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Is there enough evidence to recommend preoperative calcium and vitamin D in patients who undergo total thyroidectomy?

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See "Effectiveness of prophylactic calcium and vitamin D supplementation for preventing post-thyroidectomy hypocalcemia: a meta-analysis" by Hyeyeon Moon, Ju Won Seok, Keunyoung Kim, Hye Young Kim, Mi Kyoung Park, In Joo Kim, Kyoungjune Pak, Sunghwan Suh.

Hypoparathyroidism or hypocalcemia after total thyroidectomy occurs in up to 46% of patients temporarily and in 6.6% permanently [1,2]. It may be caused by inadvertent removal or devascularization of the parathyroid gland during surgery [3]. Therefore, surgeons should identify the parathyroid gland and preserve its vasculature to preserve the function. Even for experienced surgeons, post-thyroidectomy hypoparathyroidism is unavoidable in some cases, and the incidence may be higher for less experienced surgeons [4]. Management of post-thyroidectomy hypoparathyroidism includes supplementation of calcium and vitamin D, perorally or intravenously [5].

There have been several studies demonstrating the advantages of prophylactic calcium and vitamin D supplementation before surgery to prevent post-thyroidectomy hypocalcemia [6-9]. In a retrospective study of 65 patients who underwent total thyroidectomy, Maxwell et al. [7] presented that preoperative calcium and calcitriol supplementation for 5 days, in addition to routine postoperative supplementation, was associated with reduced incidence of symptomatic hypocalcemia, length of hospital stays, and overall charges. In the study, patients were divided into two groups; one with preoperative as well as postoperative supplementation with calcium carbonate, 1,000 to 1,500 mg, three times daily and calcitriol, 0.25 to 0.5 μg, twice daily; and the other receiving only postoperative supplementation. The authors reported that the prophylactic medication led to savings of 2,819 US dollars per patient due to lesser amount of postoperative intravenous calcium gluconate and shorter hospital stay (0.9 days). While there are limitations in its retrospective design and relatively small number of enrolled patients, this may be a representative study demonstrating the advantage of prophylactic calcium and vitamin D supplementation before total thyroidectomy. While Rowe et al. [9] reported that high dose pre-thyroidectomy vitamin D (cholecalciferol) did not reduce the overall rate of postoperative hypocalcemia, we are curious whether both calcium and vitamin D have a role in prevention of post-thyroidectomy hypocalcemia.

In this issue of *Kosin Medical Journal*, Moon et al. [10]

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support using preoperative prophylactic calcium and vitamin D supplementation with routine postoperative supplementation for patients who undergo total thyroidectomy. They concluded that such supplementation led to lower incidence of symptomatic hypocalcemia. It seems reasonable to agree that the policy may be cost-effective and relatively safe in most cases. However, I believe that we need more evidence to recommend this policy for every patient who undergoes total thyroidectomy. As the authors have insisted in their article, a small number (four) of studies enrolled for this meta-analysis and the heterogeneous characteristics between the studies seem to be critical bias. In fact, incidence of post-thyroidectomy hypocalcemia may be related to the body mass index of the patient, pathology or size of thyroid tumor, presence of diffuse parenchymal disease, and extent of surgery whether neck dissection is included or not [8,11]. Duration, amount or dosage of medication, criteria for postoperative hypocalcemia, protocols for management such as intravenous calcium infusion have not been described in the review within the article. Outcomes may have great differences related to the protocols of management of hypocalcemia other than prophylactic calcium and vitamin D supplementation itself.

While prophylactic calcium and vitamin D supplementation may be considered as effective management to reduce post-thyroidectomy symptomatic hypocalcemia, recommendations to apply this policy in everyday cases of total thyroidectomy should be given with careful informed consent and monitoring. Dosage and duration of medication before surgery, as well as contraindications or risk factors for prescription should be decided with further well-designed studies. In addition, comparative studies showing a higher preventive effect of prophylactic calcium and vitamin D for post-thyroidectomy hypocalcemia over prophylactic vitamin D only may be required. Moreover, studies to evaluate whether the outcomes differ by level of preoperative serum calcium or vitamin D may be helpful for clinical decisions. Based on such studies, we may be able to suggest evidence-based guidelines for pre-thyroidectomy prophylactic supplementation of calcium and vitamin D.

Article information

Conflicts of interest

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Review article

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Mucinous carcinoma of the breast: distinctive histopathologic and genetic characteristics

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Mucinous carcinoma is a rare histologic type of breast cancer that, when classified with favorable histology, can be treated with different therapeutic options. This study reviews the histologic findings of mucinous carcinoma that support or exclude favorable histology and emphasizes the necessity of an appropriate gross examination with radiologic findings for an accurate diagnosis. In addition, unusual findings such as micropapillary arrangements and lobular differentiation in mucinous carcinoma and their implications for prognosis and treatment are reviewed. Mucinous carcinoma involves upregulation of *MUC2*, a mucus-associated gene common in mucinous carcinoma of the breast as well as various other organs. In mucinous carcinoma, the fraction of genome altered and tumor mutation burden are lower than those of invasive carcinoma of no special type, the most common histology of breast cancer. In addition, the genetic alterations found in mucinous carcinoma are diverse, unlike the pathognomonic genetic alterations observed in other histologic types of breast cancer. These genetic features support the importance of conventional microscopic evaluations for the pathologic differential diagnosis of mucinous carcinoma of the breast in routine practice. A variety of breast lesions, including mucinous cystadenocarcinoma and mucocele-like lesions, as well as mucinous carcinoma from other organs, can mimic mucinous carcinoma of the breast. In order to obtain an accurate pathologic diagnosis, careful evaluation of the overall histopathologic characteristics and ancillary testing are required to provide information on appropriate treatment and prognosis.

Keywords: Adenocarcinoma, mucinous; Breast; Diagnosis, differential; Prognosis

Introduction

Mucinous carcinoma of the breast (MCB) is a rare histologic type of invasive breast carcinoma, accounting for approximately 2% of breast carcinomas [1]. In terms of its low rates of local and distant recurrence and high rate of disease-free survival, it has been categorized as indolent breast cancer with favorable histology [2,3]. Therefore, accurate diagnosis of MCB is important because it can avoid improper application of endocrine therapy or chemotherapy, the mainstays of breast cancer, allowing for more appropriate treatment [4].

This article reviewed the histopathologic characteristics of MCB, including uncommon findings, with an emphasis on prognostic value. In addition, the histopathologic and genetic characteristics of MCB were reviewed to determine their significance in differential diagnosis of breast lesions.

Histopathologic characteristics of mucinous carcinoma

MCB is one of the favorable histologies of breast cancer

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that can be treated differently from general breast cancer. However, favorable histology is only for pure types of MCB [4].

As a general rule for histologic types of breast cancer, pure MCB is defined as an invasive carcinoma where more than 90% of the lesion is made up of mucinous components [5]. If mucinous components constitute between 10% and 90% of the overall lesion, the diagnosis is "mixed MCB with other non-mucinous histologic types"; if mucinous components make up less than 10% of the overall lesion, it is classified as a "non-mucinous histologic type." In the latter, the focal element of the MCB should be described following pathologic diagnosis. The assessment of the proportion of mucinous components is based on the overall area occupied by the cancer. Therefore, such a proportion can only be fully assessed from surgical specimens and not small biopsy tissue. In addition, when MCB is observed in surgical specimens, careful gross examination and tissue sampling are required to ensure identification of non-mucinous histologic types [3].

Mucinous components in the majority of MCBs produce glistening, gelatinous, or mucoid features on gross examination, allowing for a large number of MCBs to be well-demarcated from surrounding breast parenchyma [6]. However, Memis et al. [7] found an association between the type of border of MCBs on mammograms and the volume of mucinous components. They reported that all cases of pure MCB with mucinous components greater than 80% were well-defined, while all cases of mixed MCB were poorly defined or spiculated. However, pure MCBs with less than 80% mucinous components exhibit both types of borders. Similar results have been reported by other investigators [8,9]. Therefore, if gross examinations reveal a glistening, gelatinous, or mucoid breast mass suggestive of MCB, pathologists should locate and sample areas with poorly defined or spiculated borders during gross examination, based on a review of the radiological findings.

Magnetic resonance imaging (MRI) can provide additional clues for an appropriate gross examination based on contrasting findings of mucinous and non-mucinous components of the MCB. MRIs for pure MCBs present with hyperintense T2 signals and fat-saturated T2-weighted sequences with low signal intensity on diffusion-weighted imaging, corresponding to a high apparent diffusion coefficient [6]. These findings are the result of abundant water molecules that diffuse freely within the mucin pools that are characteristic of MCB. In contrast, non-mucinous components of mixed MCBs present with hypointense T2 signals and lower apparent diffusion coefficient [10]. Dynamic contrast-enhanced MRIs also exhibit useful and contrasting enhancement patterns [8]. In contrast to pure MCBs, which exhibit mild or strong, gradual enhancement in the early phase and strong and heterogeneous enhancement in the delayed phase, mixed MCBs are characterized by strong and heterogeneous enhancements in both early and delayed phases.

Even if the morphological characteristics of pure mucinous carcinoma are confirmed, a therapeutic approach to favorable histology can be considered only when typical ancillary test results such estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, and human epidermal growth factor receptor 2 (HER2)-negative are confirmed [4]. A recent study using the Surveillance, Epidemiology, and End Results (SEER) database for 10,593 cases of MCB reported a high expression rate for hormone receptors (93.6% ER-positive, 84.6% PR-positive) and a low expression rate for HER2 (2.7% HER2-positive) [11]. When MCB is immunoprofiled as hormone receptor-negative or HER2-positive, it can be considered highly unusual or discordant, requiring confirmatory testing [12-14].

The prototypical histopathologic findings for MCB include small clusters of hypocellular tumor cells of low-to-intermediate nuclear grade floating in abundant extracellular mucin. However, MCBs can exhibit tumor cells of high nuclear grade, and it is unresolved if these can be classified as invasive carcinoma of no special type (NST) with mucin production [15]. However, the National Comprehensive Cancer Network (NCCN) recommends considering these cases as invasive breast carcinoma of NST in terms of therapeutic approach, even in MCB with a typical immunoprofile [4].

Capella et al. [16] reported that MCB is a heterogeneous disease consisting of types A and B, referred to as classical and endocrine variants, respectively. Unlike type A of prototypical histopathologic findings, type B exhibits distinct histopathologic features characterized by hypercellular tumor cells arranged in large clusters with a relatively small amount of extracellular mucin (Fig. 1). They described the detailed arrangement of type B as isolated or anastomosing clumps to sheet-like structures traversed by spaces, sometimes simulating cribriform structures. However, the

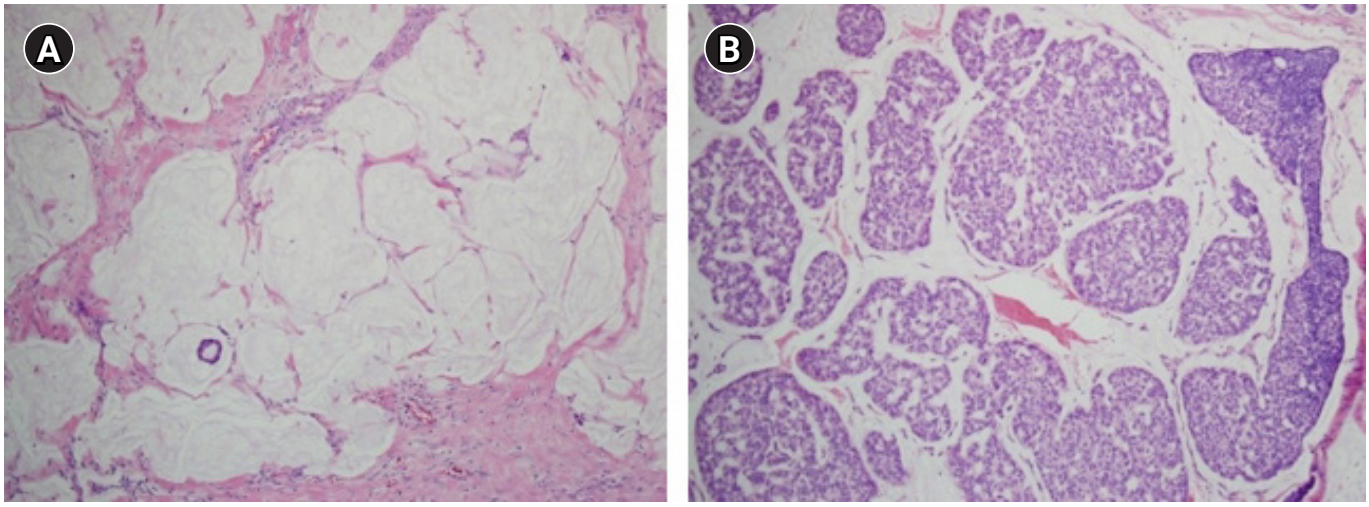

Fig. 1. A comparison of mucinous carcinoma of the breasts (MCBs) with different cellularity. (A) Type A MCB is characterized by a small number of tumor cells forming small clusters and a background of abundant extracellular mucin (H&E, ×100). (B) Type B MCB is characterized by hypercellular tumor cells forming large clusters. The mucinous background between the clusters is relatively poor compared to that of type A (H&E, ×100).

latter was distinctly different from those of type A, usually consisting of aggregates of rings or annular growth patterns associated with trabeculae and ribbons. The amount of extracellular mucin in type B was less than that in type A. However, abundant extracellular mucin accounting for more than 50% of the total volume of the lesion, a consistent finding in type A, is also found in a minority of type B. Interestingly, abundant intracytoplasmic mucin was confined to some type B, while foamy cytoplasm was confined to some type A.

