Welgene Inc. (Gyeongsan, Korea). Sodium pyruvate, fetal bovine serum (FBS), penicillin and streptomycin antibiotics, L-glutamine, and trypsin-EDTA solution were purchased from Gibco-BRL (Gaithersburg, MD, USA). Plasticware for cell culture was purchased from Corning Inc. (Oneonta, NY, USA) or SPL Life Sciences (Pocheon, Korea). All other chemicals were of the purest analytical grade.

1. Cell culture and drug treatment

The human HepG2 hepatoma cell line was a generous gift from Prof. Jiyeon Kim (Eulji University, Daejeon, Korea). HepG2 cells were maintained in high glucose (25 mM) DMEM supplemented with 10% FBS at 37°C in an atmosphere of 5% CO₂ in air and the cells were passaged every 3 days by trypsinization. For the experiments, HepG2 cells grown to 70% confluence were incubated in serum-free normal glucose (5.5 mM) DMEM in a six-well plate overnight. After further culture in serum-free normal or high glucose DMEM containing various concentrations of telmisartan for 24 h, the cells were cotreated with 100 nM insulin for a further 10 min. In some experiments, HepG2 cells were cotreated with the indicated drugs or chemicals for the specified time.

2. Dominant negative (dn)-PKCζ gene transfection

The HA-tagged rat dn-PKCζ construct (K281R) was transfected into HepG2 cells as described in our previous report [14,15] with minor modifications. Briefly, HepG2 cells were seeded in a six-well plate as described above and transfected with 1.5 μg pcDNA3.1 vector containing dn-PKCζ cDNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. For the control, equal amounts of the pcDNA3.1 vector were transfected. After incubation for 5 h at 37°C, the cells were further incubated in normal glucose DMEM containing 10% FBS overnight before use for further experiments.

3. Western blot analysis

For the western blot analysis, cells treated with telmisartan in the absence or presence of various chemicals were lysed in lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM...
EDTA, 1 mM EGTA, 1 mM PMSF, 10 mM β-glycerophosphate, 1 mM NaF, 1 mM Na3VO4, and 1× protease inhibitor cocktail [Roche Molecular Biochemicals, Indianapolis, IN, USA]]. In addition to HepG2 cells, the mouse livers used were dissected, and the proteins were extracted by chopping the tissue using iris scissors in ice-cold lysis buffer. The protein concentration was measured using a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Equal quantities of protein (20 μg) were electrophoretically separated on a sodium dodecyl sulfate-polyacrylamide gel and then transferred onto a nitrocellulose membrane (GE Healthcare Life Sciences, Pittsburgh, PA, USA). The blots were then probed with appropriate antibodies, followed by the corresponding secondary antibodies (Invitrogen), and finally developed using ECL reagents (Amersham Biosciences, Arlington Heights, IL, USA).

4. Glucose uptake assay
For glucose uptake measurements, HepG2 cells grown to 70% confluence in six-well plates were serum starved overnight and then incubated in serum-free high glucose DMEM containing various concentrations of telmisartan for 24 h. The medium was replaced with Krebs-Ringer-phosphate-HEPES (KRPH) buffer (10 mM HEPES, pH 7.4, 136 mM NaCl, 4.7 KCl, 5 mM KH2PO4, 1 mM MgSO4, 1 mM CaCl2, and 2% bovine serum albumin) for glucose starvation for 40 min. The cells were treated with 100 nM insulin for 10 min, and then 10 mM 2-deoxyglucose was added to the medium for an additional 20 min. Cell lysates were obtained, and a colorimetric glucose uptake assay was performed using a glucose uptake assay kit (Abcam, Cambridge, UK) according to the manufacturer’s instructions. The uptake of glucose was normalized to the total protein content determined from the whole-cell lysates.

5. Glucose production assay
For the glucose production assay, HepG2 cells grown to 70% confluence were serum starved overnight, and the cells were further incubated in serum-free normal or high glucose DMEM containing various concentrations of telmisartan for 24 h. The medium was then replaced with glucose production buffer containing 2 mM sodium pyruvate, 20 mM sodium lactate, and 100 nM insulin for 4 h. The glucose production buffer was collected, and glucose concentration was measured using a colorimetric glucose assay kit (Cayman, Ann Arbor, MI, USA) according to the manufacturer’s instructions. The glucose amounts were normalized to the total protein content determined from the whole-cell lysates.

6. Animal studies
Male C57BL/6 mice were purchased from Orient Bio Inc. (Seoul, Korea) and housed in a temperature-controlled facility with a 12-h light–dark cycle. The mice were provided ad libitum access to normal chow and water until 6 weeks of age, and then they were started on an HFD (D12492, 60% fat kcal; Research Diets Inc., New Brunswick, NJ, USA) for 13 weeks, followed by randomization to either the vehicle (DMSO)-treated (n=5) or telmisartan-treated (n=6; 5 mg·kg body weight⁻¹·day⁻¹) group. Animals were treated by oral gavage and fed the HFD for an additional 5 weeks. The mice were euthanized and their livers were dissected. All the experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the Soonchunhyang University (Approval no. SCH16-0002).

7. Statistical analysis
All results are expressed as the means±standard deviation (SD). Significant differences were identified using the Student’s t-test for two points. All differences were considered significant at p<0.05.

Results

1. Telmisartan increases glucose production by increasing p-IRS-1-Ser302 and decreasing p-IRS-1-Tyr632 in hyperglycemic HepG2 cells
We first examined the effect of telmisartan on glucose production in normoglycemic or hyperglycemic HepG2 cells. In the presence of insulin treatment, telmisartan dose-dependently increased glucose production in hyperglycemic but not normoglycemic HepG2 cells (Fig. 1A). Therefore, all subsequent experiments were performed under hyperglycemic conditions. Next, we assessed the expression of G6Pase-a and PEPCK that are critical enzymes for hepatic gluconeogenesis. As shown in Fig. 1B, telmisartan at 40 μM slightly increased PEPCK expression without dose-dependency, while G6Pase-a expression was not altered by telmisartan. Although hepatic glucose uptake is considered to be unchanged by insulin [3], glucose-induced nitric oxide has been reported to stimulate Glut4 synthesis and glucose uptake in hepatocytes [16]. Therefore, we determined whether telmisartan increased glucose uptake under our experimental conditions to rule out the possibility that glucose production may be affected by increased glucose uptake. Telmisartan at 40 μM decreased glucose uptake and Glut4 expression but not Glut2 (Supplementary Figs. 1A, 1B), indicating that telmisartan-induced glucose production was largely caused by enhanced hepatic gluconeogenesis rather than glucose uptake. Our results
Fig. 1. Telmisartan increases glucose production by increasing p-IRS-1-Ser\textsuperscript{302} and decreasing p-IRS-1-Tyr\textsuperscript{632} in hyperglycemic HepG2 cells. (A) HepG2 cells were treated with various doses of telmisartan (0, 10, 20, or 40 μM) for 24 h in the presence of 5.5 mM or 25 mM D-glucose and then glucose production assay was performed as described in the Methods. (B−D) HepG2 cells were treated with various doses of telmisartan (0, 10, 20, or 40 μM) for 24 h in the presence of 25 mM D-glucose and then with or without 100 nM insulin for an additional 10 min. Total proteins were obtained, and levels of PEPCK, G6Pase-α, p-IRS-1-Ser\textsuperscript{302}, p-IRS-1-Ser\textsuperscript{318}, p-IRS1-Ser\textsuperscript{1101}, p-IRS-Tyr\textsuperscript{632}, and p-IRS-Tyr\textsuperscript{896} were detected using western blot analysis. Nitrocellulose membranes were reprobed using antibody to detect actin or total IRS-1 to monitor equal sample loading. Densitometry was used to quantify PEPCK, G6Pase-α, p-IRS-1-Ser\textsuperscript{302}, and p-IRS-Tyr\textsuperscript{632} relative to the actin or the IRS-1 bands, respectively. Blots represent at least four experiments. Bar graphs show mean fold alterations above/below control (±SD). Differences were statistically significant at a) p<0.05, b) p<0.01. IRS-1, insulin receptor substrate-1; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase-α, glucose-6-phosphatase α.

showed a concentration-dependent telmisartan-induced increase in glucose production but not PEPCK expression (Figs. 1A, 1B), and a previous study reported that PEPCK expression does not exclusively contribute to hepatic gluconeogenesis [17]. These observations prompted us to investigate the molecular mechanism of the telmisartan-induced HGP by first examining phosphorylation states of Akt and GSK3β, because the increased phosphorylation of the proteins mediates insulin action [18]. Unexpectedly, telmisartan dose-dependently increased p-Akt-Ser\textsuperscript{473} and p-GSK3β-Ser\textsuperscript{9} (Supplementary Fig. 2), with insulin treatment. In an attempt to identify the signaling pathway mediating the effects of telmisartan, we examined the phosphorylation status of IRS-1, because it is essential and the critical molecule mediating insulin action, and IRS-1 activity is largely regulated by its extensive phosphorylation in the presence of insulin [4,5]. Tyrosine phosphorylation of IRS-1 at Tyr632 and Tyr896 activates insulin action, whereas serine phosphorylation at Ser302, Ser307, and Ser1101 generally attenuates it [4,5].
In accordance with glucose production, telmisartan dose-dependently increased p-IRS-1-Ser\(^{302}\) and decreased p-IRS-1-Tyr\(^{632}\) with no alterations in other phosphorylation sites of IRS-1, when HepG2 cells were stimulated by insulin (Fig. 1C, Supplementary Fig. 3). Furthermore, the phosphorylation of both sites was rarely detected in the absence of insulin (Fig. 1D). These results suggest that telmisartan impaired insulin action by altering IRS-1 phosphorylation in hyperglycemic HepG2 cells.

2. Telmisartan-induced p-PKC\(\zeta\)-Thr\(^{410}\) mediates IRS-1 phosphorylation changes that increase glucose production

Among various upstream kinases for IRS-1 phosphorylation, we investigated whether telmisartan affects the expression and phosphorylation of PKC\(\zeta\). This is because PKC\(\zeta\) acts as a negative feedback regulator in insulin-stimulated Fao cells and NIH-3T3 fibroblasts by decreasing tyrosine phosphorylation of IRS-1, which is secondarily caused by PKC\(\zeta\)-induced serine phosphorylation of IRS-1 [7,19,20]. As shown in Fig. 2A, telmisartan dose-dependently enhanced p-PKC\(\zeta\)-Thr\(^{410}\) without altering its expression. This result supports that telmisartan increased PKC\(\zeta\) activity because increased phosphorylation of PKC\(\zeta\) at Thr410 residue located in the PKC\(\zeta\) activation loop is well established to fully activate its enzymatic activity [21]. To clarify the role of PKC\(\zeta\) activation by telmisartan in IRS-1 phosphorylation and glucose production, we overexpressed dn-PKC\(\zeta\) constructs in HepG2 cells. As shown in Figs. 2B and 2C, ectopic expression of dn-PKC\(\zeta\) almost completely restored both the telmisartan-induced p-IRS-1-Ser\(^{302}\) and -repressed p-IRS-1-Tyr\(^{632}\) and the telmisartan-induced glucose production. These results clearly demonstrate that increased PKC\(\zeta\) activity mediated the effects of telmisartan on IRS-1 phosphorylation and glucose production in insulin-stimulated HepG2 cells. As expected, the minimal increase in PEPCK expression induced by telmisartan was not changed by overexpression of dn-PKC\(\zeta\) constructs (Fig. 2D), indicating that PEPCK expression did not affect glucose production.

3. Among tested ARBs, only telmisartan induces p-IRS-1-Ser\(^{302}\) and represses p-IRS-1-Tyr\(^{632}\) via PPAR\(\gamma\)-independent pathway

We demonstrated that telmisartan increased glucose production in hyperglycemic HepG2 cells by increasing p-IRS-1-Ser\(^{302}\) and decreasing p-IRS-1-Tyr\(^{632}\) thereby attenuating insulin action. Although most ARBs share some structural moieties, each ARB contains highly variable side chains [9]. This paradigm led us to examine whether ARBs other than telmisartan also change IRS-1 phosphorylation.

As shown in Fig. 3A, among the ARBs tested, including losartan and fimasartan, only telmisartan increased p-IRS-1-Ser\(^{302}\) and decreased p-IRS-1-Tyr\(^{632}\), indicating that its inhibitory effects on insulin action are specific properties. Unlike other ARBs, telmisartan is also reported to act as a PPAR\(\gamma\) partial agonist [9]. To investigate the role of PPAR\(\gamma\) in the telmisartan-altered IRS-1 phosphorylation, we performed inhibitor studies using GW9662, a specific and irreversible PPAR\(\gamma\) antagonist [22]. As shown in Fig. 3B, GW9662 did not affect telmisartan-regulated IRS-1 phosphorylation. Taken together, our results suggest that telmisartan exerts its effects on IRS-1 phosphorylation in a PPAR\(\gamma\)-independent manner.

4. Telmisartan induces p-IRS-1-Ser\(^{302}\), represses p-IRS-1-Tyr\(^{632}\), and increases p-PKC\(\zeta\)-Thr\(^{410}\) in livers of HFD-fed mice

Next, to confirm our in vitro findings in vivo, we performed animal studies by feeding mice with an HFD (60% fat kcal) for 13 weeks (Fig. 4A). In accordance with the in vitro results, the livers from telmisartan-treated mice showed significantly higher p-IRS-1-Ser\(^{302}\) and lower p-IRS-1-Tyr\(^{632}\) levels than those from vehicle-treated mice did (Fig. 4B), when insulin was injected through the portal vein. Furthermore, telmisartan treatment increased p-PKC\(\zeta\)-Thr\(^{410}\) by ~2.2-fold compared to that in vehicle-treated mice (Fig. 4C). Similar to the in vitro results, PEPCK and G6Pase expression levels were slightly increased in telmisartan-treated livers (Fig. 4D). However, telmisartan did not change Glut4 and Glut2 expression levels in the livers (Supplementary Fig. 4). These results revealed that the effects of telmisartan on phosphorylation of IRS-1 and PKC\(\zeta\) were also evident in vivo.

Discussion

One of the most important findings in this study is that telmisartan impaired insulin action in hyperglycemic hepatocytes by increasing p-IRS-1-Ser\(^{302}\) and decreasing p-IRS-1-Tyr\(^{632}\), and consequently increased HGP (Figs. 1, 4). Although the effect of telmisartan on HGP has not been reported, several clinical trials reported that telmisartan reduces incidence of new-onset diabetes despite the different magnitude of the effects between trials [23]. A recent meta-analysis data revealed that telmisartan improves insulin resistance in patients with hypertension [10]. It was also reported that telmisartan has beneficial effects on insulin resistance in skeletal muscles and adipose tissues [12,13]. Based on the previous reports, therefore, our results suggest that the beneficial effects of telmisartan on glycemic control may be
Fig. 2. Telmisartan-induced p-PKCζ-Thr^{410} mediates IRS-1 phosphorylation changes that increase glucose production. (A) HepG2 cells were treated with various concentrations of telmisartan (0, 10, 20, or 40 μM) for 24 h in the presence of 25 mM D-glucose and then 100 nM insulin for an additional 10 min. Western blot analyses were performed as described in Fig. 1. (B) After HA-tagged dn-PKCζ (K281R) constructs were transfected into HepG2 cells as described in the Methods, cells were treated with 40 μM telmisartan or vehicle for 24 h in the presence of 25 mM D-glucose and then 100 nM for an additional 10 min. Western blot analyses were performed as described in Fig. 1. (C) HepG2 cells were transfected with dn-PKCζ constructs, treated with 40 μM telmisartan for 24 h in the presence of 25 mM D-glucose, and glucose production assay was performed as described in the Methods. (D) After transfection as described above, HepG2 cells were treated with 40 μM telmisartan for 24 h in the presence of 25 mM D-glucose and then 100 nM insulin for an additional 10 min. Western blot analyses were performed as described in Fig. 1. Blots represent at least four experiments. Bar graphs show mean fold alterations above/below control (±SD). Differences are statistically significant at a) \( p < 0.05 \), b) \( p < 0.01 \). n.s., not significant. PKCζ, protein kinase Cζ; IRS-1, insulin receptor substrate-1; HA, hemagglutinin.

mediated by its actions on other insulin target organs such as the skeletal muscles and adipose tissues rather than the liver.

In the present study, we found that telmisartan increased HGP in hyperglycemic HepG2 cells (Fig. 1A) and, therefore,
we expected that telmisartan may decrease p-Akt-Ser\(^{473}\) and p-GSK3\(\beta\)-Ser\(^{9}\), because Akt and GSK3\(\beta\) have been established as important mediators of insulin action and are used as markers for insulin resistance in vitro when the phosphorylation is suppressed [18]. However, our results revealed that telmisartan clearly enhanced p-Akt-Ser\(^{473}\) and p-GSK3\(\beta\)-Ser\(^{9}\) in hyperglycemic HepG2 cells, when insulin was present (Supplementary Fig. 2). Presently, we cannot fully explain the mechanism by which telmisartan increased HGP, despite the increase of p-Akt-Ser\(^{473}\) and p-GSK3\(\beta\)-Ser\(^{9}\). In this regard, Akt2 plays a pivotal role in the maintenance of glucose homeostasis, whereas Akt1 is not essential for the regulation of glucose homeostasis and chronically regulates the transcription of gluconeogenic enzymes rather than that of acute glucose output in vivo [2,24,25]. Our results and those of the previous reports suggest that telmisartan may exert its effects on HGP via a signaling pathway mediated by other signaling molecules rather than Akt1.

Although most ARBs share some structural moieties, each ARB contains highly variable side chains [9]. In terms of structural chemistry, unlike other ARBs, telmisartan contains a carboxyl group substituted for the common tetrazole group linked to the biphenyl moiety, and two benzimidazole groups that are linked tandemly [9]. Thus, telmisartan may have various ancillary effects along with its common blood pressure-lowering effects. For example, telmisartan is reported to ameliorate vascular inflammation [26,27] and to act as a PPAR\(\gamma\) partial agonist [9]. Obermoser et al. have recently reported that the biphenyl moiety and the centered benzimidazole group directly linked to the biphenyl moiety are critical for PPAR\(\gamma\) activation [28].

Previously, Moeschel et al. reported that the PKC\(\zeta\)-mediated phosphorylation of IRS-1 at Ser318 attenuates IRS-1 function in response to prolonged insulin stimulation [29]. However, other inhibitory serine residues that phosphorylated by PKC\(\zeta\) are not fully identified. Furthermore, effects of the PKC\(\zeta\)-inhibited IRS-1 activity on its downstream effectors including PI3K and Akt are rarely

Fig. 3. Among tested ARBs, only telmisartan induces p-IRS-1-Ser\(^{302}\) and represses p-IRS-1-Tyr\(^{632}\) via PPAR\(\gamma\)-independent pathway. (A) HepG2 cells were treated with various ARBs (telmisartan, losartan, or fimasartan at 40 \(\mu\)M) for 24 h in the presence of 25 mM D-glucose and then 100 nM insulin for further 10 min. Western blot analyses were performed as described in Fig. 1. (B) After pretreatment with 5 \(\mu\)M GW9662 for 1 h, HepG2 cells were treated with 40 \(\mu\)M telmisartan for 24 h in the presence of 25 mM D-glucose and then 100 nM insulin for further 10 min. Western blot analyses were performed as described in Fig. 1. Blots represent at least four experiments. Bar graphs show mean fold alterations above/below control (±SD). Differences were statistically significant at \(p<0.01\). n.s., not significant. ARB, angiotensin II type 1 receptor blocker; IRS-1, insulin receptor substrate-1; PPAR\(\gamma\), peroxisome proliferator-activated receptor \(\gamma\).
elucidated, although PKCζ has been reported to be able to initiate a negative feedback regulation in the insulin-stimulated cells by decreasing tyrosine phosphorylation of IRS-1, which is secondarily caused by PKCζ-induced serine phosphorylation of IRS-1 [7,19,20]. In this respect, Ravichandran et al. revealed that overexpression of wild-type PKCζ reduces the activity of PI3K which is associated IRS-1 without inhibition of total PI3K activity [20] and PKCζ-induced phosphorylation of IRS-1 specifically interferes with the phosphorylation of IRS-1 at Tyr612 and Tyr632 [20]. In accordance with the report, we showed that PKCζ activated by telmisartan attenuated insulin action in hyperglycemic hepatocytes by increasing p-IRS-1-Ser302 and decreasing p-IRS-1-Tyr632 (Figs. 2, 4). Therefore, our results suggest that the PKCζ-mediated phosphorylation of IRS-1 at Ser302 plays an important role in the negative feedback regulation of IRS-1 activity. In addition, we also found that telmisartan elevated p-Akt-Ser473 that is considered as an important downstream effector of IRS-1 and PI3K which mediate insulin signaling (Supplementary Fig. 2). Based on the report and our results, we speculate that telmisartan augmented a negative feedback signaling pathway through PKCζ activation. Following insulin treatment, PI3K is activated and phosphorylates its downstream effectors including phosphoinositide-dependent protein kinase-1 (PDK-1) by which PDK-1 is activated and phosphorylates Akt1 and PKCζ. Telmisartan may have promoted PDK-1-mediated PKCζ phosphorylation and activated PKCζ increases p-IRS-1-Ser302, which reduces p-IRS-1-Tyr632. This process finally attenuates the activity of IRS-1, although activated PDK-1 could still phosphorylate Akt1, elevating p-Akt-Ser473. In support of this notion, PDK-1 is the best known upstream

Fig. 4. Telmisartan induces p-IRS-1-Ser302, represses p-IRS-1-Tyr632, and increases p-PKCζ-Thr410 in livers of HFD-fed mice. (A) Schematic diagram of animal experiments. Hyperglycemic mouse models were established as described in the Methods. (B–D) Mice were euthanized, their livers were dissected, total liver proteins were extracted, and then subjected to western blot analysis as described in Fig. 1. Blots represent at least five livers from each mouse group. Bar graphs show mean fold alterations above/below control (±SD). Differences are statistically significant at *p<0.05, **p<0.01. IRS-1, insulin receptor substrate-1; PKCζ, protein kinase Cζ; HFD, high-fat diet.

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by inducing p-PKCζ-Thr410 (Supplementary Fig. 2), and a previous study also reported that PDK-1 phosphorylates PKCζ at threonine 410 and activates it [21].

Previously, high interpatient variability in telmisartan plasma concentrations has been reported in patients with mild to moderate hypertension; mean +/- SD values for Cmax are 159 +/- 104 ng/mL for telmisartan 40 mg, 693 +/- 606 ng/mL for telmisartan 80 mg, and 1635 +/- 1406 ng/mL for telmisartan 120 mg, which are equivalent to 0.31 +/- 0.20 μM for telmisartan 40 mg, 1.35 +/- 1.12 μM for telmisartan 80 mg, and 3.12 +/- 2.73 μM for telmisartan 120 mg, respectively [30]. Similarly, Zhang et al. have also reported that mean +/- SD values for Cmax in healthy Chinese subjects are 163.2 +/- 128.4 ng/mL and 905.7 +/- 583.4 ng/mL for 40 mg and 80 mg telmisartan, which are equivalent to 0.32 +/- 0.25 μM and 1.76 +/- 1.13 μM for 40 mg and 80 mg telmisartan, respectively [31]. If telmisartan concentrations used in our in vitro experiments (10–40 μM) are simply compared to these clinical data, these seem to be higher. However, we clearly showed that telmisartan exerted its effects on HGP, p-IRS-1-Ser302, p-IRS-1-Tyr410, and p-PKCζ-Thr410 at 20 μM telmisartan, although profound effects were exhibited at 40 μM telmisartan (Figs. 1A, 1C, 2A). In addition, higher concentration of telmisartan at 100 μM has been used in other in vitro investigations [32,33]. With taking account of our results and other in vitro experiments using telmisartan, concentration of telmisartan used in the present study is considered fairly compatible with a clinically observed peak Cmax.