They found neuroendocrine differentiation in type B, which was classified as an endocrine variant. Although the criteria for endocrine differentiation vary, it has been diagnosed in cases with an argyrophilia by histochemical reaction using Bodian and/or Grimelius stains, positive immunoreactions for neuron-specific enolase, chromogranin and/or synaptophysin, and dense endocrine granules on electron microscopy [16,17]. Tse et al. [17] acknowledged that it was difficult to evaluate such significance because of the good prognosis of MCB and death from other causes not related to breast cancer during the follow-up period due to the high proportions of elderly patients. In addition, they suggested that evaluation of independent prognostic factors such as nuclear grade and lymph node metastasis would be more feasible. From a practical point of view, neuroendocrine differentiation or cellularity of MCB is not a consideration for therapeutic strategies of MCB according to the recent NCCN guidelines and Korean clinical practice guidelines for breast cancer [4,18].

The histopathologic finding of MCB that has been proposed to be relevant to prognosis is the micropapillary arrangement of the tumor cells. MCBs with micropapillary features are characterized by tight morula-like and floret-like clusters common in micropapillary carcinomas, together with abundant extracellular mucinous background typical of MCB. These findings have been reported as invasive micropapillary mucinous carcinoma, mucinous micropapillary carcinoma, and micropapillary variant of mucinous breast carcinoma, and were described as "mucinous carcinoma with micropapillary features" in the World Health Organization (WHO) classification of breast tumors, fifth edition [5]. Although it remains controversial whether these combined histopathologic findings can be interpreted as genuine micropapillary variants of MCB or the mucinous counterpart of invasive micropapillary carcinomas, investigators have reported that more than half of the tumors exhibited lymphovascular invasion, resulting in increased regional lymph node metastasis and early recurrence in the skin and chest wall [19,20]. Furthermore, large retrospective studies have reported that invasive micropapillary carcinoma exhibits higher nuclear grade, HER2 overexpression or amplification, and Ki67 index compared

to pure MCB [21,22].

Meanwhile, some investigators have reported that micropapillary arrangement does not contribute to poor prognosis [23-25]. In these studies, none of the cases studied presented with high nuclear grade or HER2-positive immunoreaction (3+). These conflicting results can be attributed to relatively small sample sizes and inconsistent diagnostic criteria.

Considering the low prevalence of MCBs, the number with micropapillary features would be limited. Fortunately, there is excellent diagnostic agreement between pathologists assessing MCBs with micropapillary features [22]. The unique histopathologic findings of MCBs with micropapillary features are the reverse polarity of tumor cells and psammomatous microcalcifications [19]. Reverse polarity describes the inside-out growth pattern in micropapillary carcinomas of various organs, with its apical pole facing outward toward surrounding clear, empty stromal spaces, rather than facing the center of the tumor cell clusters (Fig. 2). It presents with scalloped or frayed

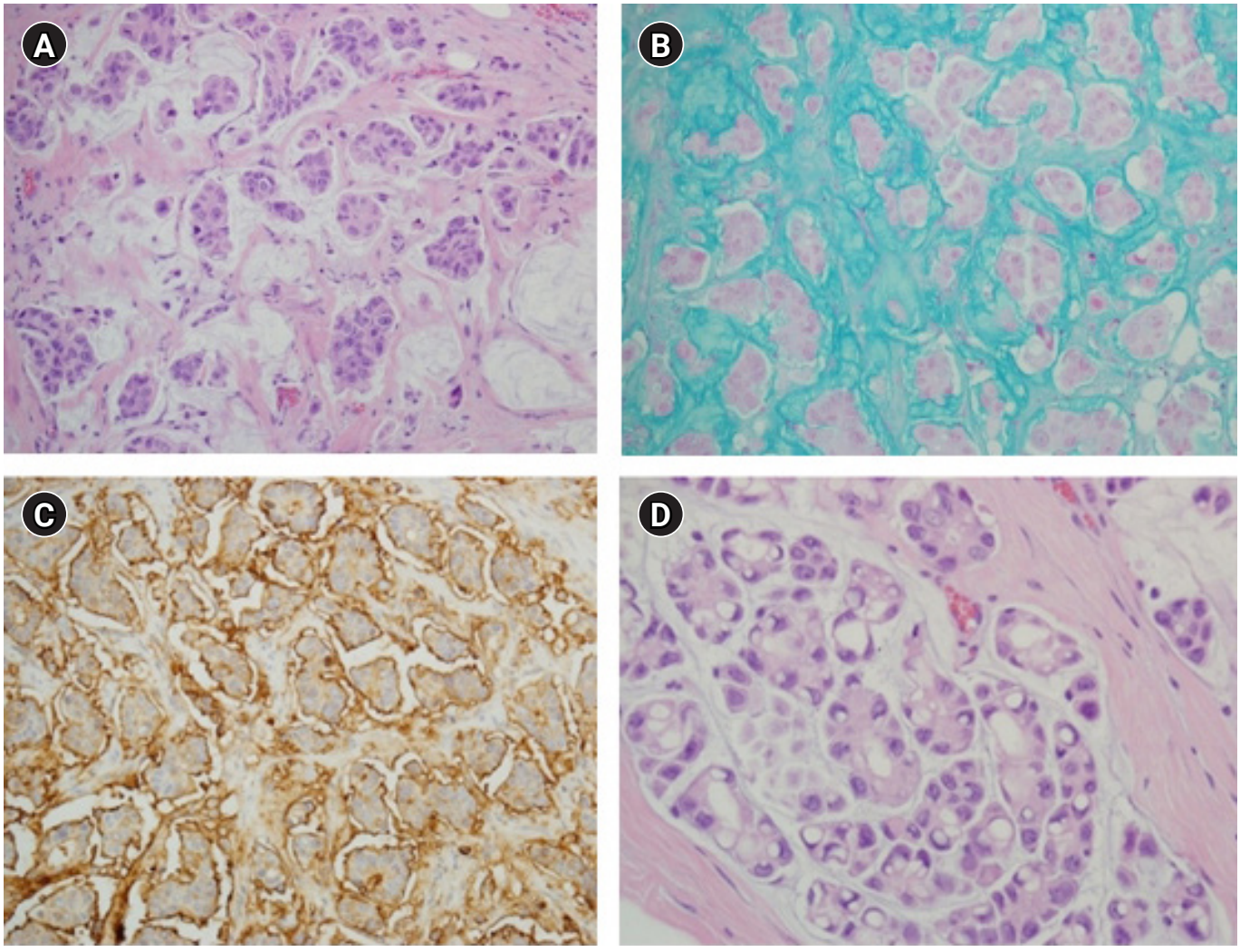

Fig. 2. Mucinous carcinoma of the breasts (MCBs) with micropapillary features. (A) Tight morula-like clusters of intermediate to high-grade tumor cells are floating within the small spaces filled with extracellular mucin (H&E, ×200). (B) Mucus filling the spaces between the tumor cells and the surrounding stromal tissue is identified by Alcian blue staining (×200). (C) Immunohistochemical staining for epithelial membrane antigen is observed on the stromal-facing peripheral membrane of the tumor cells, clearly revealing the inside-out pattern (×200). (D) Abundant intracytoplasmic vacuoles are found in the focal area of MCB with micropapillary features (H&E, ×400).

edges and apical cytoplasmic snouts at the outer cell membrane, with surface glycoprotein (EMA and MUC1) at the stromal-facing peripheral membrane, and with cell adhesion proteins (E-cadherin and p120) at the basolateral membrane [26,27]. However, an immunoreaction suggestive of reverse polarity has been reported in pure MCBs and should be carefully interpreted in conjunction with the characteristic histopathologic findings [22,28].

Liu et al. [21] reported that invasive micropapillary MCB was found in approximately 25% of pure MCBs in a large retrospective cohort, amounting to 134 cases of invasive micropapillary MCB. Additionally, other investigators indicated that a micropapillary arrangement is not an uncommon finding in MCB [19,22,24]. However, well-defined diagnostic criteria such as a cutoff value for micropapillary elements, have not been established. Moreover, definitions of MCB with micropapillary features have not been consistent in previous investigations and were based on various morphologic and immunohistochemical findings [22]. Therefore, further investigations are needed to discriminate clinically aggressive breast cancers from pure MCBs in order to provide appropriate treatment and surveillance.

Another histopathologic finding associated with the prognosis of MCB is lobular differentiation (Fig. 3). Invasive lobular carcinoma with abundant extracellular mucin exhibits both extracellular and intracytoplasmic mucins, with tumor cells positive for MUC1, MUC2, MUC5AC, and MUC6 [29-31]. Abundant extracellular mucins are an unexpected finding in invasive lobular carcinomas that have exclusively intracytoplasmic mucins, but it remains unclear whether they should be classified as a type of either invasive lobular carcinoma or MCB. Burky et al. [32] summarized 31 published cases of invasive lobular carcinoma with abundant extracellular mucin and determined that it tended to have a higher nuclear grade (2 or 3) and more frequent HER2-positive status, suggesting an aggressiveness to these cases. However, the diagnostic criteria for invasive lobular carcinoma with abundant extracellular mucin have not yet been established, and various proportions of the mucin-producing lesions are reported to vary from 5% to 80% [31]. Distinguishing them from pure MCBs requires recognition of these unusual findings, and further studies are needed on the biomedical and therapeutic approaches for invasive lobular carcinoma with abundant extracellular mucin.

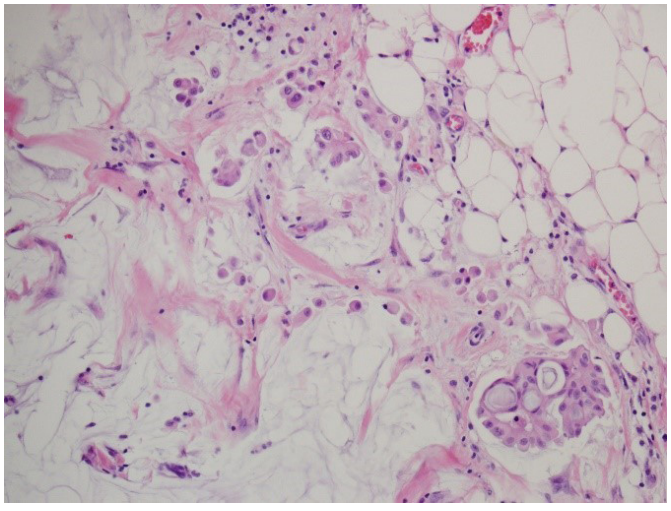

Fig. 3. Mucinous carcinoma of the breasts (MCBs) with lobular differentiation. Single tumor cells with low nuclear grade float within the mucin pool. The presented lobular differentiation is a focal finding in a case of mixed MCB (H&E, ×200).

Genetic characteristics of mucinous carcinoma

Mucins can be broadly classified into secreted gel-forming mucins and membrane-bound mucins. The mucin gene expression signatures presented in MCB are upregulation of gel-forming mucins and downregulation of membrane-bound mucins [33]. Among the various mucin-related genes, *MUC2*, which encodes the epithelial glycoprotein MUC2, is the most commonly upregulated gene. The expression of *MUC2* in MCB was negatively associated with the level of methylation of CpG mapped to *MUC2*, which has been suggested as a mechanism for the production of extracellular mucin [34]. Furthermore, Nguyen et al. [35] found a different methylation pattern in MCB, where *MUC2* is hypomethylated at the promoter and the 5'-untranslated region and hypermethylated in more downstream exons. Upregulated *MUC2* expression signatures have been reported in mucinous carcinoma of various other organs such as the colon, rectum, stomach, pancreas, gallbladder, and lung, as well as in MCB [33,36]. However, other molecular features such as increased microsatellite instability and increased *BRAF*, *KRAS*, and *PIK3CA* mutations that are commonly reported in mucinous carcinoma of non-mammary organs are not common in

MCBs [36,37]. Differences in the molecular characteristics of MCB and mucinous carcinoma of various organs can explain the wide range of biological behaviors, suggesting that their pathogeneses are not identical.

Thennavan et al. [38] characterized the transcriptomic and genomic profiles of 24 pure MCB cases using a histological annotation dataset from The Cancer Genome Atlas Breast Cancer (TCGA-BRCA) project. They identified transcriptomic features of MCB including early estrogen response, late estrogen response, and protein secretion. Enrichment of estrogen-related genes overlaps similarly with that of other special histologic types of breast cancer such as cribriform, micropapillary carcinoma, and papillary carcinoma of luminal A/B subtypes. However, this contrasts with the immune-related and mitosis-related genes found in basal-like special histologic types such as metaplastic carcinoma and invasive carcinoma with medullary features. These results indicate the dominant biologic significance of genes shared by intrinsic subtypes. When analyzed using a transcriptome-based differentiation score, MCB expressed a mature luminal signature distinct from luminal progenitor and mammary stem cell signatures.