In conclusion, we demonstrate that telmisartan increases HGP by inducing p-PKCζ-Thr410, which increases p-IRS-1-Ser302 and decreases p-IRS-1-Tyr410 in a PPARγ-independent manner. These actions consequently impair the action of insulin in hyperglycemic HepG2 cells and HFD-fed mouse livers. These results suggest that the beneficial effects of telmisartan on insulin resistance and cardiometabolic profile [10,11] may be mediated by its actions on other insulin target organs such as the skeletal muscles and adipose tissues rather than the liver.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.12701/yujm.2019.00059

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Conflicts of interest

All authors declare that no conflicts of interest exist.

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References


Non–coplanar whole brain radiotherapy is an effective modality for parotid sparing

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Background: The purpose of this study was to evaluate the efficacy and feasibility of non–coplanar whole brain radiotherapy (NC–WBRT) for parotid sparing.

Methods: Fifteen cases, previously treated with WBRT were selected. NC–WBRT plans were generated. The beam arrangement for the non–coplanar plans consisted of superior anterior, right, and left beams. After generation of the non–coplanar plans a field–in–field technique was applied to the bilateral parallel opposed beams in order to reduce maximum dose and increase dose homogeneity. The NC–WBRT plans were subsequently compared with the previously generated bilateral WBRT (B–WBRT) plans. A field–in–field technique was also used with the B–WBRT plans according to our departmental protocol. As per our institutional practice a total dose of 30 Gy in 10 fractions of WBRT was administered 5 days a week.

Results: The mean dose to the parotid gland for the two different plans were 16.2 Gy with B–WBRT and 13.7 Gy with NC–WBRT ($p<0.05$). In the NC–WBRT plan, the V5Gy, V10Gy, V15Gy, V20Gy, and V25Gy of the parotid were significantly lower ($p<0.05$) than those of the B–WBRT plan. The D$_{max}$ of the lens was also lower by 10% with NC–WBRT.

Conclusion: The use of NC–WBRT plans could be a simple and effective method to reduce irradiated volumes and improve the dose–volume parameters of the parotid gland.

Keywords: Organ at risk; Parotid gland; Whole brain radiotherapy; Xerostomia

Introduction

Whole brain radiotherapy (WBRT) is widely used with palliative intent in patients with multiple brain metastases and with prophylactic intent in patients with small cell lung cancer having good responses after primary treatment [1–7]. Issues related to WBRT–associated toxicities have been mostly neglected because the outcomes of patients requiring WBRT are generally poor. However, owing to advances in cancer therapy, survival times are much longer, and the issue of quality of life has presently gained importance [8].

The WBRT technique which employs parallel opposed fields is a simple and effective method to encompass the whole brain. During WBRT the main organs at risk are considered to be the lenses and the aero–digestive tract because these organs are particularly radiosensitive and irradiation increases the risk of cataracts and dysphagia [9,10].

Although there was not much change in target coverage in the transition from 2–dimensional radiation therapy (2D–RT) to 3–dimensional conformal radiation therapy (3D–CRT), computed tomography (CT)–based simulation made it possible to evaluate the dose distribution in the normal organs. This advantage also made it possible to discover that large volumes of the parotid gland were being irradiated to clinically meaningful...
doses during WBRT. To avoid severe xerostomia, the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) guidelines recommend that the mean dose to at least one parotid gland should be less than 20 Gy or that the mean dose to the combined volume of both glands should be less than 25 Gy [11]. However Trignani et al. showed that 28% of patients treated with conventional treatment received parotid gland doses beyond the recommended limits [12]. Noh et al. have recently showed through the normal tissue complication probability model that the parotid gland should be considered an organ–at–risk (OAR) during WBRT [13].

Several institutions have tried to reduce the dose to the parotid gland usually by modifying the lower margin of the radiotherapy field and by using intensity–modulated radiation therapy (IMRT). Modifying the field has shown good results in sparing the parotid glands but uncertainty in target coverage remains an issue [14]. IMRT is also an excellent method to spare the parotid gland but it is not cost–effective [15]. We speculated that the superior anterior beam could effectively block the parotid gland without compromising target coverage. In this study, we evaluated the efficacy and feasibility of non–coplanar WBRT by adding a superior anterior beam.

**Materials and methods**

Fifteen patients with brain metastases previously treated with WBRT were selected. The median age of this cohort was 59 years and lung cancer was found to be the most common primary tumor. The patient characteristics of this cohort are summarized in Table 1. In all these patients CT simulation was performed in the supine position using a thermoplastic mask for immobilization. We obtained CT scan images of 5 mm slice thickness and contoured the OARs, including both parotid glands and both the lenses. The brain contours were identified by auto–segmentation. The clinical target volume (CTV) included the brain parenchyma and the spinal cord up to the lower level of the atlas. The CTV was expanded by 7 mm in all directions to create the planning target volume (PTV).

The non–coplanar WBRT (NC–WBRT) plans were then generated. The beam arrangement for the non–coplanar plan consisted of superior anterior, right, and left beams. After generation of the non–coplanar plan, a field–in–field technique was applied to the bilateral parallel opposed beams for reducing the maximum dose and to increase dose homogeneity (Fig. 1). The NC–WBRT plans were subsequently compared with the previously generated bilateral WBRT (B–WBRT) plans. The field–in–field technique was also used with the B–WBRT plans according to our departmental protocol. According to our institutional practice WBRT is administered using the schedule of 30 Gy in 10 fractions for 5 days a week.

The conformity index (CI) and homogeneity index (HI) were utilized in treatment plan analysis. The CI is defined as the ratio of the volume of PTV that receives 95% of the prescribed dose to the entire PTV while the HI is the ratio of the maximum target dose to the prescribed dose. The paired t– and Wilcoxon signed rank–tests were used to compare the dosimetric outcomes including dose coverage and OAR doses between the NC– and B–WBRT plans. All the statistical analyses were performed using the IBM SPSS version 21.0 (IBM Co., Armonk, NY, USA).

**Table 1.** Patient characteristics

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<th>Variable</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
</tr>
<tr>
<td>Range</td>
<td>47-84</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td>Primary sites</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>7</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
</tr>
<tr>
<td>Ovary</td>
<td>1</td>
</tr>
</tbody>
</table>

**Results**

The CI for the B– and NC–WBRT plans were 0.80±0.06 and 0.84±0.06, respectively. The HI was similar in both plans at a value of 1.04±0.01. The dose–volume statistics of the parotid glands are summarized in Table 2 and the dose–volume histogram is shown in Fig. 2. Compared with the B–WBRT plans, the NC–WBRT plans lowered the mean dose of the right and left parotid glands from 15.6 Gy to 13.9 Gy and from 15.9 Gy to 13.6 Gy, respectively (p<0.05). The V5, V10, V15, V20, and V25 for the combined volume of both parotid glands were also significantly lower in the NC–WBRT plans (p<0.05). The parotid doses delivered by the NC–WBRT plans were significantly lower in terms of all dosimetric parameters except for the V5 and Dmax. The greatest reduction was seen with respect to V25 which showed a difference of 19% in the right and 20% in the left parotid gland, respectively (Fig. 3). The Dmax of the lens was 6.6±1.7 Gy vs. 6.1±1.7 Gy on the right and 5.9±1.4 Gy vs. 5.4±1.3 Gy on the left in the in the B–WBRT and NC–WBRT plans, respectively.

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Fig. 1. Beam’s eye view. (A) Superior anterior field of the NC–WBRT. (B) Field–in–field of B–WBRT. NC–WBRT, non–coplanar whole brain radiotherapy; B–WBRT, bilateral whole brain radiotherapy.

Fig. 2. Dose–volume histogram of parotid gland for B–WBRT and NC–WBRT. B–WBRT, bilateral whole brain radiotherapy; NC–WBRT, non–coplanar whole brain radiotherapy. PTV, planning target volume; CTV, clinical target volume; lt., left; rt., right.
Table 2. Dose–volume statistics of parotid gland with B–WBRT and NC–WBRT

<table>
<thead>
<tr>
<th>Volume</th>
<th>Dose</th>
<th>B–WBRT</th>
<th>NC–WBRT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>SD</td>
<td>Median</td>
</tr>
<tr>
<td>Parotid rt.</td>
<td>V5 (%)</td>
<td>71.2</td>
<td>11.1</td>
<td>70.4</td>
</tr>
<tr>
<td></td>
<td>V10 (%)</td>
<td>61.0</td>
<td>11.5</td>
<td>53.9</td>
</tr>
<tr>
<td></td>
<td>V15 (%)</td>
<td>53.0</td>
<td>9.7</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>V20 (%)</td>
<td>44.1</td>
<td>10.6</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>V25 (%)</td>
<td>33.2</td>
<td>9.2</td>
<td>14.0</td>
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<tr>
<td></td>
<td>Dmin (cGy)</td>
<td>86.5</td>
<td>21.4</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>Dmax (cGy)</td>
<td>2,965.4</td>
<td>31.4</td>
<td>2,919.0</td>
</tr>
<tr>
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<td>Dmean (cGy)</td>
<td>1,554.9</td>
<td>285.3</td>
<td>1,389.5</td>
</tr>
<tr>
<td>Parotid lt.</td>
<td>V5 (%)</td>
<td>72.8</td>
<td>10.6</td>
<td>70.3</td>
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<tr>
<td></td>
<td>V10 (%)</td>
<td>62.8</td>
<td>11.1</td>
<td>55.2</td>
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<td></td>
<td>V15 (%)</td>
<td>56.1</td>
<td>12.1</td>
<td>50.0</td>
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<td></td>
<td>V20 (%)</td>
<td>46.0</td>
<td>10.4</td>
<td>36.0</td>
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<td>V25 (%)</td>
<td>32.9</td>
<td>9.8</td>
<td>12.3</td>
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<td></td>
<td>Dmin (cGy)</td>
<td>92.6</td>
<td>21.4</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>Dmax (cGy)</td>
<td>2,964.8</td>
<td>27.6</td>
<td>2,922.8</td>
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<tr>
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<td>Dmean (cGy)</td>
<td>1,593.3</td>
<td>284.6</td>
<td>1,357.6</td>
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<td>Parotid both</td>
<td>V5 (%)</td>
<td>73.7</td>
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<td></td>
<td>V10 (%)</td>
<td>63.9</td>
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<td></td>
<td>V15 (%)</td>
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<td>V20 (%)</td>
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<td></td>
<td>V25 (%)</td>
<td>33.5</td>
<td>8.6</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Dmin (cGy)</td>
<td>85.0</td>
<td>17.7</td>
<td>81.3</td>
</tr>
<tr>
<td></td>
<td>Dmax (cGy)</td>
<td>2,978.8</td>
<td>26.6</td>
<td>2,925.8</td>
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<tr>
<td></td>
<td>Dmean (cGy)</td>
<td>1,618.4</td>
<td>261.9</td>
<td>1,372.4</td>
</tr>
</tbody>
</table>

B–WBRT, bilateral whole brain radiotherapy; NC–WBRT, non-coplanar whole brain radiotherapy; SD, standard deviation; rt., right; lt., left.

Discussion

The incidence of brain metastases from all cancers is 5.0 per 1,000 person–years. The major primary sites in South Korea in the order of incidence are the lung, the liver, the breast, and the colorectum [16]. WBRT is the standard palliative treatment in most patients with multiple brain metastases. It is also employed as curative treatment in cases of primary central nervous system lymphoma, and as prophylactic therapy for small cell lung cancer with showing good responses to chemotherapy [3,17,18].

The ‘helmet’ technique is usually employed because it provides good target coverage. This method consists of bilateral parallel opposed fields, which encompass the brain tissue, the skull, and the spinal cord to the lower level of the atlas. In the 2D era, the lens and the aero–digestive tract were considered the organs at risk. However, after the transition to the 3D era, by virtue of CT–simulation, it was possible to identify normal tissue dose distributions. Trignani et al. compared the 2D technique with the 3D technique for WBRT, and discovered that 28% of patients received a mean dose in excess of 20 Gy to the parotid gland when using 2D–RT [12]. Noh et al. also reported that mean parotid doses in 64 individual cases were in excess of 20 Gy and 25 Gy in 34.4% and 6.3%, respectively [13]. In view of the recommended dose limits of the parotid gland by the QUANTEC guidelines [11], they concluded that the parotid glands could be regarded as organs–at–risk during WBRT. Burlage et al. have reported that salivary flow rates drop dramatically during the first 2 weeks of RT. In their study cohort, when the total cumulative dose was 20 Gy, the flow rate had decreased by about 80% of the initial flow [19]. Considering that the most common treatment schedule of WBRT is 30 Gy in 10 fractions, the possibility of parotid gland dysfunction after WBRT cannot be excluded.

Some efforts have been made to reduce higher doses to irradiated parotid volumes. Most of these attempted to achieve this by adjusting the radiation fields. Fiorentino et al. reported on the dosimetric data of the parotid gland for 30 patients who received WBRT [20]. The median V20 and V25 of the right parotid gland were 3.5% (range, 0–44.5%), and 1.85% (range, 0.3–18.0%), respectively. For the left parotid gland, median V20 and V25 were 3.1% (range, 0–44.5%) and 1.8% (range, 0–32.2%), respectively. They had only revised WBRT with

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the treatment fields encompassing the brain tissue, and the skull. Compared to our results, the volume of parotid gland irradiated to higher doses in their study was very low. Cho et al. have shown that a modified field (MF) customized for the brain tissue is an effective technique for sparing the parotid gland, compared with conventional fields (CF) [14]. The mean parotid doses with CF and MF were 17.4 Gy and 8.7 Gy, respectively. The V20 of both plans were 48.4% and 18.2%, respectively. In our study, the median parotid dose was 13.7 Gy and V20 was 36.4% in NC–WBRT, which was better than that of CF. However, although parotid sparing was better with the MF technique, there have been some concerns about target coverage with the MF because the radiation field is restricted to the parotid gland region. Cho et al. also reported that target coverage with MF was statistically lower than that of CF although this was not clinically significant [14].

All techniques used in the studies mentioned above including our study met the dose constraints of the parotid gland as per the QUANTEC recommendations. This guideline is based on the relationship of the incidence of severe xerostomia with the dose–volume parameters of the parotid. Deasy et al. in their study, found that at doses below the range of 10 to 15 Gy the reduction of parotid function was minimal but this gradually increased, when the received doses were in the range of 20 to 40 Gy [11]. The reduction in function of the parotid gland could lead to problems such as poor dental hygiene, oral infections and difficulty in chewing, and swallowing [21]. These problems contribute to a poor quality of life. It is therefore important to reduce doses to the parotid gland as far as possible for better salivary flow.

IMRT which provides improved dose homogeneity and conformity, is a useful technique for lowering the dose in complex–shaped organs; studies on hippocampus–sparing WBRT using IMRT, have recently been performed [15,22,23]. The efficacy of IMRT in sparing the parotid gland has already been demonstrated in head and neck cancers [24–26] and
holds promise in WBRT. However, IMRT is nearly twice as expensive as 3D–CRT and requires a longer preparation time. It is therefore not employed in all whole brain treatments, because most of the patients who undergo WBRT have a short life expectancy and the intent of therapy is essentially palliative. The rate of IMRT utilization in Korea has steadily increased since 2011 accounting for about 25% of all RT treatments in 2016. According to the Korean Health and Insurance Review and Assessment Service data, fewer IMRT treatments were noted in those areas that are geographically situated away from the state capital [27]. Owing to the limitations in IMRT services reduction of OAR–doses using 3D–CRT may be a more practical solution.

The lens is well–known for its radiosensitivity; small radiation doses may lead to cataract formation as a late complication. The recommended limit to the lens is around 5 Gy delivered in a fractionated schedule [28]. Our study showed that the NC–WBRT plans had reduced the $D_{max}$ of the lens by 10%.

**Conclusion**

NC–WBRT could be an effective and simple alternative to B–WBRT offering comparatively lower irradiated parotid volumes and improved lens dosimetry without compromising target coverage.

**Conflicts of interest**

No potential conflicts of interest relevant to this article were reported.

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**References**


Comparison of the removal torque and a histomorphometric evaluation of the RBM treated implants with the RBM followed by laser treated implants: an experimental study in rabbits

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Background: In the osseointegration of dental implants, the implant surface properties have been reported to be some of the most important critical factors. The effect of implant’s surfaces created by resorbable blast media (RBM) followed by laser ablation on bone tissue reactions was examined using the removal torque test and histomorphometric analysis.

Methods: Two types of dental implants, RBM-laser implants (experimental group) and RBM implants (control group) (CSM implant system, Daegu, Korea; L=6 mm, diameter=3.75 mm) were placed into the right and left distal femoral metaphysis of 17 adult rabbits. Six weeks after placement, removal torque was measured and histomorphometric analysis was performed.

Results: The mean removal torque was 24.0±10.2 Ncm and 46.6±16.4 Ncm for the control and test specimens, respectively. The experimental RBM-laser implants had significantly higher removal torque values than the control RBM implants (p=0.013). The mean values of total and cortical bone to implant contact (BIC) were respectively 46.3±10.8% and 65.3±12.5% for the experimental group, and 41.9±18.5% and 57.6±10.6% for the control group. The experimental RBM-laser implants showed a higher degree of total and cortical BIC compared with RBM implants, but there was no statistical significance (p=0.482, 0.225).

Conclusion: The removal torque and BIC of the test group were higher than those of the control group. In this study, the surface treatment created by RBM treatment followed by laser ablation appears to have a potential in improving bone tissue reactions of dental implants.

Keywords: Dental implants; Laser ablation; Osseointegration; Resorbable surface media

Introduction

Osseointegrated dental implants have been widely accepted as the method for the functional and esthetical restoration of missing teeth. Their successful long-term stability has been reported in a large number of clinical studies [1-4]. The following factors are critical to the success of dental implant treatments: the mechanical properties of the implant material, the implant design, the implant surface configuration, the quality of the host tissue, the surgical technique, and the loading pattern [5]. Implant surfaces treated with resorbable blast media (RBM) have a higher removal torque and superior interfacial bone contact.
than machined implants, providing more benefits on early bone formation and initial stability [6-9]. Laser processing has been reported to be a new method for treating implant surfaces to produce a high degree of purity with sufficient roughness for good osseointegration [10].

The purpose of this study was to evaluate the synergistic potential of implant surfaces created by RBM treatment followed by laser ablation (RBM-laser implants) on bone tissue reactions compared to routine RBM surface-treated implants (RBM implants).

**Materials and methods**

1. **Implant surface preparation**
   Thirty-four commercially pure titanium (Ti) dental implants (CSM implant system, Daegu, Korea) were used. The implants were 6 mm long with an outer diameter of 3.75 mm.

   First, the 34 implants, which included the control group (17 implants), were blasted with 10 µm hydroxyapatite (HA) powder using an Index Type Auto Blast M/C (Korea Shot Blast Co., Ltd, Siheung, Korea). Thereafter, only 17 implants, the experimental group, were modified by laser ablation. A Nd: YAG laser (JENOPTIK Laser Optik Systeme GmbH, Jena, Germany), 15 kHz laser beam (10 W, 2 μsec in pulse width) was used.

2. **Experimental design**
   Thirty-four implants (L=6 mm; d=3.75 mm) were placed in the femurs of the rabbits (17 animals) and evaluated after 6 weeks of healing. As the experimental and the control groups, each implants were placed respectively in the right and the left femurs of the same rabbits.

3. **Surgical procedures**
   Permission was obtained from the Bioethics Committee for Animal Experimentation of the Yeungnam University College of Medicine (permission no. YUMC-2009-021) and the animal experiments were carried out in accordance with the guidelines. (1) Seventeen adult white rabbits, weighing 2.8-3.3 kg, were used. (2) Prior to surgery, the operation site was cleaned with a mixture of iodine and 75% ethanol. The femoral metaphysis was exposed by a full thickness flap and the bone was denuded. The sites were prepared with progressive drilling under saline cooling. The test and control implants were placed in each of the distal femoral metaphyses on the right and left sides, respectively. (3) Immediately after surgery, the rabbits were kept in separate cages. (4) They were allowed post-surgical full-weight bearing and movement. Six weeks after surgery, the animals were sacrificed.

4. **Scanning electron microscope**
   A topographic evaluation was performed by field emission scanning electron microscopy (FE-SEM, Hitachi S-4300 and 2DX-350, Tokyo, Japan) to compare surface structure on both implant groups. The peaks, valleys and flanks were measured in two implant samples. A ×500 magnification was used. Energy dispersive spectrometry (EDS) was used to evaluate the surface's chemical composition.

5. **Surface roughness**
   Surface roughness was measured on thread-tops, flanks and valleys-selected at random on the implant surface using an Optical Profiler (Wyko NT8000, Veeco, Tucson, AZ, USA). It was calculated as arithmetic average height (Ra), root mean square height (Rq), maximum height (Rt), and developed surface ratio (Sdr).

6. **Removal torque test**
   The removal torque of the implants was measured using a manual torque gauge (MGT12, Mark-10 Corporation, NY, USA). The result was recorded measuring the maximum removal torque at which fracture occurred between the implant and the bone. The anticlockwise movement to remove the implant was performed trying to avoid forces in directions different from the vertical.

7. **Histomorphometrics**
   Histologic specimens including the implant and its surrounding tissue were obtained and observed under natural and fluorescent light using an optical microscope (BX-51T, Olympus, Tokyo, Japan). A fluorescent marker (tetracycline) was injected intramuscularly to the animals at 4 and 5 weeks after placement. Digital images with magnifications of ×20 and ×100 were captured using a digital camera. Measurements, such as the entire length of the implant, length of bone to implant contact (BIC) and degree of BIC were calculated using software (iMTechnology, Daejeon, Korea) analyses of digital optical microscopy images at ×20 magnification.

8. **Statistical analysis**
   Statistical analyses of removal torque, total BIC and cortical BIC values between the two groups were performed using the Wilcoxon rank sum test (SPSS version 14.0; SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered significant.

**Results**

1. **Scanning electron microscope**
The surface morphology of the specimens was observed by FE-SEM. Images of both original samples (fixture) and removed, torqued samples (fixture) were taken at magnifications ×500 (Figs. 1, 2).

Figs. 1, 2. FE-SEM image of the RBM-laser sample (original magnification, ×500). RBM-laser implant (experimental group) reveals precise porous structures dispersed uniformly throughout the implant surfaces. These porous structures are about 20–40 μm in size. FE-SEM, field emission-scanning electron microscopy; RBM, resorbable blast media.

Figs. 3, 4. EDS spectrum of the RBM and laser treated implants. The EDS analysis of RBM and laser treated implants showed considerable surface contamination with oxygen (O). The RBM-laser implants showed a clean surface with Ti and O peaks.

The RBM-laser implants (experimental group) showed pronounced porous structures but these were irregularly dispersed and their size was only 1-5 μm (Fig. 2).

Figs. 3, 4 present the results of the EDS analysis. EDS analyses of the RBM implants showed considerable surface contamination with oxygen (O). The RBM-laser implants showed a clean surface with Ti and O peaks.

2. Surface roughness analysis

The surface roughness was measured using an Optical Profiler (Wyko NT8000, Veeco). In the experimental group, the Ra at the top, valley and incline was 9.96 μm, 6.78 μm, and 6.97 μm, respectively. The Rq at the top, valley and incline was 12.07 μm, 9.00 μm, and 8.68 μm, respectively. The Rt at the top, valley and incline was 72.83 μm, 72.80 μm, and 63.29 μm, respectively. In the control group, the Ra at the top, valley and incline was 1.08 μm, 0.87 μm, and 1.19 μm, respectively. The Rq at the top, valley and incline was 1.41 μm, 1.14 μm, and 1.53 μm, respectively. The Rt at the top, valley and incline was 18.74 μm, 16.26 μm, and
22.45 μm, respectively. The Sdr was 230.76% and 55.00% for the experimental and control groups, respectively (Table 1).

Table 1. Surface roughness and developed surface ratio (Sdr) of the control and experimental group samples

<table>
<thead>
<tr>
<th>Surface roughness</th>
<th>Ra (μm)</th>
<th>Rq (μm)</th>
<th>Rt (μm)</th>
<th>Sdr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBM-laser Top</td>
<td>9.96</td>
<td>12.07</td>
<td>72.83</td>
<td></td>
</tr>
<tr>
<td>Valley</td>
<td>6.78</td>
<td>9.00</td>
<td>72.80</td>
<td>230.76</td>
</tr>
<tr>
<td>Incline</td>
<td>6.97</td>
<td>8.68</td>
<td>63.29</td>
<td></td>
</tr>
<tr>
<td>RBM Top</td>
<td>1.08</td>
<td>1.41</td>
<td>18.74</td>
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</tr>
<tr>
<td>Valley</td>
<td>0.87</td>
<td>1.14</td>
<td>16.26</td>
<td>55.00</td>
</tr>
<tr>
<td>Incline</td>
<td>1.19</td>
<td>1.53</td>
<td>22.45</td>
<td></td>
</tr>
</tbody>
</table>

Surface roughness value (Ra, Rq, Rt) and developed surface ratio (Sdr) of the control and experimental samples.