In comparison to ER-positive invasive carcinoma of NST, MCB has distinct genomic features. MCB is characterized by a low fraction of genome altered and presents as a diploid with neither chromosome 1q gain nor 16q loss, which are prevalent in ER-positive/HER2-negative, low-grade invasive carcinoma of NST [22,33,34,39]. In addition, a lower tumor mutation burden has been reported in MCB with less frequent *TP53* or *PIK3CA* mutations compared to ER-positive/HER2-negative, low-grade invasive carcinoma of NST [34,39,40]. Interestingly, Pareja et al. [37] suggested that the mucinous and ductal components of mixed MCB were clonally related through either clonal selection or parallel evolution. However, the significance of the result was limited due to the small sample size (seven cases), and further investigations of clonality are needed to help elucidate the pathogenesis of mixed MCB.

As high-throughput sequencing becomes more accessible, some investigators have reported genomic features of MCB (Table 1). A low frequency of *PIK3CA* and *TP53* mutations was common, as previously described. However, the genetic alterations frequently found in each study were diverse, and no pathognomonic mutations or genomic alterations have been identified in MCB. These discrepant findings might be influenced by the small sample size due to the relative rarity of MCB. However, they also might indicate the genomic diversity of MCB. The heterogeneity of the genomic drivers of MCB was also suggested in a genomic stratification study of 1,643 cases of breast cancer in the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) cohort [42]. That study found that the integrated clusters (IntClust) allocated by genomic and transcriptomic profiling and copy number analysis varied in MCB, in contrast to other histologic types of breast cancer with significant associations of specific IntClust.

Differential diagnosis of mucinous carcinoma

A mucinous background of breast lesions is a typical finding of MCB, but it can also be observed in a variety of other lesions of the breast. In terms of the absence of pathognomonic genomic findings in MCB, the pathological differential diagnosis of MCB in daily practice is within the scope of conventional microscopic evaluations.

Mucinous cystadenocarcinoma is exceptionally rare, with fewer than 30 cases in the literature, and was recently

Table 1. Genetic alterations of pure mucinous carcinoma in previous studies

Author	Tests (cases studied)	Frequently altered genes
Yim et al. [41]	WES (8)	Missense and multi hit *HYDIN* (88%) missense *IGSF3* (38%), *ASPM* (25%), *ERBB2* (25%), *FAM83G* (25%), *HNRNPCL1* (25%), *KIAA2026* (25%), *KIF25* (25%), *LGR6* (25%), *LHX9* (25%), *MAGEF1* (25%), *MC1R* (25%), *OBSCN* (25%), *PIEZO1* (25%), *PKHD1L1* (25%), *REEP4* (25%), *ZNF469* (25%), missense and frameshift *FLG2* (25%), missense and nonsense *LAMA3* (25%)
Sun et al. [22]	WES (11)	Missense and in-frame indel *TTN* (27.2%)
Nguyen et al. [35]	LP-WGS (30)	Deletion *RB1* (38.1%), *BRCA2* (38.1%), *EGFR* (28.6%), *CDH1* (23.8%), *TP53* (23.8%), *MAP2K4* (23.8%), *PGR* (23.8%), amplification *ZNF217* (19.4%), *FGFR1/ZNF03* (9.5%)
Pareja et al. [37]	WES and RNA-seq (25)	Frameshift *GATA3* (23.8%), missense or truncating *KMT2C* (19.0%), *MAP3K1* (14.3%), missense *XIRP2* (14.3%)

WES, whole-exome sequencing; LP-WGS, low-pass WES; RNA-seq, RNA sequencing.

listed as a separate histologic type in the fifth edition of the WHO classification [43,44]. On macroscopic evaluation, most mucinous cystadenocarcinomas present as large cystic masses filled with gelatinous content, reminiscent of MCB [45]. However, this disease can be distinguished from MCB based on characteristic tumor cells lining multicystic spaces, creating stratification, tufting, or intracystic papillary structures that are not accompanied by myoepithelial cells. These tumor cells are tall columnar cells with abundant cytoplasmic mucin and basally located nuclei with various nuclear pleomorphisms. These tumor cells exhibit a triple-negative, basal-like phenotype that is ER-negative, PR-negative, HER2-negative, CK5/6-positive, and EGFR-positive, in contrast to the luminal phenotype of MCB [46]. Kim et al. [45] described tight clusters of tumor cells floating within abundant stromal mucin pools typical of mucinous cystadenocarcinoma. Therefore, tumor cells floating in mucin pools are not an absolute criterion for MCB, and comprehensive pathologic evaluation of the overall histologic findings in combination with immunohistochemical results is required. In addition, the characteristic tumor cells of mucinous cystadenocarcinoma are similar to those of mucinous cystadenocarcinoma of pancreatobiliary trees or ovaries [47]. To differentiate these diseases, it would be helpful to determine the histopathologic findings of concomitant ductal carcinoma *in situ* and establish a negative immunoreaction for CK20 and CDX2, in addition to obtaining clinical information on its systemic status [44].

Mucocele-like lesions are characterized by an acellular extracellular mucin pool that corresponds to extravasated mucin from dilated cysts. When mucocele-like lesions have epithelial strips floating in pools of mucin, they can mimic MCB. Since the epithelial strips involve epithelial lining dislodged from dilated cysts, determining the myoepithelial cells adhered to the epithelial cells might provide helpful diagnostic findings [15]. However, complicated pathologic findings such as various degrees of atypical epithelial proliferation, including atypical ductal hyperplasia, ductal carcinoma *in situ*, and invasive carcinoma, are not uncommon in mucocele-like lesions, hindering accurate diagnosis [48]. Despite reports that radiologic findings play a diagnostic role in epithelial proliferative lesions associated with mucocele-like lesions, the confirmatory diagnosis is based on histopathologic evaluation [49-52]. When an attenuated or proliferative epithelial lining appears along an extracellular mucin pool, the diagnosis favors mucocele-like lesions over MCB with prominent luminal locations of tumor cells floating within the mucin pool [52]. When the epithelial strips are small, deeper histologic sections and immunohistochemistry are helpful to differentiate mucocele-like lesions from epithelial proliferative lesions. Tan et al. [15] described that determining epithelial clusters in mucocele-like lesions can be difficult in some cases. Therefore, they suggested that it may be appropriate to acknowledge the uncertainty of these findings and the possibility of minute invasive foci.

Breast tissue is not an obligate origin of mucinous carcinoma, which can occur in various extramammary organs such as the gastrointestinal tract, pancreatobiliary trees, female genital tract, urinary bladder, and lung. A recent study based on data from TCGA analyzed 902 patients with mucinous carcinoma of colorectal, breast, pulmonary, gastric, endocervical, or pancreatic origin [33]. They found that mucinous carcinomas of independent tumor origin shared transcriptomic similarities such as upregulation of gel-forming *MUC2*, *SEC16A*, and *CRACR2A* and complementary genes involved in MUC2 packing, folding, and transport, suggesting pan-cancer biomarkers of mucinous histology. In addition, genomic similarities of fewer DNA copy number alterations in mucinous carcinoma have been reported across tumor origins. Therefore, histopathologic, transcriptomic, and genomic characteristics of mucinous carcinomas can be similar, irrespective of tumor origin.

Mucinous carcinoma that has metastasized from extramammary organs is rare in the breast, where primary breast lesions dominate [53]. Nevertheless, if a patient exhibits a clinical history of malignancy, especially with mucin-producing adenocarcinoma, pathological differential diagnosis should be considered. It is important to collect sufficient clinical information and perform a careful pathological evaluation for the differential diagnosis. When a site of primary origin is specified, it is helpful to correlate expected or known histopathologic and immunohistochemical findings of primary origin with those of breast lesions, allowing efficient and rapid diagnosis through relatively small numbers of immunohistochemical markers. On the other hand, if the breast lesion is suspicious of a metastatic lesion, but the primary origin has not been identified, an approach using multiple immunohistochemical markers that can cover a broad range of anatomic areas is required

Table 2. Mucinous carcinomas from various anatomic origins and their immunoreactivity for representative immunohistochemical markers

	Breast	Colorectal	Appendix	Stomach	Pancreaticobiliary	Ovary	Uterine cervix	Bladder	Lung
CK7	100 (18)	16 (204)	34 (44)	78 (50)	92 (62)	84 (161)	100 (15)	60 (5)	95 (66)
CK20	0 (18)	90 (229)	96 (48)	73 (55)	63 (76)	44 (158)	47 (15)	100 (5)	15 (66)
CDX2	0 (18)	88 (229)	85 (48)	71 (55)	67 (58)	27 (140)	0 (7)	60 (5)	5 (37)
SATB2		49 (88)	78 (9)	20 (15)	0 (15)	2 (123)			
WT1	64 (151)	0 (56)	0 (28)	0 (17)	0 (29)	0 (31)	0 (7)	0 (5)	0 (16)
PAX8	32 (34)	9 (160)	4 (45)	9 (44)	9 (54)	34 (338)	20 (5)	0 (4)	0 (16)
ER	89 (18)	0 (75)	0 (28)	3 (32)	0 (44)	21 (39)	43 (7)	0 (5)	0 (16)
GATA3	100 (24)					10 (20)			
Mammaglobin	50 (24)								
GCDFP15	58 (24)								
TTF1									68 (65)
Napsin A									52 (65)

Values represent the percentage of immunoreactive cases (total number of cases studied). These results were calculated by integrating the individual results from the literature.

to identify a primary origin. Moreover, a single immunohistochemical marker is insufficient to define a specific primary origin. Therefore, a panel-based approach that integrates overall clinical, radiological, and microscopic findings is essential [54,55]. Table 2 summarizes the representative immunohistochemical markers for mucinous carcinomas from various primary origins [54,56-70]. However, the cases in the literature were selected according to various morphological criteria, and there were many differences in immunohistochemical details such as clones and interpreted criteria of immunohistochemical markers.

Conclusion

For an accurate diagnosis of MCB, which is a prerequisite for appropriate treatment, each pathological evaluation process such as macroscopic, microscopic, genetic, and ancillary examinations should be properly coordinated on basis of appropriate correlations with radiologic findings and medical history. In this study, the pathologic and genetic findings relevant to the prognosis of MCB were reviewed with a focus on diagnostic significance in daily practice.

Article information

Conflicts of interest

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How to conduct well-designed clinical research

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Clinicians and healthcare decision-makers conduct their clinical practice based on the results of clinical trials. However, some health problems remain unresolved; in such cases, further research is required. To ensure reliable research results, it is important to understand the study design and conduct well-designed clinical trials. Many study designs can be chosen within the two broad categories of observational and interventional. Clinical studies have a variety of designs, including case series, case-control, cross-sectional, and prospective and retrospective cohort studies. Well-designed clinical studies can clarify important differences between treatment options and provide data on long-term drug efficacy and safety. Interpreting the results of clinical trials can be difficult because weaknesses in research design, data collection methods, analytic methods, and reporting can compromise their value and usefulness. However, although randomized controlled trials are limited owing to ethical and practical issues, they are optimal for investigating the effects of therapy and establishing causality. Here we present an overview of different clinical research designs and review their advantages and limitations.

Keywords: Clinical study; Clinical trial; Research; Research design

Introduction

In the era of evidence-based medicine, clinicians and healthcare decision-makers treat patients based on the results of clinical trials [1]. However, information presented in clinical trials is only useful if the trial is well planned. Thus, well-designed study plans and appropriate definitions of eligible participants are required to minimize confusion and other biases [2]. An accurate understanding of the research design is critical to the professional interpretation and determination of the validity and generalizability of the results [2]. Trial participants are randomized to experimental or control regimens [3].

In a real clinical setting, researchers frequently encounter situations lacking exact solutions to patients' health problems. Although many studies have investigated various diseases, some health problems remain unsolved. As a result, physicians and researchers have started reviewing related clinical trials to find applicable solutions. If useful results have not yet been published, a major conference may be a source of valuable information. However, if a definite solution is unavailable, further clinical research is desirable. Similar study designs can be found at clinicaltrials.gov. If a newly conceived clinical study has not yet been published, the potential new study may have sufficient value. Thus, one can create a simple synopsis of the proposed clinical research, contact pharmaceutical companies for new drugs, and obtain research funding. The proposed clinical research is reviewed by the global review committee. The proposed clinical study will proceed upon receiving ap-

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proval. However, various difficulties can be encountered in the process of obtaining valuable research results.

This study aimed to help researchers who have started clinical studies present their research results more effectively. It provides an overview of different research designs and comments on the strengths and limitations of each. The characteristics of the study designs used in the clinical trials are summarized in Table 1.

Retrospective study

It is difficult for novice researchers to start prospective studies such as randomized controlled trials (RCTs) [4]. Therefore, retrospective studies are more suitable for these researchers as they are easily conducted. This involves collecting information from previously treated patients and deriving results through statistical analyses of the content. Retrospective studies can be divided into case reports or case series, cross-sectional, case-control, and cohort studies according to the data collection method [5].

1. Case reports and case series

Case reports and case series have a profound impact on the literature in medicine and continue to advance our medical knowledge [6]. The authors reported a possible association between specific exposure and the observed results on the clinical records of one or more subjects. These studies may be the first to reveal a new disease or adverse treatment effects. The results of cost-effective studies help generate hypotheses that may later be explored using more

Table 1. Characteristics of study designs used in clinical research

Study design	Characteristics	Advantage	Limitation
Retrospective study designs			
Case series	Detailed description of cases	Fast and inexpensive Hypothesis-generating	Very limited potential to establish causal effects Selection bias
Cross-sectional study	Exposure and outcome measured at the same time point Subjects with and without outcomes are compared	Useful for describing disease prevalence Fast and inexpensive Hypothesis-generating	Very limited potential to establish causal effects Selection bias Survival bias
Case-control study	Cases (those with the outcome of interest) are compared with controls (those without the outcome of interest) with respect to exposure	Efficient Suitable for studying rare outcomes and multiple exposures Relatively inexpensive Hypothesis-generating	Some potential to establish causal effects Can only study one outcome Choice of the control group can be difficult Selection bias Recall bias
Retrospective cohort study	A cohort of subjects free of the outcome is followed and compared based on the exposure	Suitable for studying multiple exposures, rare exposures, and multiple outcomes Hypothesis-generating High generalizability	Some potential to establish causal effects Selection bias
Prospective study designs			
Prospective cohort study		The most accurate and objective method of collecting information from numerous patients	Can take a long time Can be expensive
Randomized controlled trial	Randomization: allocation of subjects to experimental or control group by chance	Gold standard in establishing causal effects in studies on therapy Suitable for studying more than one intervention	Very expensive Can take a long time Not suitable for studying rare events Can be unethical Often low generalizability due to strict selection criteria

Each study design may suffer from a specific type of bias. These are explained in the manuscript.

advanced research designs; however, causality is seldom established [5]. However, owing to the potential existence of weak inferences and the bias associated with such case reports, researchers are not passionate about developing frameworks for assessing, evaluating, synthesizing, and applying the evidence derived from the results of case reports or case series [6].

2. Cross-sectional studies

Most cross-sectional studies using descriptive methods based on data from a population or representative group aim to estimate a prevalence [7]. Most cross-sectional studies account for the prevalence of disease in a population or treatment in specific patient groups [5]. However, since exposures and outcomes are identified simultaneously, authors and readers should not infer causality unless it can be safely assumed that the exposure is stable over time and unaffected by the outcome [7].

3. Case-control studies

Case-control studies can achieve significant scientific findings with little cost, time, and effort relative to other study designs. This fast road to research results attracts many young researchers [8]. This study type selects participants based on outcome variables and compares participants with conditions (cases) to participants without conditions (controls). Previous studies compared cases and controls based on exposure [5]. However, case-control studies tend to be more sensitive to bias than other comparative studies [8].

4. Retrospective cohort studies

Cohort studies are observational studies in which a cohort of individuals sharing some characteristics is followed over time and the outcomes are measured at certain time points. Cohort studies can be categorized as prospective or retrospective [9]. A cohort study allows researchers to investigate multiple outcomes and exposure variables [5]. A major advantage of a cohort study is its ability to examine multiple results that can be related to single or multiple exposures in a single study [9]. In longitudinal cohort studies, measuring changes in exposure levels and outcomes over time can provide insight into the dynamic relationship between exposure and outcomes [9]. In addition, registry cohort studies collect data retrospectively and prospectively. Several retrospective cohort studies have used data collected previously for other purposes. As a result, investigators have little control over the data collection process. Thus, the measurement of variables may be inaccurate or inconsistent, resulting in information bias. However, this research method is useful for analyzing the results of unusual or occupational exposure [9].

Retrospective cohort studies use big data from healthcare companies. The potential of big data in the healthcare field depends on the ability to detect specific patterns and convert high volumes of data into practical knowledge for decision-makers and precision medicine [10]. In healthcare systems, big data and data collection are valuable. The establishment of a big data platform will enable easy operation, remote consultation, and low cost; strengthen global cooperation to improve clinical practice, education, and scientific research; and support the global application of precision medicine and emerging health management models [11]. However, the major drawbacks of relying on large datasets to guide healthcare decision making have been well documented. The sensitive nature of stored and analyzed big healthcare data poses a unique challenge [12].

Prospective study

Since retrospective studies use previously collected data, it is necessary to recognize the possibility of selection bias and acknowledge the limitations of accepting data based on statistical results. RCTs are considered the gold standard for evaluating treatments and other interventions. A definite advantage of RCTs over observational studies is that they provide evidence of causality and are unlikely to have selection bias and prognostic selection [13,14].

1. Prospective cohort studies

A prospective registry cohort study is the most accurate and objective method to collect information from numerous patients. However, it requires a long follow-up period of waiting for events to occur; thus, it features a high risk of loss to follow-up.

2. Phases of clinical trials

Clinical trials can be divided into stages at which a new drug is tested [4].

1) Phase I trial

A phase I trial, which is usually conducted in healthy volunteers [15], aims to test the safety of a new drug in humans and determine its ideal administration method. A phase I trial is usually not randomized or controlled and does not include a control group. It mainly consists of a series of cases in which participants are administered the drug progressively while being monitored carefully by the research team [1].

2) Phase II trial

Once a drug's safety has been evaluated in a phase I trial, a phase II trial attempts to determine the efficacy of various doses and frequencies of its administration in a small, non-randomized group [16]. If the drug is ineffective or excessively virulent in a small group of patients, no further testing is performed. If the drug is effective and its side effects are tolerable, the researcher can proceed to a phase III trial [1,16].

3) Phase III trial

A phase III trial, which is usually considered a full-scale RCT, is a comparative definitive study that compares the effectiveness of a new drug with that of a standard drug [16,17]. Phase I trials enr oll a large number of patients, often in the thousands, to determine whether the new treatment is more effective and less toxic than the standard treatment [17,18]. Because it requires reliable results from earlier clinical trials, a phase I trial is the ultimate test of a new treatment [5]. Although RCTs have powerful study designs, they are costly because of the large number of enrolled patients and interventions. Moreover, it is unethical to expose patients to an intervention considered inferior to the standard treatment [4].

4) Phase IV trial

A phase IV trial, usually called post-marketing surveillance, is a large-scale study that attempts to monitor the adverse effects of a new treatment after marketing approval. However, the sample size is often insufficient to identify rare adverse reactions [19]. Also, since this is a non-interventional study lacking close monitoring, reports of adverse reactions may be omitted.

Tips for conducting well-designed clinical research

We offer young researchers the following tips for designing clinical trials: when you have an idea about a study, check clinicaltrials.gov to see if similar studies are already underway. Examine how other researchers have planned and conducted their studies. If a newly conceived clinical study has not yet been published, the potential new study has sufficient value.

- Researchers initially perform a retrospective study. From case reports or case series to cohort studies, an appropriate study design is chosen based on the available data and research ideas.
- Meaningful results are often obtained from multicenter prospective studies. Start by participating in multicenter studies, then lead a multicenter study.
- Combining clinical research and cell line-based experimental research compensates for the limitations of this research. When confirming the results of clinical research using clinical data alone, the results are best supplemented with cell line research. A factor that influences good clinical research is the researcher's ability to improve upon basic experimental research or collaborate with a basic researcher for a study [20].

Conclusion

For successful clinical research, it is important that one starts with a retrospective study, advances to a prospective study, participates in and then leads multicenter studies, and finally cooperates with experimental research teams.

Article information

Conflicts of interest

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

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Dignity therapy for effective palliative care: a literature review

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Dignity therapy for terminally ill patients in end-of-life care helps improve their psychological and spiritual well-being. In this study, the effectiveness and feasibility of dignity therapy in terminally ill patients were analyzed by reviewing previous studies. The review's findings show that dignity therapy alleviates psychological distress and improves patients' spiritual well-being and dignity. In addition, many patients and their families found emotional support in generativity documents created through dignity therapy. Finally, the possibility of applying dignity therapy to palliative care in Korea in the future was explored. The findings indicate the influence of Eastern culture on recognizing death in patients who receive dignity therapy. Thus, dignity therapy shows promise as a contribution to improving palliative care; however, additional studies are needed to implement effective dignity therapy in the Korean context.

Keywords: Death; Hospices; Palliative care; Psychotherapy

Introduction

Palliative care refers to medical services that relieve or prevent the pain caused by the various physical and psychological issues faced by patients with terminal diseases, and their families [1,2]. In particular, psychotherapeutic interventions have been used to relieve distress. Among these interventions, dignity therapy has shown potential as a new therapeutic regimen for improving patients' emotional states toward the end of their lives [3,4]. First proposed by Canadian psychiatrist Chochinov et al. in 2002 [3], dignity therapy is a brief form of narrative psychotherapy designed to enhance dignity and alleviate psychological distress in terminally ill patients through reminiscence on their lives [5]. It aims to alleviate patients' psychological distress and assist in leading assist them in a more dignified ending period by allowing them to reflect on meaningful moments in their lives [6]. In end-of-life care, dignity is important because it is a human right to be guaranteed and is closely related to well-being [7-9]. Chochinov [6] suggested three major factors affecting dignity: illness-related concerns, a dignity-conserving repertoire, and a social dignity inventory. Illness-related concerns include themes related to suffering from disease and autonomy [6]. The dignity-conserving repertoire focuses on the spiritual state of patients and includes themes such as hopefulness, continuity of self, and generativity [6,10]. The social dignity inventory is related to relationships with other people surrounding the patient. Themes such as social support and quality of care, burden to others, and aftermath concerns are included in the social dignity inventory [6].

In this study, the feasibility and effectiveness of dignity

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therapy in patients with terminal diseases are investigated. Furthermore, its potential applications in Korea were examined by identifying cases from Eastern cultures. In this narrative review, an in-depth examination of qualitative and quantitative research on the effectiveness and feasibility of dignity therapy is conducted.

Taxonomy of dignity therapy

1. Implementing dignity therapy

Before the interview, patients are introduced to the process of dignity therapy, and presented with the questions from the Dignity Therapy Question Protocol (DTQP) so that they could consider their responses to the interview questions in advance. Subsequently, they are interviewed using the DTQP, consisting of nine questions. DTQP questions are presented in Table 1.

During the interviews, the therapists present the questions to the participants, flexibly, according to the situation. The interviews typically last approximately 60 minutes, and all interviews are recorded. Afterwards, the recorded content is transcribed and produced in document form. The "generativity document," containing the interview details, is cross-checked by each participant for any omissions or errors. The revised generativity document is then, delivered to patients or those designated by the patient.

2. Characteristics of the generativity document

The four main characteristics of the generativity document describing the interviews with patients in dignity therapy are generativity, care tenor, hopefulness, and continuity of self. Generativity originates from the messages that patients wish to leave for their family or friends after their death, and the generativity document, which contains such messages, serves as a "legacy." The characteristics of dignity therapy, in which patients are allowed to disclose their narratives during the interview in an atmosphere of respect and empathy, serve as the sources of care tenor [3,11]. Hopefulness derives from a positive view of their future and by discovering meaning and purpose of their lives through therapy sessions [11,12]. Continuity of self is derived from the opportunity to reflect on their feelings and self-images that the therapy gives.

Methods

PubMed, PsycINFO, Google Scholar, and KoreaMed databases were used to search for studies published up to December 31, 2021. "Dignity therapy," "efficacy," "feasibility/acceptability," "adaptation," and "culture" were the search keywords, and Boolean operators (e.g., OR, AND, NOT) were used in the search. The inclusion criteria were as follows: (1) publications on the effectiveness, feasibility, acceptability, or cultural adaptation of dignity therapy, and (2) published in English or Korean. The exclusion criteria were (1) papers not related to dignity therapy, (2) duplicate papers, and (3) papers presented at conferences or symposiums. The first author conducted the initial and secondary comprehensive literature review in 2021. After screening the abstracts, the full text of the relevant articles was reviewed. The first search identified 40 distinct publications. Eighteen papers were excluded from the review,

Table 1. Dignity Therapy Question Protocol

Questions
1. Tell me a little about your life history; particularly the parts that you either remember most or think are the most important? When did you feel most alive?
2. Are there specific things that you would want your family to know about you, and are there particular things you would want them to remember?
3. What are the most important roles you have played in life (family roles, vocational roles, community-service roles, etc.)? Why were they so important to you and what do you think you accomplished in those roles?
4. What are your most important accomplishments, and what do you feel most proud of?
5. Are there particular things that you feel still need to be said to your loved ones or things that you would want to take the time to say once again?
6. What are your hopes and dreams for your loved ones?
7. What have you learned about life that you would want to pass along to others? What advice or words of guidance would you wish to pass along to your (son, daughter, husband, wife, parents, other[s])?
8. Are there words or perhaps even instructions that you would like to offer your family to help prepare them for the future?
9. In creating this permanent record, are there other things that you would like included?

including those that were not relevant to dignity therapy, duplicate papers, and papers presented at conferences or symposiums. Thus, 22 papers were finally chosen for the narrative review. The information in each publication was summarized by author's name, year of publication, research purpose, research subjects, study design, outcome measurements, and study results in the selection process.