RBM, resorbable blast media; Ra, arithmetic average height of surface roughness; Rq, root mean square height of surface roughness; Rt, maximum height of surface roughness; Sdr, developed surface ratio of surface roughness.

3. Removal torque measurement

Table 2 shows the removal torque values obtained 6 weeks after implant placement. The mean removal torque in the experimental and control group was 46.6±16.4 Ncm, 24.0±10.2 Ncm, respectively.

Statistical analysis of the removal torque revealed that the experimental RBM-laser implant values were significantly higher than those of the control RBM implants (p=0.013).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Right (experimental group)</th>
<th>Left (control group)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>No.1</td>
<td>35.7</td>
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<td>No.2</td>
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<td>No.3</td>
<td>58.9</td>
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<tr>
<td>No.10</td>
<td>41.3</td>
<td>25.1</td>
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</tr>
<tr>
<td>Mean±SD (Ncm)</td>
<td>46.6±16.4</td>
<td>24.0±10.2</td>
<td>0.013</td>
</tr>
</tbody>
</table>

RBM-laser implant was statically significant superior to the removal torque test (p=0.013).

SD, standard deviation; RBM, resorbable blast media.
<sup>a</sup>p<0.05 between the groups by Wilcoxon rank sum test.

4. Histomorphometric analysis

In the experimental group, newly formed bone on the rough surface of the implant was observed indicating a favorable BIC.

Fluorescence microscopy observations revealed a faint fluorescent signal along part of the subperiosteum. This means that the newly formed, peri-implant bone consists of mature bone gently remodeled (Fig. 5) with ongoing bone formation, as expected at 4 or 5 weeks after surgery. There are no cementing lines or lamellar bone present. In the control group, newly formed bone into the peri-smooth implant surface was also

Fig. 5. Histological image of the experimental group after 6 weeks of healing (left image, original magnification, ×20; A-D, original magnification, ×100). On the optical microscopic observation, favorable bone to implant contact by newly formed bone into the peri-implant rough surface was observed (A, B). On the fluorescence microscopic observation, the fluorescent line was seen faintly in some of subperiosteum (C, D).

Fig. 6. Histological image of the control group after 6 weeks of healing (left image, original magnification, ×20; A-D, original magnification, ×100). On the optical microscopic observation, newly formed bone into the peri-smooth implant surface was also observed but was observed that fibrous tissue development went, so a bony union was lost partially (A, B). On the fluorescence microscopic observation, the fluorescent line was seen near the peri-implant surface (C, D).
observed, which means a relatively favorable BIC. On the other hand, vessel development occurred and a bony union was partially lost. On fluorescence microscopy, a fluorescent line was observed near the peri-implant surface, suggesting that a new bone apposition had progressed (Fig. 6).

Six weeks after surgery, the mean values of total BIC were 46.3±10.8% for the experimental group and 41.9±18.5% for the control group (Table 3). And the mean values of cortical BIC were 65.3±12.5% for the experimental group and 57.6±10.6% for the control group (Table 4). Implants with RBM-laser surfaces showed a higher degree of total and cortical BIC compared to RBM implants; however, this difference did not reach statistical significance (p=0.482, 0.225).

**Table 3.** Total BIC of the experimental and control groups after 6 weeks of healing

<table>
<thead>
<tr>
<th></th>
<th>RBM-laser (experimental group)</th>
<th>RBM (control group)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BIC (%)</td>
<td>38.3</td>
<td>37.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41.9</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.4</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64.1</td>
<td>36.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.2</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>53.5</td>
<td>70.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.5</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td>Mean±SD (%)</td>
<td>46.3±10.8</td>
<td>41.9±18.5</td>
<td>0.482</td>
</tr>
</tbody>
</table>

The RBM-laser implant exhibited higher degree of total BIC, but there was no statistical significance (p=0.482).

BIC, bone to implant contact; SD, standard deviation; RBM, resorbable blast media.

*a* p<0.05 between the groups by Wilcoxon rank sum test.

**Table 4.** Cortical BIC of the experimental and control groups after 6 weeks of healing

<table>
<thead>
<tr>
<th></th>
<th>RBM-laser (experimental group)</th>
<th>RBM (control group)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical BIC (%)</td>
<td>76.1</td>
<td>66.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73.7</td>
<td>65.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.5</td>
<td>44.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67.9</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63.4</td>
<td>65.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73.6</td>
<td>64.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62.8</td>
<td>54.7</td>
<td></td>
</tr>
<tr>
<td>Mean±SD (%)</td>
<td>65.3±12.5</td>
<td>57.6±10.6</td>
<td>0.225</td>
</tr>
</tbody>
</table>

The RBM-laser implant exhibited higher degree of cortical BIC, but there was no statistical significance (p=0.225).

BIC, bone to implant contact; SD, standard deviation; RBM, resorbable blast media.

*a* p<0.05 between the groups by Wilcoxon rank sum test.

**Discussion**

Our results indicated that part of the research hypothesis could be accepted. This study showed that the experimental RBM followed by laser treatment achieved a higher removal torque than that of the control RBM implants. Removal torque measurements are used as biomechanical indicators of osseointegration of implants. A positive correlation was observed between the removal torque of the implant and the amount of BIC [11], a higher removal torque might be interpreted as improved osseointegration [12].

RBM surface blasting with coarsely ground calcium phosphate gives the implant’s surface a coarse appearance without leaving residues. RBM implants have shown significantly higher removal torque values and BIC percentages than machined implants [7,8,13]. Also, laser-treated implants achieve higher removal torque values than machined implants [12].

The RBM implants showed 0.5-3.0 μm porous structures that were dispersed irregularly throughout the implant surface, whereas the RBM-laser implants had many 20-40 μm porous structures dispersed uniformly over the implant surface. These coarse porous structures contained hundreds of fine porous structures within them, which play an important role in the growth of bone tissue around the implant surface area. This indicates that active bone appositions with strong bone-to-implant attachment were achieved on the RBM-laser implant surfaces, which might be the reason for their higher removal torque.

The degree of surface titanium contamination determines the mechanical stability and the osseoconductive quality of the implant [14]. Laser processing had been reported to produce implant’s surfaces with a high degree of purity [10].

EDS analyses of the RBM implants showed considerable surface contamination with several foreign elements. However, RBM-laser implants showed less contamination with Ti and O peaks. This showed that RBM-laser treatment of the implant’s surface led to a surface roughness with a higher degree of purity than that of RBM implants.

Faeda et al. [15] reported that the surface roughness of laser-treated implants was 1.38±0.23 μm, which was ten times higher than that of machined implants. Wennerberg et al. [16] reported that screw-shaped implants with an average surface roughness of about 1.5 μm were found to be optimal for bone growth, based on removal torque test experiments. In contrast, Rønold et al. [17] found that an optimal surface roughness for bone attachment was in the range of 3.62 to 3.90 μm. Other studies state that the optimal surface roughness of laser-treated implants
remains unknown [18] and that optimal roughness values may differ depending on the surface modification employed on the implant [19].

Surface roughness was measured at the top, valley and incline using an Optical Profiler (Wyko NT8000, Veeco). The experimental group revealed a considerably higher surface roughness than the control group. This suggests that a higher surface roughness increased the implant’s surface area and affected the bone-implant bonding. Moreover, considering the correlation between surface roughness and Sdr, the surface roughness of the experimental group increased significantly compared to the control group. This was attributed to the porous structures resulting from the surface treatment process of the experimental implants. In addition, the increased surface roughness of the RBM-laser implants leads to greater mechanical shear strength, resulting in the need of a higher torque for the implant to be removed.

Surface modifications through a range of processes have resulted in increased BIC and biomechanical bone bonding compared to the smooth surface of machined implants [5,20,21]. Interfacial bone formation may also be promoted by a roughened surface, as a significantly greater percentage of BIC has been observed adjacent to micro rough titanium surfaces, compared to the percentage of BIC observed on machined or polished titanium surfaces [22].

In this study, the RBM-laser-treated surfaces had a higher BIC than the control RBM surfaces. However, these differences were not statistically significant. Nevertheless, the experimental group showed higher total BIC than the control group. This might indicate that RBM-laser-treated surfaces acquire a more convenient surface topography.

Laser treatment of implants has been demonstrated to be a fast, clean, and easy method for implant’s surface modification [23]. Rong et al. [18] reported that laser-treated and acid-etched surfaces had better osteoconductivity than laser-treated surface. Recently, it was demonstrated that associating laser-treated implants with HA coatings could reduce the implant’s healing period [15]. Also, implant’s surfaces produced by dual treatment with laser etching and microarc oxidation resulted in enhanced bone responses compared to those obtained using pure titanium machined implant surfaces [24].

Within the limitation of this study, the experimental group showed a higher percentage BIC and removal torque \((p=0.013)\), favorable surface roughness. Therefore, RBM followed by laser surface treatment can promote a favorable osseointegration. Nevertheless, further studies of RBM and laser-treated surfaces are still needed.

**Conflicts of interest**

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

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Eun-Kyong Kim, https://orcid.org/0000-0001-9582-1415

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Periodontol 2008;38:467–73.
Awareness during general anesthesia occurs in approximately 0.1–0.2% of cases; nevertheless, particular attention is required because it can lead to critical complications including insomnia, depression, anxiety, and post-traumatic stress disorder. To prevent these complications, bispectral index (BIS) and end-tidal anesthetic gas (ETAG) concentration monitoring are commonly used to examine patient consciousness during surgery. In the present case, an 80-year-old man was scheduled for total gastrectomy. Anesthesia was maintained using desflurane 4.0–5.0% vol, oxygen, and nitrous oxide. The authors simultaneously monitored BIS, which was maintained between 37 and 43, and ETAG, which was maintained between 0.9 and 1.2 minimum alveolar concentration (MAC). After the operation, however, the authors were surprised to learn that the patient complained of awareness during anesthesia. Although BIS and ETAG concentration monitoring are useful in preventing awareness during anesthesia, they cannot be completely trusted. Even though BIS was maintained at approximately 40 and ETAG at 0.7–1.3 MAC, awareness during anesthesia occurred.

Keywords: Anesthesia; Awareness; Consciousness monitors

Introduction

Awareness during anesthesia occurs in 0.1–0.2% of patients who undergo surgery requiring general anesthesia [1,2]. Awareness during anesthesia is rare, but when it occurs, it can have grave consequences for patients because it can trigger wide-ranging and even serious complications, such as insomnia, depression, anxiety and nightmares, and post-traumatic stress disorder [3]. Many anesthesiologists use diverse methods to monitor patient consciousness during anesthesia to prevent such awareness during anesthesia. Among the equipment used for this purpose, bispectral index (BIS) and end-tidal anesthetic gas (ETAG) concentration monitoring are widely used. Avidan et al. [4] reported an occurrence rate of 0.24% for anesthesia awareness in a BIS group, in which BIS was maintained between 40 and 60, and 0.07% in a group in which ETAG was maintained between 0.7 and 1.3 minimum alveolar concentration (MAC). Therefore, we believed that the simultaneous combination of these two monitoring methods would be more effective than if used alone for preventing awareness during anesthesia. Herein, we report our experience of patient awareness during anesthesia that occurred despite the simultaneous combination of BIS and ETAG concentration monitoring. Written consent was obtained from the patient for publication of anonymized case details.

Case

The patient was an 80-year-old man (weight 49.7 kg, height 1.60
m), who was scheduled to undergo subtotal gastrectomy due to stomach cancer. No specific findings were noted in his medical history, and his preoperative chest x-ray and electrocardiogram were normal.

Additionally, all of the patient’s laboratory test results were in the normal range. He had a history of total hip replacement under spinal anesthesia; however, he did not experience awareness during anesthesia for that procedure. This particular operation (gastrectomy) was his first time under general anesthesia.

His blood pressure (BP) was 100/60 mmHg, with a heart rate of 78 beats/min, and a body temperature of 36.5°C; all vital signs were within the normal range in the waiting room before the operation. Premedication of 0.2 mg of glycopyrrolate was administered by intramuscular injection. After arrival to the operating room, the patient’s pre-induction BP was 110/65 mmHg, heart rate 72 beats/min, and oxygen (O₂) saturation 98%, which were all in the normal range; BIS (BisVISTA, Aspect Medical Systems, USA) was >95.

Anesthesia was induced by inhaling 8 L/min of 100% O₂ for 2 min through a face mask, followed by an intravenous injection of propofol 100 mg. The loss of eyelid reflex was confirmed before intravenous administration of rocuronium bromide 50 mg and fentanyl 50 μg. After confirmation of muscle relaxation, an endotracheal tube was inserted, which was ventilated 10 times/min using a tidal volume of 8 mL/kg while end-tidal carbon dioxide was maintained in the range 35 to 36 mmHg. Thereafter, ETAG concentration was measured while anesthesia was maintained with desflurane 4.0–5.0% vol, O₂ (1.5 L/min), and nitrous oxide (1.5 L/min). Post-induction BP was approximately 85/45 mmHg. Therefore, desflurane was maintained at 4.0% vol, and BIS at the time was maintained in the range 35 to 37, and ETAG was 0.9 MAC. From approximately 50 min after the initiation of anesthesia, the patient’s BP slowly escalated to approximately 120/80 mmHg. Subsequently, fentanyl 50 μg and rocuronium bromide 10 mg were additionally administered, and desflurane was maintained at 5.0% vol. At that time, the BIS remained in the range 37 to 43, and the ETAG was 1.2 MAC.

Multiple metastatic lesions were found on the abdominal wall, as well as on the omentum, during the operation. Hence, the patient’s family member was called in approximately 1 h after the initiation of anesthesia and was informed that due to severe cancer metastasis, it was not possible to continue the operation any further. Subsequently, the operators immediately closed the incision, and the patient exhibited stable vital signs approximately up to 120/80 mmHg until completion of the surgery. No sudden increase in BP or heart rate was observed (Table 1). The total duration of anesthesia was 2 h and, because the patient was able to sufficiently breathe on his own, the endotracheal tube was removed and the patient was transferred to the post-anesthesia care unit (PACU). After completely recovering consciousness in the PACU, he did not exhibit any specific expression of awareness during anesthesia. However, immediately after he was moved back to his in-patient room, he complained of awareness during anesthesia by telling his guardian that he overheard the conversation between the operator and his caregiver, felt the pain, and experienced inability to move during a short period of time. The patient did not exhibit any specific sequelae, despite the episode of awareness during anesthesia, and was able to tolerate further treatment processes.

**Table 1. Change of BP, pulse, BIS value, and ETAG concentration**

<table>
<thead>
<tr>
<th>Variable</th>
<th>00:00</th>
<th>00:15</th>
<th>00:30</th>
<th>00:45</th>
<th>01:00</th>
<th>01:15</th>
<th>01:30</th>
<th>01:45</th>
<th>02:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td>110/65</td>
<td>85/50</td>
<td>85/45</td>
<td>90/45</td>
<td>110/65</td>
<td>120/80</td>
<td>120/75</td>
<td>120/70</td>
<td>120/70</td>
</tr>
<tr>
<td>Pulse (beats/min)</td>
<td>88</td>
<td>70</td>
<td>77</td>
<td>80</td>
<td>78</td>
<td>78</td>
<td>82</td>
<td>78</td>
<td>65</td>
</tr>
<tr>
<td>BIS value</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>37</td>
<td>39</td>
<td>43</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>ETAG concentration</td>
<td>0</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

BP, blood pressure; BIS, bispectral index; ETAG, end-tidal anesthetic gas; MAC, minimum alveolar concentration.

**Discussion**

Pollard et al. [5] reported that the incidence of awareness during anesthesia has been reduced to 0.007% owing to advances in anesthetic monitoring equipment and the accumulated experiences of anesthesiologists. However, Errand et al. [6] reported that the occurrence rate of awareness during anesthesia is relatively higher—by 1%—in high-risk groups, which have a history of awareness during anesthesia. It has been reported that female sex, young age (in adults), obesity, previous awareness, emergency operations, type of surgery (cardiac, thoracic and obstetric operation), and use of neuromuscular blockade were risk factors for awareness during anesthesia [7]. In the present case, we used neuromuscular blockade for proper muscle relaxation for abdominal surgery; there were no other risk factors for awareness during anesthesia. Pandit et al. [7] recommended to avoid or
minimize the use of neuromuscular blockade, and to always use a nerve stimulator before allowing emergence from anesthesia to reduce awareness during anesthesia.

To prevent awareness during anesthesia, the use of benzodiazepine during premedication, induction and maintenance of anesthesia, and maintenance of optimal depth during anesthesia through BIS monitoring and/or maintenance of volatile anesthetics with appropriate ETAG concentrations have been recommended [5]. In the present case, we applied both BIS and ETAG concentration monitoring, but did not use benzodiazepine, which may have been the cause of his awareness during anesthesia.

BIS equipment is widely used to prevent awareness during general anesthesia to avoid overdose of anesthetics by using an optimal dose of anesthetics depending on each patient, to minimize any side effects, and to facilitate rapid postoperative recovery [8]. Usually, the BIS value needs to be in the range of 40-60 to render the patient unconscious during the operation under general anesthesia [9]. However, when the BIS value is approximately 50, it is not sufficient to fully prevent awareness during anesthesia; however, when it is approximately 40, it can significantly reduce the incidence of awareness during anesthesia [10,11]. Therefore, in the present case, we maintained BIS at approximately 40 (i.e., 37–43). Myles et al. [12] reported that the implementation of BIS monitoring has reduced awareness during anesthesia by 82% in high-risk groups. Additionally, Avidan et al. [4] reported that awareness during anesthesia occurred in 7 of 2,861 patients (0.24%), even in the group in which BIS was maintained at values ranging from 40 to 60. Other than BIS, McLeskey [13] recommended the use of ETAG concentration monitoring for accurate assessment of the relationship between the dose of anesthetics and awareness during anesthesia. When ETAG was in the range 0.7–1.3 MAC, the occurrence of awareness during anesthesia was significantly mitigated [1,14]. In the latest study from Avidan et al. [4], the group that had ETAG maintained between 0.7–1.3 MAC exhibited an anesthesia awareness occurrence rate of 0.07%.

In the present case, despite an abdominal incision and surgical stimulation, the patient’s BIS value was maintained in the range 37 to 43 without sudden changes, and ETAG was maintained in the range 0.9–1.2 MAC. At the moment of awareness that the patient specifically recalled, the ETAG was 1.1–1.2 MAC.

When any awareness during anesthesia occurs, it can cause sympathetic nervous system activation that manifests as symptoms such as sweating, tachycardia, hypertension, and mydriasis, among others. In the present case, none of these symptoms were observed; however, normotension (110/65 mmHg) manifested at the time of awareness.

Even though anesthesia was maintained at the optimal depth in the present case, with BIS maintained at approximately 40 and ETAG concentration maintained at 0.9–1.2 MAC, and vital signs were stable, awareness during anesthesia occurred. This indicates that although BIS and ETAG concentration monitoring could be good options to prevent awareness during anesthesia, we should recognize that awareness during anesthesia can still occur, even at BIS values and ETAG concentrations of optimal anesthesia depth. It is commonly believed that BIS monitoring, combined with additional monitoring, such as ETAG, should reduce the incidence of awareness during anesthesia. However, to date, there have been no reports describing the simultaneous combination of BIS and ETAG monitoring, or that combined they are more effective for preventing awareness during anesthesia than used alone. Nevertheless, further investigation is warranted.

In conclusion, awareness during anesthesia is a rare occurrence. Even if proper vital signs, BIS value (40–60) and ETAG concentration (0.7–1.3 MAC) are maintained, it is not possible to completely prevent awareness during anesthesia.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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References


Estrogen-secreting adrenocortical carcinoma

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³Department of Urology, Korea Cancer Center Hospital, Korea Institute of Radiological and Medical Sciences, Seoul, Korea

Adrenocortical carcinoma is a rare type of endocrine malignancy with an annual incidence of approximately 1–2 cases per million. The majority of these tumors secrete cortisol, and a few secrete aldosterone or androgen. Estrogen-secreting adrenocortical carcinomas are extremely rare, irrespective of the secretion status of other adrenocortical hormones. Here, we report the case of a 53-year-old man with a cortisol and estrogen-secreting adrenocortical carcinoma. The patient presented with gynecomastia and abdominal discomfort. Radiological assessment revealed a tumor measuring 21×15.3×12 cm localized to the retroperitoneum. A hormonal evaluation revealed increased levels of estradiol, dehydroepiandrosterone sulfate, and cortisol. The patient underwent a right adrenalectomy, and the pathological examination revealed an adrenocortical carcinoma with a Weiss’ score of 6. After surgery, he was treated with adjuvant radiotherapy. Twenty-one months after treatment, the patient remains alive with no evidence of recurrence.

Keywords: Adrenal gland neoplasm; Adrenocortical carcinoma, Estrogen; Gynecomastia

Introduction

Adrenocortical carcinoma is a very rare type of tumor reported to occur in approximately 1–2 per million people per year [1,2]. Adrenocortical carcinomas can be divided into functional or nonfunctional tumors according to the hormone secretion status; however, most adrenocortical tumors secrete cortisol and rarely secrete androgens and aldosterone [3,4]. International reports have rarely described adrenocortical carcinomas that secrete estrogen; although a few existing reports have used the term feminizing adrenal tumors (FATs) to indicate adrenocortical carcinomas that secrete estrogen in men, such cases have not previously been reported in Korea [5]. We report a case of estrogen-secreting adrenocortical carcinoma in a 53-year-old man who presented with abdominal discomfort and gynecomastia.

Case

Patient: A 53-year-old man.

Chief complaint: Gynecomastia and abdominal discomfort.

Present illness: A 53-year-old man with a history of hypertension visited the Department of Family Medicine at our hospital with the complaint of a 6-month history of bilateral breast enlargement, as well as right-sided flank pain and abdominal discomfort. After abdominal computed tomography (CT) revealed an abdominal mass, he was referred to an endocrinologist for a hormone assessment and further examination.

Past history: He had been diagnosed with hypertension 5 years earlier and was taking medication.
**Physical examination:** The patient's height and weight were 173 cm and 72 kg, and his body weight had increased by approximately 10 kg over the last 6 months. His blood pressure was 120/80 mmHg, body temperature was 36.5°C, pulse rate was 68 beats/minute, and respiration rate was 18 breaths/minute. His consciousness was clear, and he had no symptoms such as palpitation, sweating, or headache. His conjunctiva and sclera were normal, and a head and neck examination detected no moon face, flushing, or other specific symptoms. During a chest examination, a 4-cm-sized gynecomastia was observed bilaterally with tenderness. The patient's respiratory sounds were normal, and no heart murmurs were detected. An abdominal examination revealed no purple striae but did reveal tenderness in the right upper quadrant and a palpable hard, fixed mass with an approximate size of 15 cm. The patient's bowel sounds were normal, and he exhibited no edema or tenderness in his limbs. There were no skin rashes or specific findings of the joints. A lower limb test revealed normal sensory and motor functions. The patient denied erectile dysfunction and reported a normal shaving frequency of once every 2 days.

**Laboratory findings:** A complete blood cell count during blood analysis revealed a leukocyte count of 9,330/mm³ (neutrophils, 75.3%), hemoglobin level of 11.4 g/dL, and platelet count of 329,000/mm³. A comprehensive metabolic panel indicated the following levels: aspartate aminotransferase and alanine aminotransferase, 31 and 23 IU/L, respectively; alkaline phosphatase, 80 IU/L; total protein, 6.2 g/dL; albumin, 3.3 g/dL; calcium, 8.8 mg/dL; blood urea nitrogen, 9.0 mg/dL; and creatinine, 0.7 mg/dL. A hormonal assessment revealed increases in the levels of dehydroepiandrosterone, estradiol, and 24-hour urine cortisol but decreases in the levels of follicle-stimulating hormone, testosterone, and adrenocorticotropic hormone. The patient's serum cortisol and luteinizing hormone levels were normal (Table 1). His morning plasma cortisol level was 16.3 μg/dL (range, <5 μg/dL), and this was not suppressed during an overnight dexamethasone suppression test. The patient rejected an additional planned low-dose dexamethasone suppression test. A 24-hour urine test yielded normal vanillylmandelic acid, metanephrine, and epinephrine levels.