Results

1. Effectiveness of dignity therapy

Chochinov et al. [3] conducted a quasi-experimental study to examine the effects of dignity therapy on psychosocial and existential distress, as well as determine its feasibility. The study included 100 patients with terminal diseases whose life expectancy was ≤6 months. To assess patients' symptom levels, a questionnaire assessing anxiety, depression, and dignity, a quality-of-life instrument (two-item), and the revised Edmonton Symptom Assessment Scale (ESAS) were administered.

Approximately 90% of the participants stated that they were satisfied with the therapy. In addition, the therapy had a significant effect on reducing pain and depression in patients with cancer and increased their willingness to live.

In a subsequent randomized controlled trial [13], the effectiveness of dignity therapy was confirmed in 441 patients with terminal diseases who received palliative care with a life expectancy of ≤6 months to investigate whether the therapy can contribute to alleviating the distress of the patients and providing better end-of-life care. The participants were assigned to dignity therapy (n=108), client-centered care (n=107), and standard palliative care (n=111). The counseling therapy received by participants assigned to client-centered care was different from that received by those assigned to dignity therapy and helped clients focus on their current condition. The Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being (FACIT-Sp) scale, which measures spiritual well-being, and the Patient Dignity Inventory (PDI), which measures a patient's sense of dignity, were implemented to evaluate the effectiveness of the therapy. The Hospital Anxiety and Depression Scale (HADS) was used at baseline and post-intervention to examine the anxiety and depression experienced by hospital patients. In addition, the ESAS and a 7-item Structured Interview for Symptoms and Concerns (SISC) were used. This study found that dignity therapy helped participants reduce psychological distress such as depression and anxiety; however, according to the surveys, no statistically significant differences between the groups across all measurements.

In a study in the United Kingdom [5], 45 patients with advanced-stage cancer were randomly assigned to one of two groups: one receiving standard palliative care and dignity therapy (n=22), and the other receiving only standard palliative care (n=23). The PDI was used at baseline, and the following measurements were used at 1 and 4 weeks after completion of the intervention: the Herth Hope Index (HHI), HADS, and EuroQol 5-dimension (EQ-5D) to measure quality of life and two 10-point Likert scales. The participants' level of psychological distress was low at baseline, and there was no significant effect of any other index on the participants who received dignity therapy at the 1-week follow-up; however, the only positive effect was an increase in participants' levels of hope.

Juliao et al. [14] conducted a randomized controlled trial of 80 patients with terminal illnesses. The intervention group (n=39) received both standard palliative care and dignity therapy, whereas the control group (n=41) only received standard palliative care. Participants' psychological symptoms were measured using the HADS at baseline and on days 4, 15, and 30 after the intervention. The results showed that participants who received dignity therapy had significantly lower anxiety and depression scores than the control group at all time points. Additionally, the average survival period of the control group was only 20.8 days compared to that of the dignity therapy group, which was 26.1 days [15]. Therefore, researchers have speculated that dignity therapy may help patients live longer lives.

In a study of 70 patients with advanced diseases and a life expectancy of less than 12 months [16], participants were randomly assigned to one of three groups: a group receiving dignity therapy, those receiving a life review intervention, and a waitlist control group. In contrast to the other groups, participants in the dignity therapy group received legacy documents [17] containing memories or words meaningful to their families and could send the documents to anyone. The Brief Generativity and Ego-Integrity Questionnaire, PDI, Functional Assessment of Cancer Therapy-General (FACT-G), and treatment evaluation questionnaires were used to measure the outcomes. Although there were no significant differences in physical or mental health

Table 2. Summary of the literature review on the effectiveness of dignity therapy

Author (year)	Purpose	Study design	Participants	Outcome measurements	Results
Chochinov et al. (2005) [3]	Determine the viability of DT and its impact on various psychosocial and existential distress measures	Quasi-experimental study	Terminally ill cancer patients (n=100)	Depression, dignity, anxiety, pain, hopefulness, willingness to die, suicide, and sense of well-being questionnaires Quality of life (2 items) ESAS	91% were satisfied with DT. 76 % expressed a heightened sense of dignity. Significant improvements in suffering, reduced depressive symptoms, and so on, were observed in post-intervention measures.
Chochinov et al. (2011) [13]	Investigate whether DT can reduce distress or improve patients' quality of life	Randomized controlled trial	Patients receiving palliative care (n=441) DT (n=165) CCC (n=136) SPC (n=140)	FACIT-Pal, PDI, HADS, SISC (7 items), ESAS	No significant difference in distress before and after intervention in any group. Patients reported that DT was more likely to be perceived as helpful.
Hall et al. (2011) [5]	Evaluate the effect of DT on reducing distress in advanced cancer patients	Randomized controlled trial	Advanced cancer patients (n=45) DT (intervention; n=22) SPC (control; n=23)	Primary outcome: PDI Secondary outcomes: HHI, HADS, quality of life (EQ-5D), Likert scales, surveys for feedback	No significant difference in dignity-related distress between groups. The intervention group reported higher hopefulness than the control group at both follow-ups.
Juliao et al. (2014) [14]	Determine the impact of DT on depression and anxiety in highly distressed inpatients with a terminal illness	Randomized controlled trial	Terminally ill patients (n=80) Intervention (DT+SPC; n=39) Control (SPC; n=41)	HADS	DT was associated with a significant decrease in depression and anxiety scores at all follow-ups.
Vuksanovic et al. (2017) [16]	Evaluate the effects of legacy documents of DT comparing the intervention group (DT) with LR and WC groups	Randomized controlled trial	Patients with terminal diseases (n=70) DT (n=23) LR (n=23) WC (n=24)	Brief Generativity and Ego-Integrity Questionnaire, PDI, FACT-G, questionnaires for treatment evaluation	Unlike LR and WC groups, DT recipients demonstrated significantly increased generativity and ego-integrity scores at study completion. No significant changes in dignity-related distress or physical, social, emotional, and functional well-being in any groups.

DT, dignity therapy; ESAS, Edmonton Symptom Assessment Scale; CCC, client-centered care; SPC, standard palliative care; FACIT-Pal, Functional Assessment of Chronic Illness Therapy-Palliative Care; PDI, Patient Dignity Inventory; HADS, Hospital Anxiety and Depression Scale; SISC, Structured Interview for Symptoms and Concerns; HHI, Herth Hope Index; EQ-5D, EuroQol 5-dimension; LR, life review; WC, waitlist control; FACT-G, Functional Assessment of Cancer Therapy-General.

among the three groups, participants who received dignity therapy tended to have higher ego-integrity and generativity than others.

A summary of the reviews on the effectiveness of dignity therapy is presented in Table 2.

2. Satisfaction of dignity therapy

Hall et al. [12] identified the subjective benefits of dignity therapy as reported by participants in a randomized trial of elderly people aged ≥65 years, living in nursing facilities. Participants in both the dignity therapy (n=25) and control (n=24) groups experienced positive changes in their outlook on life, and increased self-esteem and self-efficacy. However, significant improvements in the interaction between participants and their families were only found in the dignity therapy group. Some participants reported that the legacy documents created during therapy sessions helped them recall pleasant memories and share their life stories with their families. Talking to therapists who empathized with their feelings made them feel important. In another study [18], both the intervention and control groups observed the formation of positive self-values and a sense of purpose, whereas only those who received the intervention demonstrated the generativity effect of dignity therapy. The participants stated that therapy allowed them to leave a legacy even after death.

A pre-and post-intervention study of 10 patients with metastatic cancer and their families [19] used the Beck

Depression Inventory-II (BDI-II) and the Functional Assessment of Chronic Illness Therapy-Palliative Care (FACIT-Pal). Measurements were used to assess participants' quality of life and psychological state. Consequently, 75% of participants who completed the post-measures expressed satisfaction with dignity therapy. Participants also stated that the intervention made their lives more meaningful, and that it benefitted them and their families. Families who had lost a loved one stated that legacy documents were a source of comfort.

In a study of 60 family members of terminally ill patients who received dignity therapy [20], more than 70% of the participants reported that it improved their dignity and sense of purpose. It was observed that generativity documents dealing with the lives and memories of patients contributed to reducing the distress caused by the patient's death and assisted the patients' families in overcoming their grief.

Montross et al. [21] conducted a transverse study on the effects of dignity therapy on 18 hospice workers. According to 92% of the staff members at hospice care, it was considered meaningful because it relieved patients' pain and provided care to their families. Furthermore, it helped the hospices to develop relationships with patients and instilled a sense of pride in their profession.

A summary of the reviews on the satisfaction with dignity therapy is presented in Table 3.

3. Feasibility and acceptability of dignity therapy

A randomized controlled trial of 60 nursing facility residents aged ≥65 years revealed the feasibility and acceptability of dignity therapy [22]. To determine feasibility, the study measured the number of patient visits by a therapist for intervention as well as the duration of therapy. To determine acceptability, the researchers asked participants whether the intervention helped them or their families. Though the

Table 3. Summary of the literature review on satisfaction with dignity therapy

Author (year)	Purpose	Study design	Participants	Outcome measurements	Results
Hall et al. (2013) [12]	Investigate and contrast participants' perspectives on participating in DT	Qualitative study	Nursing home residents (1-week follow-up; n=49, 8-week follow-up; n=36)	Semi-structured interviews	Six themes, including refocusing, interaction with the researcher or therapist, and diversion, were shown in the intervention and control group interviews. Only the intervention group interview included responses on the generativity document, generativity, and reminiscence themes.
Hall et al. (2013) [18]	Investigate intervention and control participants' views of the advantages of participating in DT	Qualitative study	Cancer patients 1-week follow-up (n=29), 4-week follow-up (n=20) Family members of the intervention group (n=9)	Semi-structured interviews	Five themes, including continuity of self, hopefulness, and care tenor, appeared in the interviews. The intervention group interviews included reminiscing and a "pseudo-life review."
Johns (2013) [19]	Explore the implementation of DT in clinical practice	Pre-post evaluation	Metastatic cancer patients (n=10) Family members of patients (n=6)	Questionnaires on distress, BDI-II, FACIT-Pal, surveys for feedback from patients and their families	Participants considered DT feasible and acceptable. 75% of patients reported that DT was helpful to their families, and all family members agreed that the generativity document was beneficial to them.
Montross et al. (2013) [21]	Explore the effect of DT from the viewpoints of hospice staff	Qualitative study	Hospice staff members (n=18)	Individual interviews	DT was reported to be beneficial to patients and able to provide positive end-of-life experiences.
McClement et al. (2007) [20]	Investigate the opinions of family members on the influence of DT on patients and themselves	Qualitative study	Family members of deceased patients who participated in DT (n=60)	Individual interviews Feedback questionnaires	The majority of participants reported that DT reduced patients' distress, as well as helped patients' family members cope with grief.

DT, dignity therapy; BDI-II, Beck Depression Inventory; FACIT-Pal, Functional Assessment of Chronic Illness Therapy-Palliative Care.

researchers anticipated that dignity therapy would take longer to be implemented, most participants completed all therapy sessions in a timely manner. Furthermore, participants who received therapy demonstrated a positive change in their attitude toward life compared to the control group. They reported that dignity therapy made their lives more meaningful and supported their families.

Dignity therapy was implemented in a different study by Chochinov et al. [23] with frail elderly participants without cognitive problems (n=11) and those with cognitive impairment who received therapy with family support (n=12). Based on the findings, it was proposed that dignity therapy could be widely used in the elderly population.

In a study of 29 patients with motor neuron disease (MND) [24], it was found that dignity therapy can be helpful. Since MND is an incurable disease that restricts movement and has limited treatment options, it can cause physical and psychological suffering in MND patients, thereby threatening their well-being. Dignity therapy's acceptability was investigated using a 25-item feedback questionnaire that examined whether it improved participants' spiritual well-being and quality of life. Furthermore, the feasibility of dignity therapy was investigated based on its duration and the effect of MND symptoms on participation in the intervention. Participants' self-reports indicated that the intervention was effective and, provided a solid foundation for acceptability. According to the researchers [24], when dignity therapy is administered to patients with MND, the intervention may take longer than it does for cancer patients, and communication issues may arise. They remarked that overcoming these barriers could benefit patients undergoing MND.

Johnston et al. [25] found that dignity therapy is effective in patients with early-stage dementia. In this mixed-method study using interviews and measures for patients in the early stages of dementia, dignity therapy, improved their quality of life and, relieved their psychological distress. The intervention was also performed without major difficulties. Given the symptoms of early dementia, it is expected that the therapy may have a greater impact if it is modified such that participants can easily recall their memories.

Nonetheless, dignity therapy may be difficult to implement in clinical practice because of its inefficiency in terms of time and money [19]. To overcome these constraints, Bentley et al. [26] conducted a study on patients with terminal illnesses to determine whether dignity therapy can be implemented online to save money and time. The research team confirmed its feasibility and applicability over the Internet, while highlighting the technical issues caused by online delivery.

A summary of the review in the feasibility and acceptability of dignity therapy is presented in Table 4.

4. Dignity therapy in East Asian cultures

A Japanese study [27] suggested that the low participation rate in dignity therapy among patients with terminal illnesses was due to differences between Western and Japanese cultures. According to this study, Japanese patients with terminal diseases prefer situations where death is not recognized. Furthermore, because Japanese culture values nonverbal communication, Japanese patients may be hesitant to talk explicitly about death with their family members. Lee and Rhee [28] also found a tendency to avoid death due to fear of death in patients among terminal cancer in Korea. Negative attitudes toward death have reportedly prevented patients from accepting death and reflecting on their lives.

In a Chinese study, Wang et al. [29] investigated the efficacy and limitations of family participatory dignity therapy (FPDT) in patients with hematological cancer and their families. The DTQP was modified in the FPDT, and the treatment target was extended to the patient's family. The overall treatment was similar to Chochinov's treatment procedure [3] for dignity therapy. However, the FPDT includes a step in which patients and their families choose photos and music to create and appreciate audiovisual materials. The patients and their families reported that the intervention improved their emotional and health status, allowing them to communicate with their families. The researchers also discovered that communication between patients and their families was difficult due to the Chinese culture, which forbids discussions regarding cancer and death. They proposed that FPDT, which facilitates communication with patients and their families and prepares them for death, may help solve these issues. In a randomized study in China [30], it was found that dignity therapy improved psychological health and hopefulness in patients. However, there were difficulties in implementing dignity therapy because the patients preferred not to express their thoughts about death.

Table 4. Summary of the literature review on the feasibility and acceptability of dignity therapy

Author (year)	Purpose	Study design	Participants	Outcome measurements	Results
Hall et al. (2012) [22]	Evaluate the feasibility, acceptability, and potential efficacy of DT in reducing distress in the elderly in nursing facilities	Randomized controlled trial	Care home residents aged 65 or older (n=60) Intervention (DT; n=31) Control (n=29)	Potential efficacy: PDI Potential effectiveness: GDS (15 items), HHI, etc. Feasibility: The number of visits by therapists, time taken to deliver the therapy, etc. Acceptability: Ratings of participants' views on DT	No significant differences in potential effectiveness at any time. Reduction in dignity-related distress in both groups. The intervention group outscored the control group on all the acceptability items at both follow-ups. Significant ratings for the efficacy of DT in increasing the meaningfulness of life for patients and helping families overcome distress caused by their deaths.
Chochinov et al. (2012) [23]	Determine the feasibility of DT for the elderly	Transversal study	Cognitively intact (n=12) Cognitively impaired (n=11) Families (n=24) HCPs (n=12)	Feedback questionnaires	All participants completed DT sessions. Most of the cognitively intact and proxy residents found DT to be helpful. HCPs reported the benefits of DT in terms of positively changed perceptions toward residents.
Bentley et al. (2014) [24]	Evaluate the feasibility, acceptability, and potential effectiveness of DT for MND patients	Pre-test post-test design	MND patients (n=29)	Effectiveness: HHI, PDI, FACIT-Sp-12 Feasibility and acceptability: Feedback questionnaires, the time for therapy sessions, reasons for non-completion, etc.	Changes in hopefulness were observed on the individual level. Better family relationships, a stronger sense of self, and greater acceptance were reported to be advantages of DT.
Johnston et al. (2016) [25]	Explore the feasibility, acceptability, and potential effects of DT on early-stage dementia patients	Mixed-methods study	Early-stage dementia patients (n=7) Family members (n=7) Stakeholders (n=7) Focus group members (n=6)	HHI, PDI, quality of life ratings	DT was found to be feasible, acceptable, and potentially effective for patients with dementia in terms of improving quality of life and sense of dignity. Patients had no problems in completing DT sessions and reported that the therapy was helpful to them.
Bentley et al. (2020) [26]	Assess the feasibility and acceptability of DT delivered online	Pre-test post-test design	Patients with terminal illnesses (n=6)	HADS, HHI, FACIT-Pal, feedback questionnaires	High levels of acceptability, efficacy, and convenience were reported. The time for therapy was cut by approximately 40%.

DT, dignity therapy; PDI, Patient Dignity Inventory; GDS, Geriatric Depression Scale; HHI, Herth Hope Index; HCPs, healthcare providers; MND, motor neuron disease; FACIT-Sp-12, Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being 12 Item Scale; HADS, Hospital Anxiety and Depression Scale; FACIT-Pal, Functional Assessment of Chronic Illness Therapy-Palliative Care.

A Korean study [31] investigated the effect of a short-term life review that partially applied the dignity therapy protocol on distress in patients with terminal illnesses. Patients were first interviewed using items from the DTQP in the first session of the short-term life review. After the patients confirmed the documented interview a week later, they created a photo album to reflect on their lives in the second session, using their chosen photographs. After the intervention, anxiety and depression were significantly reduced in the experimental group compared to the control group, and spiritual well-being was significantly improved. However, some patients were not allowed to receive intervention because of objections from their families [31], which is assumed to be related to the family's negative perception of dignity therapy.

A summary of the review on the implementation of dignity therapy in East Asian cultures is presented in Table 5.

Table 5. Summary of the literature review on dignity therapy implemented in East Asia

Author (year)	Purpose	Study design	Participants	Outcome measurements	Results
Akechi et al. (2012) [27]	Explore the feasibility of DT in Japan	Transversal study	Adults with terminal cancer (n=11)	The DT participation rate, feedback questionnaire	86% refused to participate in DT. 78% reported the usefulness of DT for the sense of well-being. 67% reported the usefulness of DT for improving dignity. 56% reported the benefits and usefulness of DT in terms of overall well-being.
Wang et al. (2020) [29]	Explore the feasibility and advantages of FPDT	Mixed-methods study	Hematologic cancer patients (n=10) and their family members (n=10)	HHI, FACIT-Sp, EORTC QLQ-C30, semi-structured interviews	HHI, FACIT-Sp, and EORTIC QLQ-C30 scores tended to increase after DT. DT was shown to be meaningful in improving the well-being of both patients and their family members according to the interviews.
Chen et al. (2021) [30]	Investigate the satisfaction and effectiveness of DT with cancer patients in China	Randomized controlled trial	Hematologic cancer patients (n=66) DT group (n=32) Control group (n=34)	FACIT-Sp-12, HHI, EORTC QLQ-C30, Likert scale for investigating satisfaction with DT	Significant increases were found in spiritual well-being and hope scores at the 1-week and 4-week follow-ups. The majority of participants reported that they were satisfied with DT.
Ahn et al. (2012) [31]	Explore the effects of a short-term life review on the spiritual well-being and distress of patients with terminal cancer	Quasi-experimental design	Terminal cancer patients (n=32) Experimental group (n=18) Control group (n=14)	FACIT-Sp-12, HADS	Significant improvements in spiritual well-being and decreased levels of depression and anxiety were shown in the experimental group compared to the control group.

DT, dignity therapy; FPDT, family participatory dignity therapy; HHI, Herth Hope Index; FACIT-Sp, Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being; EORTC QLQ-C30, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire; FACIT-Sp-12, Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being 12 Item Scale; HADS, Hospital Anxiety and Depression Scale.

Discussion

In some of the studies reviewed, dignity therapy was shown to be effective in reducing psychological distress in end-of-life patients [5,13,14,16]. Subjective reports of improved spiritual well-being, increased hope, and dignity were also reported in patients who participated in the therapy [3,5,13]. Furthermore, the results of several quantitative findings revealed that dignity therapy has the ability to alleviate negative moods or improve emotions, which contributes to the improvement in the quality of life of patients with terminal illnesses, through self-integration and generativity, among others [14,16].

However, there are some limitations to these previous studies. In some studies, the baseline scores of the study participants were low in the factors to measure the effect of dignity therapy (e.g., dignity-related distress). Therefore, the ceiling and floor effects did not show statistically significant differences after therapy [5,13,32]. One study reported that the measurements used had limitations in capturing the changes in patients' psychological state after dignity therapy [13]. Regarding quality of life, it is argued that existing measurements have difficulty in properly measuring the quality of life in patients receiving palliative care [33,34]. To evaluate the positive benefits of dignity therapy on end-of-life patients' quality of life and emotional improvement, further research is needed to provide evidence for implementing this therapy. The development of adequate scales and research design to capture the effects of therapy would be required. To develop tools that can successfully find dignity therapy's effects, we suggest referring to qualitative studies of dignity therapy and creating suitable items as shown in various qualitative studies. For example, items can be designed to identify changes in the sense of self-integrity based on participants reporting that they have gained an opportunity to perceive their life and self in a positive way through dignity therapy [16]. In addition, questionnaires could also be developed to understand the emotional connection and generativity with the family that may arise in the process of sharing records of dignity thera-

py with family members [18,20,30].

Subjective satisfaction with dignity therapy was shown to be high in studies conducted on patients and their family members [12,18,19,21]. Generativity documents were viewed as a crucial component in promoting high satisfaction with the therapy. The patient can use generativity documents to reflect on their lives and give them significance by recalling the answers to the questions in the DTQP questions. In addition, the patients' loved ones could also build emotional relationships with the patient and find consolation in the life narratives they provided by the patient in generative documents. Therefore, the document was shown to contribute to the therapeutic effects of the therapy. We suggest to investigating the therapeutic features of the document and exploring how its receivers can more effectively embrace its content and meaning (e.g., making artwork based on a patient's joyful memories) in further studies.

Participants in early investigations of dignity therapy were patients with terminal cancer having a life expectancy of approximately 6 months [3,5,13]. However, studies with a broad scope of therapy have begun to be conducted [22-25]. It has been administered to patients with advanced cancer, MND, or early-stage dementia, and the elderly [22-25]. Previous studies that assessed dignity therapy's feasibility and acceptability for these patients supported its benefits. Factors such as the patient's cognitive abilities, as well as the intervention and cooperation of the patient's family in the treatment process, must be taken into consideration for the patient to receive the therapy. In addition, a feasibility study of its online implementation has been performed [26]. Given the high practicality and acceptability of online therapy shown in the study, as well as the advantages of reducing the time for the therapy, it is presumed that dignity therapy in an online environment merits cost-effectiveness. However, for appropriate and efficient online delivery, patients' objections to the online environment and personal information protection issues should be carefully considered.

To examine the possibility of implementing dignity therapy in Korea, we analyzed studies that investigated its effectiveness and feasibility in Japan, China, and Korea [27,29-31]. These studies indicated that patients often avoid direct discussion of death, and it was difficult for patients toward the end of their lives to accept death. In Korea, there is a traditional tendency to perceive death negatively, fearing death, or try to distance it from life [28,35,36]. Thus, for effective dignity therapy in Korea, which belongs to the East Asian cultural context, a protocol that can assist people in accepting death and positively reflecting on life should be applied.

We suggest modifying the DTQP to successfully implement dignity therapy in Korea. Some questions can be modified or reduced, or the order can be changed, rather than directly translating the DTQP's nine questions and using them in the interview. We expect such changes to ease the burden on patients who have difficulties with long interview and to make it easier for them to understand the questions. For example, the first question ("Tell me a little about your life history; particularly the parts that you either remember most or think are the most important? When did you feel most alive?") can be divided into two, as follows: "Please tell me about happy and pleasing moments in your life." and "Please tell me about difficult moments of your life." In addition, because questions 2, 5, and 6 express a similar line of questioning, a new question can be created by integrating these. Considering Korean culture, which regards humility as a virtue, patient responses may be elicited more successful if the expressions "proud of" in question 4 is translated into "feeling great" rather than "boastful."

Furthermore, the therapeutic potential may be strengthened in future studies by properly adapting the dignity therapy protocol to the unique concept of the "good death" that each culture shares. The Institute of Medicine [37] defines good death as "one that is free from avoidable suffering for patients, families, and caregivers in general accordance with the patients' and families' wishes." However, these definitions are not absolute, and may vary from culture to culture or individual. The Western view of the good death shows an individualistic tendency to value autonomy and the right to make decisions about one's own situation [38]. In the Muslim cultural context, for example, good death has been shown to be related to religious beliefs and appearances to relatives [39]. On the other hand, in Japan, unlike in other cultures, patients tend to perceive unawareness of death as a good death rather than accurately recognizing their own physical situation [40]. In China, the cultural characteristics of valuing family bonds affect patients' perception of forming good relationships with their families as a determinant of a good death [41]. Similarly, Koreans value their affiliation with their families

[42]. Considering this perspective, integrating the characteristics that determine a good death in the Korean context with dignity therapy, such as creating a framework with more family participation, is expected to result in higher efficacy and satisfaction in Korea.

Conclusion

This study reviewed previous research on the effectiveness, feasibility, and acceptability of dignity therapy and, psychological treatment in palliative care. The review findings indicate that dignity therapy may help reduce psychological distress, such as depression and anxiety in patients with terminal illnesses, thereby enhancing their end-of-life experience. Legacy documents created during the therapy sessions can provide emotional support to patients and their families. An examination of dignity therapy implemented in East Asian cultures revealed that it has the potential to be adapted to the Korean context if it is preceded by an understanding of the culture in which patients recognize death and considering of their perception of a good death.

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Identification of the transcriptome profile of *Miamiensis avidus* after mebendazole treatment

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Background: The scuticociliate *Miamiensis avidus* is a major pathogenic agent that causes significant economic losses in the flounder aquaculture industry. Many different types of drugs are being tested to control this disease, including mebendazole, which is a broad-spectrum antiprotozoal agent. The purpose of this study was to determine whether mebendazole worked *in vitro* against *M. avidus* and to explore its mechanism of action.