**Table 1. Hormone study**

<table>
<thead>
<tr>
<th></th>
<th>Pre op.</th>
<th>Post op. 3 mon</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEA-S (μg/dL)</td>
<td>578.30</td>
<td>152.50</td>
<td>51.8-470.7</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>820.34</td>
<td>70.32</td>
<td>15-47</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>1.07</td>
<td>5.25</td>
<td>1.3-8.4</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>1.35</td>
<td>3.50</td>
<td>1.0-5.3</td>
</tr>
<tr>
<td>Testosteron (ng/mL)</td>
<td>0.70</td>
<td>2.64</td>
<td>2.6-10.1</td>
</tr>
<tr>
<td>Cortisol (μg/dL)</td>
<td>16.30</td>
<td>11.70</td>
<td>5-27</td>
</tr>
<tr>
<td>ACTH (pg/mL)</td>
<td>4.50</td>
<td>22.50</td>
<td>10-60</td>
</tr>
<tr>
<td>17-α-OH progesterone (ng/mL)</td>
<td>2.74</td>
<td>-</td>
<td>0.5-3.4</td>
</tr>
<tr>
<td>Aldosteron (ng/dL)</td>
<td>11.10</td>
<td>-</td>
<td>1-16</td>
</tr>
<tr>
<td>Plasma renin activity (ng/mL/hr)</td>
<td>0.60</td>
<td>-</td>
<td>0.1-2.3</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>11.06</td>
<td>-</td>
<td>1.8-15.9</td>
</tr>
<tr>
<td><strong>24 hr urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free cortisol (μg/day)</td>
<td>134.20</td>
<td>-</td>
<td>6-75</td>
</tr>
<tr>
<td>VMA (mg/day)</td>
<td>2.09</td>
<td>-</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Metanephrine (mg/day)</td>
<td>0.28</td>
<td>-</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>Epinephrine (μg/day)</td>
<td>2.95</td>
<td>-</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Norepinephrine (μg/day)</td>
<td>40.24</td>
<td>-</td>
<td>&lt;80</td>
</tr>
</tbody>
</table>

op., operation; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle stimulating hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; 17-α-OH progesterone, 17-α-hydroxy progesterone; VMA, vanillylmandelic acid.

**Radiological findings:** Simple chest radiography yielded normal findings. Abdominal CT showed a heterogeneously well-defined single mass with dimensions of 18×11×18 cm between the liver and right kidney (Fig. 1). On renal CT, the mass was not observed to have infiltrated the liver, kidney, and surrounding blood vessels, and lymph node metastasis and ascites were not observed. A chest CT scan and bone scan showed no metastasis.

**Treatment and progress:** The patient underwent open surgery under general anesthesia to remove the right adrenal tumor. The tumor was found to have adhered to the liver, kidney, and inferior...
vena cava, and a partial liver resection was performed. Inferior vena cava and kidney involvement were not observed. There were no postoperative complications. The final tumor classification was pT2N0M0, stage 2, and the patient received additional adjuvant radiation therapy. Postoperatively, he was given replacement therapy with hydrocortisone for suspected adrenal insufficiency. Three months after surgery, his estradiol level was 70.32 pg/mL and the gynecomastia had almost disappeared. The patient remains in good health without any local recurrence or metastasis at 21 months after diagnosis and treatment, and the gynecomastia has almost resolved.

Pathological findings: A gross pathology examination of the surgically resected tumor revealed a size of 21×15.3×12 cm and a smooth and partially fibrous outer surface. An analysis of the tumor cross-section revealed encapsulated yellowish-brown nodules and widely distributed necrotic and hemorrhagic lesions (Fig. 2).

A microscopic examination revealed tumor necrosis and readily apparent mitosis. The histopathologic examination revealed necrosis, diffuse architecture, a high nuclear grade, high mitotic rate, atypical mitotic figures, and a clear cell frequency of <25% (Fig. 3). The immunohistochemistry results included positive staining for inhibin α, MART-1, and calretinin and a Ki67 proliferation index of 20% (Fig. 4). No tumor invasion was observed in the resected mass.

Discussion

Feminizing adrenal carcinomas are very rare, accounting for 1–2% of all adrenocortical carcinomas [5]. According to one study, fewer than 100 cases were reported prior to 1994, and only a few cases have since been reported. Additionally, no previous report has described a case of a FAT in Korea [6].

Clinical manifestations of FATs include gynecomastia, testicular atrophy, and a loss of sexual desire; we note that our patient complained of gynecomastia and loss of libido [7]. Although gynecomastia often occurs in men as a consequence of adverse drug reactions, renal failure, and cirrhosis [8], it is very rarely caused by the direct secretion of estrogen from an adrenocortical carcinoma.

In our patient, we observed no tumor involvement in

![Fig. 1](https://example.com/fig1.png)

Contrast-enhanced abdominal computed tomography reveals a right adrenal mass. Axial (A) and coronal (B) images of a well-defined, heterogeneously enhancing mass measuring 18×11×18 cm between the liver and right kidney.

![Fig. 2](https://example.com/fig2.png)

Gross appearance of the resected adrenal tumor. The mass was large, solitary, and circumscribed tumor (21×15.3×12 cm). The cut section is yellowish-tan in color, with a variegated appearance. Many areas of necrosis and hemorrhage are visible.
the surrounding organs or metastasis. However, functional adrenocortical carcinomas are frequently malignant, especially if estrogen is secreted [9-11]. Adrenocortical carcinoma is diagnosed through gross examination, microscopic examination, and immunohistochemical evaluation. The Weiss score is used as a histologic criterion, and a diagnosis of malignancy is made if at least three of nine criteria are met. In our case, six of nine criteria were satisfied, and the patient was diagnosed with a malignant tumor [12] (Table 2).

Adrenocortical carcinoma is an aggressive tumor with a very poor prognosis. A study conducted at the Memorial Sloan Kettering Cancer Center reported 5-year survival rates of 60% for stage 1–2 adrenocortical carcinomas and 10% for stage 3–4 cases [13]. No statistical analyses of data regarding the prognosis of FAT have been reported; however, only two patients have survived for more than 10 years after diagnosis, irrespective of treatment [5]. Among cases of adrenocortical carcinoma, prognostic factors include age, mitotic count and the Ki-67 index. However, staging and the possibility of surgical resection are the most important factors [14-16]. Although locally advanced stage 1–3 adrenocortical carcinomas can be cured by surgical resection, the possibility of local recurrence and metastasis within 2 years is approximately 65% among patients who underwent a radical resection [17]. Laparoscopic adrenalectomy is considered safe, but open surgery is preferred as a curative treatment option. In our case, the patient underwent a radical resection via open surgery, which addressed the large tumor size and the adhesions to the liver, kidney, and inferior vena cava [18,19].

According to the National Comprehensive Cancer Network

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**Fig. 3.** Microscopic tumor findings. (A) The tumor exhibits a diffuse solid growth pattern with necrosis on the right side (hematoxylin and eosin [H&E] stain, x40). (B) The tumor cells are round-to-oval in shape with high-nuclear-grade nucleoli. Mitoses are frequently observed (H&E stain, x400).

**Fig. 4.** Immunohistochemical staining of the tumor. The Ki-67 index of the tumor cells is 20% (x400).

**Table 2.** Weiss score

<table>
<thead>
<tr>
<th>Present case</th>
<th>HPF, high power field.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear grade III or IV based on Fuhman criteria</td>
<td>1</td>
</tr>
<tr>
<td>Mitotic index &gt;5/50 HPF</td>
<td>1</td>
</tr>
<tr>
<td>Atypical mitoses</td>
<td>1</td>
</tr>
<tr>
<td>Clear or vacuolated cells &lt;25%</td>
<td>1</td>
</tr>
<tr>
<td>Diffuse architecture &gt;33%</td>
<td>1</td>
</tr>
<tr>
<td>Microscopic necrosis</td>
<td>1</td>
</tr>
<tr>
<td>Venous invasion</td>
<td>0</td>
</tr>
<tr>
<td>Sinusoid invasion</td>
<td>0</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td>0</td>
</tr>
<tr>
<td>Total Weiss score</td>
<td>6</td>
</tr>
</tbody>
</table>
guideline, the delivery of external radiation therapy to the tumor removal site is recommended if the case is considered to be at a high risk of local recurrence after radical resection. In our case, the massive tumor size and Ki-67 index of 20% indicated a high-risk status, and surgical resection was followed by radiotherapy. Previous studies have reported that radiotherapy could significantly reduce the recurrence rate in cases of adrenocortical carcinoma [20]; however, FATs are rare and the evidence regarding treatment remains at a case-centered level.

In conclusion, we diagnosed an adrenocortical carcinoma with estrogen secretion in a male patient with gynecomastia, which was treated via surgery and radiation therapy and monitored through continuous follow-up visits. As noted, FATs are rarely reported in the international literature and have not previously been reported in Korea, and therefore, we have presented our case in the context of a literature review.

Conflicts of interest
No potential conflicts of interest relevant to this article were reported.

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References
Isolated tubal torsion in the third trimester of pregnancy managed with simultaneous salpingectomy and cesarean section

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Isolated tubal torsion is an uncommon cause of acute abdomen in pregnancy. Tubal torsion may occur in the absence of adnexal disease. Diagnosing tubal torsion is especially difficult in pregnancy because no precise preoperative radiological and biochemical investigations have been conducted. Most patients are diagnosed during surgery. Here, I present a case of isolated tubal torsion in a pregnant woman at 35 weeks and 6 days of gestation that was managed with salpingectomy and cesarean section simultaneously.

Keywords: Abdominal pain; Pregnancy; Torsion abnormality

Introduction

Isolated tubal torsion is an extremely rare event with a reported incidence of 1 in 1.5 million women. Most of the cases occur in nonpregnant women but approximately 12% of isolated tubal torsion were diagnosed during pregnancy [1]. The first case of tubal torsion in women was reported in 1890 by Bland-Sutton [2]. Diagnosing isolated tubal torsion is difficult because of the wide range of differential diagnoses including many other emergency causes for abdominal pain such as ovarian torsion, appendicitis, pelvic inflammatory disease, ectopic pregnancy, renal colic, and diverticulitis. The predisposing factors are either extrinsic abnormalities, including paratubal mass, peritubal adhesions, and uterine enlargement compressing the fallopian tubes, or intrinsic tubal abnormalities, including hydrosalpinx, tubal ligation, or endometriosis [3,4]. The risk increases in pregnancy particularly in the presence of the above-mentioned predisposing factors.

Here, I report a case of isolated tubal torsion in a pregnant woman at 35 weeks and 6 days of gestation that was managed with salpingectomy and cesarean section simultaneously.

Case

A 36-year-old, gravida 2, para 1, patient at 35 weeks and 6 days of gestation was referred to our hospital because of acute right abdominal pain that began 2 days prior to visit. However, she had no vomiting, diarrhea, constipation, or fever. In the right lower quadrant, direct tenderness without rebound tenderness was the only abnormal finding in her physical examination. She had
no medical history and the surgical history did not reveal any abnormalities except that she gave birth to a full-term, healthy baby boy by cesarean section 2 years earlier. Her vital signs at the time of admission were normal and the laboratory tests showed the following results: hemoglobin 10.8 g/dL (range, 12-15.9 g/dL), white blood cells 9,330/µL (range, 4,000-10,000/µL), and platelets 213,000/µL (range, 140,000-400,000/µL), with C-reactive protein level increased to 42 mg/L (range, 0-5 mg/L). The non-contrast-enhanced abdominopelvic computed tomography (CT) performed in the secondary hospital prior to referral to our hospital revealed a homogeneous cystic mass measuring 44×25 mm in the right lower quadrant; no other abnormal findings were observed (Fig. 1A).