Methods: Transcriptome and gene ontology analyses were conducted to investigate the specifically expressed gene profile. We confirmed the cytotoxic effect of mebendazole against *M. avidus* when it was applied intermittently for a total of three times. We also identified differentially expressed genes using transcriptome analysis.

Results: Most of the upregulated genes were membrane transport-related genes, including Na^+/K^+-ATPase. Most of the downregulated genes were categorized into three groups: tubulin-related, metabolism-related, and transport-related genes. The expression levels of glucose uptake-related genes decreased due to the inhibition of tubulin polymerization, but this was not statistically significant.

Conclusions: Our results demonstrate that intermittent treatment with mebendazole has a significant cytotoxic effect on *M. avidus*. Furthermore, mebendazole induces downregulation of the tubulin-alpha chain and metabolism-related genes. It is presumed that this leads to a glucose shortage and the death of *M. avidus*. Transcriptome analysis will provide useful clues for further studies on mebendazole applications for scutica control.

Keywords: Differentially expressed genes; Mebendazole; *Miamiensis avidus;* Transcriptome analysis

Introduction

Scuticociliatosis, a parasitic disease caused by invasive ciliates (class: Scuticociliatida), has the largest detrimental impact on the fish industry. In South Korea, scuticociliatosis was first identified in an olive flounder farm on Jeju Island in the 1990s, and is now known to cause serious economic damage to olive flounders in farms nationwide every year [1,2]. Since the first report of scuticociliates as parasites of seahorses, they have been reported to infect

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various species of marine animals, causing serious damage [3]. Among closely related pathogenic ciliate species, *Miamiensis avidus* was identified as the dominant species with the strongest pathogenicity in olive flounders and other cultured fish [4].

Although several drug candidates are currently in trials, the Korean government had granted item permissions for the use of formalin (37% formaldehyde) in 2006 and hydrogen peroxide (35% hydrogen peroxide) in 2015 for olive flounder farms. The two agents have proven to be effective drugs for the treatment of scuticociliate infection in multiple studies, and they were subsequently commercialized [5]. However, there is a need for drugs that can effectively treat scutica without adversely affecting the host fish or marine environment.

Benzimidazole derivatives, such as mebendazole and albendazole, have been used as anthelmintics worldwide, in both human and veterinary medicine, for the treatment of various helminth infections [6,7]. Recently, mebendazole and albendazole have been repositioned as promising anti-cancer agents [8]. Among these benzimidazole derivatives, mebendazole has a similar therapeutic effect as albendazole, but has been found to induce only milder oxidative stress than albendazole in the hosts, and it was presumed to be the drug of choice for the treatment of parasitic protozoa and helminths [9]. Mebendazole has also been reported to have antiparasitic effects on monogeneans and scuticociliates that are parasitic on the gills or tissues of various aquatic species [10,11].

Therefore, we evaluated the efficacy of mebendazole against *M. avidus in vitro* and investigated its gene expression profile during the killing process of mebendazole treatment via transcriptome analysis.

Methods

1. Parasite strains and cultivation

The ciliates used in this study were obtained from Pukyong National University (Busan, Korea), which were identified to be *M. avidus* using species-specific oligonucleotide primers reported [12]. *M. avidus* was inoculated into a culture medium with 2% peptone (BD Biosciences, Franklin Lakes, NJ, USA), 1% yeast extract (BD Biosciences), 0.5% sodium chloride (BD Biosciences), 10% fetal bovine serum (Biowest, Riverside, MO, USA), and 1% penicillin-streptomycin (Gibco, Carlsbad, CA, USA) for 3 to 5 days at 22°C.

2. *In vitro* anti-*M. avidus* activity of mebendazole

Mebendazole (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at a concentration of 3,000 ppm. For the antiparasitic activity test, 5 mL (2×10^6) of *M. avidus* was dispensed into a 25 cm^2 flask, and 10, 20, and 30 ppm concentrations of mebendazole were added. Control cells were treated with DMSO only. Then, the number of *M. avidus* was counted after 1, 2, 4, 6, and 8 hours. The amount of DMSO did not exceed 1% of the total volume. Our preliminary test revealed that DMSO (<1%) did not show any harmful effect against *M. avidus* (data not shown).

Among the tested concentrations, 30 ppm showed the highest cytotoxic effect against *M. avidus*. After 8 hours of treatment with 30 ppm of mebendazole, 50%–60% of *M. avidus* species were killed, but the number of *M. avidus* cells began to increase after 8 hours. To increase the cytotoxicity to 100%, *M. avidus* was treated with 30 ppm mebendazole at 4 and 8 hours. We counted the number of live *M. avidus* (actively moving with cilia) after 24 hours.

3. Library preparation and strand-specific RNA-sequencing

When we treated *M. avidus* with 30 ppm of mebendazole for 8 hours, the mortality rate was found to be 50%–60%. Therefore, we used this group as the treated group.

First, 10–20 mL of scutica culture was pelleted by centrifugation at 3,500 ×*g* for 10 minutes at room temperature. Then, 2–4 mL of RNAprotect (Qiagen, Hilden, Germany) was added to the pellet and mixed by vortexing. The pellet was re-precipitated by centrifugation at 3,500 ×*g* for 10 minutes at room temperature, and the supernatant was discarded. The standard TRIzol (Life Technologies, Carlsbad, CA, USA) protocol was then applied to extract the total RNA from cell pellets. To ensure that the DNA was completely removed, DNase digestion was performed, and the total RNA samples were further purified using acidic phenol–chloroform. The sequencing libraries were prepared using an RNA-seq Library Preparation kit (Epicentre Biotechnologies, Madison, WI, USA) with rRNA-depleted samples, and all of the libraries were sequenced by Illumina HiSeq 2000 following the strand-specific sequencing protocol for 100 cycles.

4. Reads processing and expression calculation

The first six bases of reads and adaptors were removed using in-house-developed pipelines. The transcripts were assembled using the default parameters. All transcripts were annotated with coverage and identity greater than or equal to 0.8. Overlapped annotations on transcripts were further combined if they overlapped with each other by at least 70% of their lengths. Based on the gene annotations of the transcripts, the reads per kilobase per million mapped reads (RPKM) values were calculated to determine the gene expression levels.

5. Differential expression determination

Differentially expressed genes (DEGs) between the mebendazole-treated and control groups were determined using the following set. To be considered as an upregulation under drug treatment, the normalized expression value of the gene in the treated sample at mid-log phase must be larger than or equal to 50 RPKM. To be considered as a downregulation, the normalized expression value of the gene in the control at mid-log phase must be greater than or equal to 50 RPKM. For upregulation under drug treatment, the normalized expression value of the gene in the treated group at mid-log phase must be at least 2-fold larger than that in the control; for downregulation under mebendazole treatment, the normalized expression value of the gene in the control group at mid-log phase must be at least 2-fold larger than that in the treated group.

6. GO and KEGG enrichment analyses of DEGs

Gene ontology (GO) enrichment analysis of DEGs was performed using the GO seq R package, in which gene length bias was corrected. GO terms with corrected *p*-values <0.05, were considered to be significantly enriched in DEGs. KOBAS 2.0 was used to test the statistical enrichment of DEGs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

Results

1. Anti-*M. avidus* activity of Mebendazole *in vitro*

The concentration of 30 ppm mebendazole showed the highest killing effect against *M. avidus* after 8 hours of incubation (Fig. 1). However, the number of *M. avidus* began to increase after 8 hours. After the application of mebendazole at 4 and 8 hours after the first treatment, the killing effect of more than 99.9% was confirmed after 24 hours (Fig. 2). This confirmed that mebendazole was effective in killing *M. avidus in vitro*.

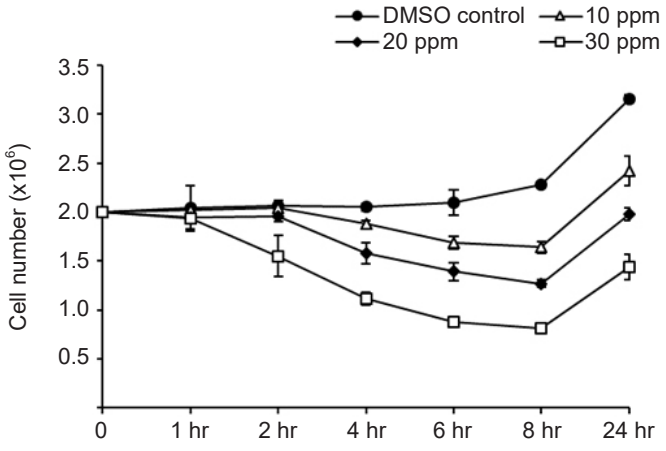

Fig. 1. Cytotoxic effects of different concentrations of mebendazole on *Miamiensis avidus*. Among the three concentrations that were tried, 30 ppm mebendazole showed the highest cytotoxic activity.

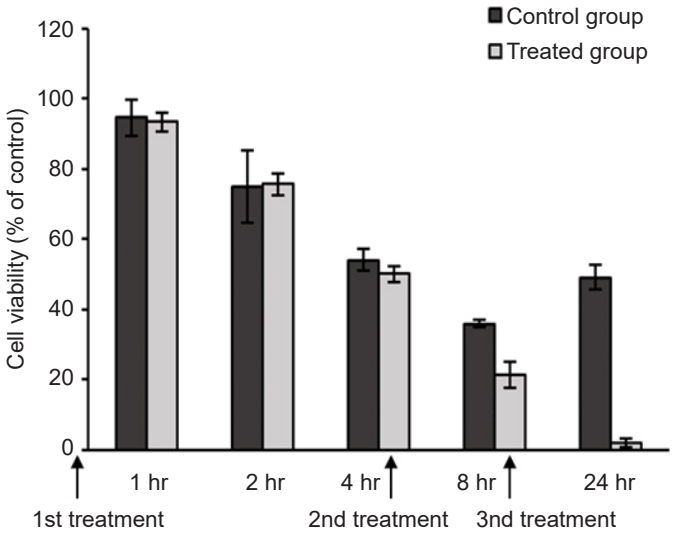

Fig. 2. Cytotoxic effects after three administrations of 30 ppm mebendazole. A higher cytotoxic effect on *Miamiensis avidus* was confirmed by repeating treatment with mebendazole at a concentration of 30 ppm at 0, 4, and 8 hours. Control group: not treated with mebendazole.

2. RNA sequencing and aligning to the reference genome

To investigate the gene expression profile associated with the cytotoxic effect of mebendazole against *M. avidus*, transcriptome analysis was carried out. The two cDNA libraries (control and mebendazole-treated groups) were sequenced on an Illumina HiSeq 4000 platform. A total of 98,172,410 and 53,616,548 raw reads were generated from the control and mebendazole-treated groups' databases, respectively (Tables 1, 2). After removing the low-quality reads, 95,425,100 and 52,123,958 clean readings were obtained, mapping 97.2% and 97.2%, respectively.

3. Gene functional annotation

The presumptive annotation of these transcripts was performed using BLASTX. The putative functions of 11,253 (64.8%) sequences of 17,346 unigene sequences in the control group and 12,167 (53.8%) sequences among 22,631 unigene sequences in the mebendazole-treated group were confirmed (Tables 1, 2).

4. Specific gene expression after mebendazole treatment

There were 204 DEGs with significant differences among 10,621 DEGs. Among them, 48 DEGs were upregulated, while 156 DEGs were downregulated. The most abundantly expressed genes in the control (untreated) and treated groups were the 40S and 60S ribosomal protein genes (Table 3). Transcripts related to the channel and transport proteins, such as Na^+/K^+ ATPase alpha subunit, aquaporin, polyol transporter 2, transmembrane amino acid transporter protein, and granule lattice protein 3 precursor, were upregulated (Table 4). Table 4 also shows the top 20 ranked downregulated genes. Among them, three categories of gene groups known to be associated with the action mechanism of mebendazole were identified (Table 5). In addition, the expression of pyruvate carboxylase related to glucose metabolism was downregulated. Catalase and peroxidase, which are important enzymes that protect the cells from oxidative damage by reactive oxygen species, were also downregulated (Table 5). Glucose transport and immune response-related genes were also identified (Table 6). However, most of them were not significant statistically.

5. GO enrichment analysis of DEGs

GO enrichment is commonly used to explain the biological roles of genes and their products. All DEGs were mapped to GO database terminology and compared to the overall transcriptome background to determine the functionality of the DEGs. All DEGs were categorized into three major functional categories: biological processes (565 unigenes), cellular components (769 unigenes), and molecular functions (210 unigenes) (Fig. 3).

The cellular process (124 unigenes), metabolic process (116 unigenes), and single-organism process (93 unigenes) represented the majority category of biological processes. The majority of cellular components were composed of cells (144 unigenes), cell parts (144 unigenes), membranes (98 unigenes), and organelles (127 unigenes).

Binding (100 unigenes) and catalytic activity (76 unigenes) accounted for the largest portion of the molecular function categories.