Ultrasonography performed in our hospital revealed a single live fetus at 36 weeks of gestation with normal amniotic fluid and placenta as well as a 37-mm sized hypoechoic cystic mass in the right lower quadrant (Fig. 1B). The nonstress test did not provoke any uterine contractions and showed normal accelerations and variability of the fetal heart rate. Because the pain in her right lower abdomen did not subside and tended toward exacerbation within 24 hours of admission, I suspected right adnexal torsion, appendicitis, or rupture of right ovarian cyst. Exploratory laparotomy was performed to prevent complications such as tissue necrosis caused by adnexal torsion or panperitonitis caused by appendicitis. Given the 36 weeks of gestation and normal fetal status as well as the history of cesarean section I decided to perform a simultaneous cesarean section. A healthy baby girl weighing 2,500 g was born. There was no abnormal finding such as adhesion during cesarean section but torsion of midportion of the right fallopian tube and necrosis measuring 6×4 cm size was observed (Fig. 2A). The cystic mass observed on CT scan and ultrasonography was hydrosalpinx which was thought to be the result of ischemic or traumatic tubal injury due to torsion. The contralateral fallopian tube both ovaries and the appearance and size of the appendix were normal. A right salpingectomy was performed and the operative specimen revealed an ovoid shaped, purple-brown colored, diffusely congested fallopian tube (Fig. 2B). The surgery was completed without any complication. The postoperative recovery of the patient and follow-up of her baby were uneventful. A histological examination revealed that the wall of the fallopian tube was severely edematous and congested.

**Discussion**

Isolated tubal torsion is one of various conditions accounting for lower abdominal pain that usually affects reproductive women. The condition is extremely rare (i.e., 1 in 1.5 million women), and has been described as a rare cause of acute abdomen in pregnancy. As far as I know, approximately 30 cases of isolated tubal torsion during pregnancy seem to have been reported in English literatures [5-11]. The lack of specific clinical...
presentation, laboratory findings, and imaging features makes it difficult to identify an isolated tubal torsion preoperatively and subsequently delays prompt surgical intervention to prevent irreversible vascular changes in the fallopian tube [12].

Torsion generally occurs in abnormal fallopian tubes; however, it might also develop in normal ones. Structural abnormalities such as hematosalpinx, hydrosalpinx, cyst of Morgagni, previous tubal ligation or surgery, peritubal adhesions, and tubal or ovarian neoplasm are believed to play a role in the development of torsion. Moreover, hormonal medications which result in hypermobility or tubal spasm by affecting the normal physiology, trauma to the fallopian tube and varicose veins in the mesosalpinx are also reported to be other etiological factors [13].

In this case, the only obvious etiological factor for the development of the twisted fallopian tube was assumed to be pregnancy which can play a role as a rotational force resulting from changes in the abdominal cavity and uterine size. Any adhesions related to the previous cesarean section could also be a possible etiological factor, but there was no abnormal finding in this case. A paratubal cyst could not be diagnosed on pathological examination. Tubal torsion occurs more commonly on the right side than on the left. Presumably, the possibility of developing torsion on the right side is higher because of the movement of the appendix and small bowel on the right side while the mesentery of the sigmoid colon is attached to the left side [14].

Imaging findings in isolated tubal torsion are nonspecific and clinical correlation is necessary. In the presence of acute pain, the ultrasonographic findings of a dilated tube with a normal-appearing ipsilateral ovary should point to the possibility of isolated tubal torsion. Ultrasonographic signs of a dilated fallopian tube are a hyperechoic wall a folded configuration and foci of echogenicity protruding into the lumen, and a tapering end broad toward the uterine cornua. In such situations, the fallopian tube is examined for a whirlpool mass in close proximity. This mass is usually smaller and less obvious than those seen in ovarian torsion [15]. In this case, the patient underwent CT scan to evaluate acute pain at another hospital. However, performing a CT scan on the mother is not appropriate because it exposes the fetus to high radiation. Magnetic resonance imaging is considered to be more appropriate than CT scan if additional imaging test is required after first performing an ultrasound.

Surgery by laparoscopy or laparotomy is the standard treatment for isolated tubal torsion and leads to a definitive diagnosis. Laparoscopy remains the standard procedure for diagnosis and treatment as early diagnosis may allow detorsion/salvage of the tube in nonpregnant patients and patients in early pregnancy. In view of technical difficulties of the laparoscopic access to adnexa, laparotomy is generally preferred in advanced gestation [5]. In addition, most surgeons consider laparotomy appropriate in advanced third-trimester pregnancy in view of the possible option of delivering the fetus as seen in this patient. A patient with tubal torsion without any ischemic damage can be successfully treated with tubal detorsion which is preferred in the reproductive age group. If the tube is gangrenous, a malignancy can be suspected, and if the tube is diseased or if the patient does not pregnancy in the future, salpingectomy is preferred. In this case, since the tube was necrotic and no further pregnancies were planned, salpingectomy was performed.

In conclusion, isolated tubal torsion in pregnancy is an extremely rare cause of acute abdomen. However, it should be included in the differential diagnosis of acute abdomen in pregnancy. Early decision regarding surgical intervention will
prevent obstetric complications and may allow preservation of the tube.

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**Conflicts of interest**

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**References**

Malignant peripheral nerve sheath tumor (MPNST) is rare, accounting for 5–10% of all soft tissue sarcomas [1-3]. Half of MPNSTs occur in patients with neurofibromatosis type I (NF1), and the remainder are mostly sporadic MPNSTs (sMPNSTs). NF-MPNSTs have aggressive clinical features and a poor prognosis; after radical excision, NF-MPNSTs have high rates of recurrence rate and metastasis [2]. Most MPNSTs are single lesions; distant metastasis to skin tissue, soft tissue, or lymph nodes is rarely reported [3]. The most common site of lesion is the extremities, nerve root, or pelvic plexus [2], although MPNSTs can arise at any site of the human body [2]. Magnetic resolution imaging is very useful for diagnosing MPNSTs, but most MPNSTs are diagnosed with histologic testing after excision. Superficial MPNST in the cutaneous or subcutaneous tissue is very rarely found without NF1. Moreover, recurrent neurofibroma that transforms to MPNST is extremely rare, with only three cases reported by Chen et al. [4].

Case

A 62-year-old man attended our hospital because of a recurrent neurofibroma in the left upper quadrant (LUQ) area of the abdominal subcutaneous tissue since 2009. With the exception of a prior myocardial infarction, he had no other diseases. Since 2009, he had had excision and biopsy of neurofibroma in the subcutaneous tissue of the LUQ area of the abdomen three times; after the fourth, the patient had recurrence so he was referred to our hospital. On physical examination, there were no specific lesions, except an old operation scar and palpable mass in the LUQ of the abdomen (Fig. 1). Laboratory tests are non-
specific. On computed tomography (CT), a 5×6-cm tumor was observed between the skin and subcutaneous tissue in the LLQ of the abdomen (Fig. 2). Under suspicion of neurofibroma, the patient was admitted and scheduled for operation. Under general anesthesia, the lesion was radically excised, and a flap and skin graft were done. The flap failed 17 days after the first operation, so a local advanced flap and skin graft were performed. Nineteen days after the second operation, the patient was discharged. Histologic testing showed tissue with specific spindle cells that were longitudinal and wavy, transformed from neurofibroma with low cellularity to MPNST with increased cellularity and pleomorphic cells (Fig. 3). On immunochemistry testing, an S100 protein test was weakly positive, and H3K27me3 staining was negative (Fig. 4); based on these results, we made a definite diagnosis of MPNST. The patient was regularly followed up in the departments of hemato-oncology and radio-oncology and treated with radiation therapy; he had no recurrence at 3 months.

Discussion

MPNSTs were previously named malignant schwannomas, for Schwann cells. MPNSTs are known to originate from a complex including Schwann cells, fibroblasts, and perineural cells. In 1978, malignant schwannomas, malignant nerve sheath tumors, neurosarcoma, and neurofibrosarcomas were grouped together as MPNSTs by the World Health Organization [5]. MPNST is a malignant soft tissue sarcoma originating from peripheral nerves.
or soft tissues, and they can arise in any site of the body [2,3,6,7]. MPNSTs comprise 5–10% of all sarcomas [1–3], and they occur in 1 person per million per year. In addition, MPNST of the skin or subcutaneous layer is very rare [8]. NF1 MPNSTs account for about half of all MPNSTs; the remainder are nearly all sMPNSTs [1,4]. The median age of patients with sMPNSTs is between 30 and 60 years [2]; the size of these tumors is smaller, and the stage shows less progression than NF-MPNSTs [1]. In the present case, the patient was 62 years old, close to the median age range for sMPNSTs. This was a very rare case of superficial sMPNST from recurrent neurofibroma in the subcutaneous layer. In 2015, Feng et al. investigated 13 patients admitted to Taipei Veterans General Hospital from 1999 to 2014 with superficial MPNST; of these, only three patients had body lesions [9]. Thomas et al. reviewed 230 cases of malignant spindle cell lesions of the skin, and only 2% of lesions were cutaneous MPNST [10]. However, there are no official statistics regarding MPNST occurrence, and most published articles are case reports.

Neurofibroma is a benign lesion that originates on the peripheral nerve sheath, the same as MPNST [11]. The most common sites of neurofibromas are cutaneous nerves of the body, neck, and head. A single lesion of neurofibroma has no specific symptoms [11]. The treatment of choice is excision; the prognosis of neurofibroma is better than that of MPNST, and postoperative malignant changes or local recurrences are very rare [11]. However, our patient showed both local recurrence and a malignant change.

Specific histologic features are very important in the diagnosis of MPNST. Neurofibroma, atypical neurofibroma, and MPNST are continuous progression lesions, making it difficult to distinguish them [7]. However, three features can be used to distinguish MPNST from neurofibromas: nuclear atypia, hypercellularity, and mitotic activity [7]. Both neurofibromas and MPNSTs comprise spindle cells, but neurofibromas have more collagen fibers than MPNSTs, which serves as another point of distinction between them [12,13]. In the present case, there was a specific transformation zone from the neurofibroma to the MPNST that showed increased cellularity and nuclear atypia.

Despite advancements in immunochemistry and molecular biology of sarcoma, diagnosis of MPNST remains difficult [13]. Recently, S100 protein has been reported as an important single marker for diagnosing MPNST. S100 protein has a nonspecific expression pattern but it has strong reproducibility [5]. However, there is a limitation that S100 protein is often negative in high-grade MPNST [14]. S100 protein is weakly positive in 50% of MPNSTs, but it is diffuse and shows a strong positive reaction in neurofibromas. Therefore, when S100 protein is diffuse and demonstrates a strong reaction, we can exclude possible MPNST [2,7]. Due to PRC2 inactivation in 70–90% of MPNSTs, tumor differentiation and progression is promoted, and demethylation of H3K27me3 (histone H3 lysine 27 trimethylation) develops [9]. Some researchers have highlighted demethylation of H3K27me3 in diagnosing MPNST with negative expression of H3K27me3. In about 51% of MPNSTs, H3K27me3 shows negative expression [9]. Studies of SOX10, EGFR, p53, and other markers are ongoing [15,16], but there is no single marker that can be used for a definite diagnosis MPNST. In this case, expression of S100 protein was weakly positive and H3K27me3 was negative, so these were helpful in diagnosing MPNST.
This patient had had surgery three times, and biopsy results had repeatedly revealed neurofibroma. We initially believed he might have neurofibroma, but in a fourth excision and biopsy, we determined that his diagnosis was superficial MPNST from recurrent neurofibroma. Both neurofibroma and MPNST are rare diseases. Gross morphologies and images of both diseases are difficult to distinguish, and both have similar histologic features, so differential diagnosis of these diseases is very difficult. Because MPNST has poor prognosis, it is necessary to distinguish these pathologies. Especially for superficial MPNSTs in the cutaneous or subcutaneous layer, it is easier to find lesions than for deep MPNSTs. Early detection and treatment will lead to better prognosis [8]. Generally, superficial tumors in the cutaneous or subcutaneous layers are easy to see in clinical environments. For more precise diagnosis and treatment, excision is necessary, to yield a negative specimen margin. In patients with recurrent tumor, malignant changes should be considered, and after excisional surgeries, physicians should perform additional testing, such as immunochemistry and so on.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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