Table 1. Quality parameters of transcriptome sequencing of the control and mebendazole-treated group of *Miamiensis avidus*

Name	Raw reads	Raw bases	Raw bases (>Q30)	Clean reads	Clean base pairs	Low-quality reads
Control	98,172,410	9,915,413,410	9,420,795,592	95,425,100 (97.2)	9,599,291,653 (96.8)	2,172,456 (2.2)
Mebendazole	53,616,548	8,096,098,748	7,579,514,339	52,123,958 (97.2)	7,655,407,067 (94.6)	1,361,044 (2.5)

Values are presented as number only or number (%).

Table 2. Quality parameters of transcriptome assembled unigenes of the control and mebendazole-treated group of *Miamiensis avidus*

Name	Gene				Gene (>FPKM 1.0)			
	Expressed	Known	Novel	Unexpressed	Expressed	Known	Novel	Unexpressed
Control	17,346	11,253	6,093	25,411	16,661	10,708	5,953	21,553
Mebendazole	22,631	12,167	10,464	20,126	22,173	11,893	10,280	16,041

FPKM, fragments per kilobase of transcript sequence per million base pairs sequenced.

Table 3. The top 20 most abundantly expressed genes in the control and mebendazole-treated groups

No	Control		Mebendazole	
	Name	Description	Name	Description
1	MTR_5g051170	Hypothetical protein	ALF2_PEA	Fructose-bisphosphate aldolase, cytoplasmic isozyme 2
2	LOC310926	Hypothetical protein LOC310926	TTHERM_00047480	40S ribosomal protein S3a
3	GL50803_114813	VSP	-	-
4	TTHERM_00047480	40S ribosomal protein S3a	MTR_5g051170	Hypothetical protein
5	RPS15	40S ribosomal protein S15	LOC310926	Hypothetical protein LOC310926
6	Rps14	40S ribosomal protein S14	GAPC1	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic
7	EF-1-alpha	Elongation factor 1-alpha	RPS15	40S ribosomal protein S15
8	RPL15	60S ribosomal protein L15	RPS16	40S ribosomal protein S16
9	RPS5	40S ribosomal protein S5	RPL17	60S ribosomal protein L17
10	RPL17	60S ribosomal protein L17	RPS17C	40S ribosomal protein S17-3
11	RPS16	40S ribosomal protein S16	rps30a	40S ribosomal protein S30
12	cyn-1	Peptidyl-prolyl cis-trans isomerase 1	RPL15	60S ribosomal protein L15
13	RPL27	60S ribosomal protein L27	rps19a	40S ribosomal protein S19-A
14	RPS6	40S ribosomal protein S6	RPS26	40S ribosomal protein S26
15	RPL23	60S ribosomal protein L23	RPL18A	60S ribosomal protein L18a
16	rps28a	40S ribosomal protein S28	RPL19	60S ribosomal protein L19
17	RPS8	40S ribosomal protein S8	RPS8	40S ribosomal protein S8
18	RPL7	60S ribosomal protein L7	Rps14	40S ribosomal protein S14
19	EF-1-alpha	Elongation factor 1-alpha	RPS5	40S ribosomal protein S5
20	RpS7	40S ribosomal protein S7	RPS27B	40S ribosomal protein S27-2

Discussion

Here, we report the profile of DEGs and GO analysis after mebendazole treatment of *M. avidus*. Our data clearly indicate that mebendazole has significant deleterious effects on *M. avidus*.

When *M. avidus* ciliate was treated with mebendazole (30 ppm) for 4 hours, more than 50% of scutica was killed and the survival rate dropped to less than 40% at 8 hours. However, the number of live cells started to increase thereafter. In order to increase the killing effect of mebendazole, the drug was treated twice at 4 and 8 hours after the first mebendazole treatment. After 24 hours, most of the scutica cells were killed. These results suggest that the appropriate intermittent administration of mebendazole can be effective for the control of scutica. However, the low efficacy of mebendazole in seawater seems to be one of the most important challenges in its application [13].

The mechanism underlying the antiparasitic action of mebendazole is known to inhibit tubulin polymerization and the formation of microtubules. Glucose transporter (GLUT)-2 is also blocked by mebendazole, which prevents glucose uptake by parasites in the intestines [14,15]. In addition, mebendazole has been reported to activate the mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway in tubulin-activating drugs [16]. We also confirmed the changes in the gene expression patterns of three key categories: membrane transport-related, tubulin-related, and metabolic processes. The characteristic changes in these three categories are in good agreement with the previously known the action mechanism of mebendazole.

Among the upregulated genes, expression of membrane channel and transport-related genes, such as Na^+/K^+ ATPase alpha subunit, apolipoprotein, aquaporin, polyol transporter 2, and transmembrane amino acid transporter protein, were confirmed. Granule lattice protein precursor transcripts are highly upregulated, and these genes are known to be involved in protein sorting and solubility [17,18]. Mebendazole is thought to primarily affect the membrane. Membrane damage and permeability changes caused by mebendazole treatment have been confirmed by antifungal activity screening in previous reports [19,20]. It seems that the expression of related genes was increased to

Table 4. The top 27 most upregulated and 20 most downregulated genes between the control and mebendazole-treated groups

Name	Description	Val_1	Val_2	log_2 (FC)	*p*-value
PTMB.66	Na+\K+ ATPase alpha subunit	0	2,222.10	16.90	0.00057
ALB	Serum albumin	0	792.00	15.40	0.00173
ACA1_015510	Replication factor a protein 1 (rpa1) subfamily protein	0	183.56	13.30	0.03071
APOB	Apolipoprotein B-100	0	166.38	13.10	0.03792
IMG5_197820	Major facilitator superfamily protein, putative	0	158.83	13.10	0.04190
Gvin1	Interferon-induced very large GTPase 1	3.67	1,735.07	8.89	0.00240
ANO10	Anoctamin-10	1.83	424.19	7.85	0.01239
TTHERM_00310510	Phosphatidylinositol-4-phosphate 5-kinase family protein	1.83	401.65	7.78	0.01372
UTY	Histone demethylase UTY	3.67	502.56	7.10	0.01431
Ogfr	Opioid growth factor receptor	1.83	213.95	6.87	0.04353
HSFA1E	Heat stress transcription factor A-1e	5.50	470.94	6.42	0.02231
TTHERM_01070340	Protein kinase domain-containing protein	3.67	259.04	6.14	0.04582
speH	S-adenosylmethionine decarboxylase proenzyme	3.67	250.94	6.10	0.04847
PIP2-4	Aquaporin PIP2-4	31.17	2,002.68	6.01	0.01959
PLT2	Putative polyol transporter 2	62.33	4,023.52	6.01	0.02657
CG8135	LMBR1 domain-containing protein 2 homolog	7.33	460.62	5.97	0.02982
TTHERM_00277160	Transmembrane amino acid transporter protein	60.50	3,650.58	5.92	0.02726
SAV_6332	Alpha-L-arabinofuranosidase	56.83	3,410.31	5.91	0.02655
TTHERM_00378890	Granule lattice protein 5 precursor, putative	34.83	1,617.00	5.54	0.02687
ATL36	Putative RING-H2 finger protein ATL36	11.00	508.75	5.53	0.03692
PHGPx	Probable phospholipid hydroperoxide glutathione peroxidase	34.83	1,475.99	5.41	0.02943
TTHERM_00676930	PAS domain S-box family protein	20.17	750.06	5.22	0.03760
CAMKK2	Calcium/calmodulin-dependent protein kinase kinase 2	18.33	638.69	5.12	0.04327
C33A12.1	Probable NADH dehydrogenase [ubiquinone] 1 alpha Gubcomplex subunit 5	33.00	990.27	4.91	0.04441
TTHERM_00624730	granule lattice protein 3 precursor, putative	64.17	1,895.44	4.88	0.04761
IMG5_197400	Snf7 family protein, putative	51.28	1,493.86	4.86	0.04590
gghB	Gamma-glutamyl hydrolase B	36.67	1,037.44	4.82	0.04722
LHCB1.3	Chlorophyll a-b binding protein 1, chloroplastic	6,169.50	0	−15.70	0.00020
RBCS	Ribulose bisphosphate carboxylase small chain, chloroplastic	3,431.50	0	−14.80	0.00017
IMG5_150220	Scramblase family protein, putative	3,018.80	0	−14.70	0.00017
ART2	Putative uncharacterized protein ART2	11,709.60	0.69	−14.10	0.00077
SE0112	Pyridoxal-deC	1,178.50	0	−13.30	0.00028
RCA	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	925.83	0	−13.00	0.00040
LHCB5	Chlorophyll a-b binding protein CP26, chloroplastic	863.50	0	−12.90	0.00045
pyc	Pyruvate carboxylase	889.20	0	−12.90	0.00043
RBCS F1	Ribulose bisphosphate carboxylase small chain F1, chloroplastic	847.50	0	−12.80	0.00047
PSBO1	Oxygen-evolving enhancer protein 1-1, chloroplastic	729.67	0	−12.60	0.00062
ABHD17A	Alpha/beta hydrolase domain-containing protein 17A	669.51	0	−12.50	0.00074
CAB36	Chlorophyll a-b binding protein 36, chloroplastic	658.17	0	−12.50	0.00076
DHFR-TS	Bifunctional dihydrofolate reductase-thymidylate synthase	634.41	0	−12.40	0.00082
CAB8	Chlorophyll a-b binding protein 8, chloroplastic	591.54	0	−12.30	0.00095
CAT3	Catalase-3	542.68	0	−12.20	0.00114
TPRP-F1	36.4 kDa proline-rich protein	548.17	0	−12.20	0.00112
PER42	Peroxidase 42	498.67	0	−12.10	0.00137
CB12_PETHY	Chlorophyll a-b binding protein, chloroplastic	495.00	0	−12.00	0.00139
PSBP	Oxygen-evolving enhancer protein 2, chloroplastic	485.85	0	−12.00	0.00145
THI1	Thiamine thiazole synthase, chloroplastic	463.83	0	−12.00	0.00161

log_2 FC, log_2 value of fold changes.

Table 5. Three major categories of downregulated genes in the mebendazole-treated group[a)]

Category	Name	Description	log_2 (FC)
Tubulin-related	TUBA3	Tubulin alpha-3 chain	−10.60
	TBA_TETTH	Tubulin alpha chain	−5.18
	CGB_B6140C	Tubulin-binding protein	−5.02
	GLO1	Lactoylglutathione lyase	−10.10
Metabolism-related	SHM1	Serine hydroxymethyltransferase 1, mitochondrial	−10.40
	pyc	Pyruvate carboxylase	−12.90
	PGK1	Phosphoglycerate kinase 1	−10.40
	PGH1	Enolase	−10.30
Transport-related	TTHERM_00473210	Sodium/calcium exchanger protein	−8.24
	dhc-1	Dynein heavy chain, cytoplasmic	−5.73

log_2 (FC), log_2 value of fold changes.
[a)] p-value <0.05.

Table 6. Two categories of downregulated genes in the mebendazole-treated group[a)]

Category	Name	Description	log_2 (FC)
Glucose transporter	At5g16150	Plastidic glucose transporter 4	−0.32
	TBA_TETTH	Glucose transporter type 1	−2.08
	SLC2A3	Solute carrier family 2, facilitated glucose transporter member 3	−2.29
Immune response-related	SOD1	Superoxide dismutase (Cu-Zn)	−3.10
	Sod-1	Superoxide dismutase (Cu-Zn)	−3.16
	CSD2	Superoxide dismutase (Cu-Zn) 2, chloroplastic	−10.00
	kat	Catalase	−2.73
	CAT2[a)]	Catalase-2	−5.35
	CAT3[a)]	Catalase-3	−12.60

log_2 (FC), log_2 value of fold changes.
[a)] p-value <0.05.

restore the damaged membranes and altered permeability.

The downregulated genes were categorized into three main groups: microtubule-related, metabolism-related and transport-related genes. Genes belonging to the microtubule-related group were microtubule constituent genes, tubulin-binding protein transcript, lactoylglutathione lyase, and dynein. Lactoylglutathione lyase is known to be associated with microtubule assembly, and dynein is a protein family responsible for the movement of cilia and flagella by moving along microtubules [21,22]. Our results on microtubule-related genes were consistent with those of previous reports.

Expression levels of genes belonging to the energy metabolism-related group, such as serine hydroxymethyltransferase 1, pyruvate carboxylase, phosphoglycerate kinase 1, enolase, and sodium/calcium exchanger protein, were significantly decreased. It is well known that inhibition of tubulin polymerization by mebendazole induces the loss of cytoplasmic microtubules, which leads to decreased glucose uptake and increased use of stored glycogen [23,24]. Genes related to glucose uptake in humans are known as the *GLUT* gene family, and it has been reported that the *Caenorhabditis elegans*-facilitated glucose transporter (*FGT*) gene in *C. elegans* performs functions similar to those of *GLUT* in humans [25,26]. However, there are no reports on *GLUT or FGT*-like genes in protozoa, including ciliates. In our study, the expression levels of *GLUT1* and *SLC2A3* (solute carrier family 2, facilitated glucose transporter member 3 genes) in humans and *At5g16150* (the plastidic glucose transporter 4) gene in *Arabidopsis thaliana* were found to be decreased. However, the changes of expression levels of these genes were not statistically significant (p>0.05). The statistically non-significant downregulation in the expression of these genes is presumed to be related to

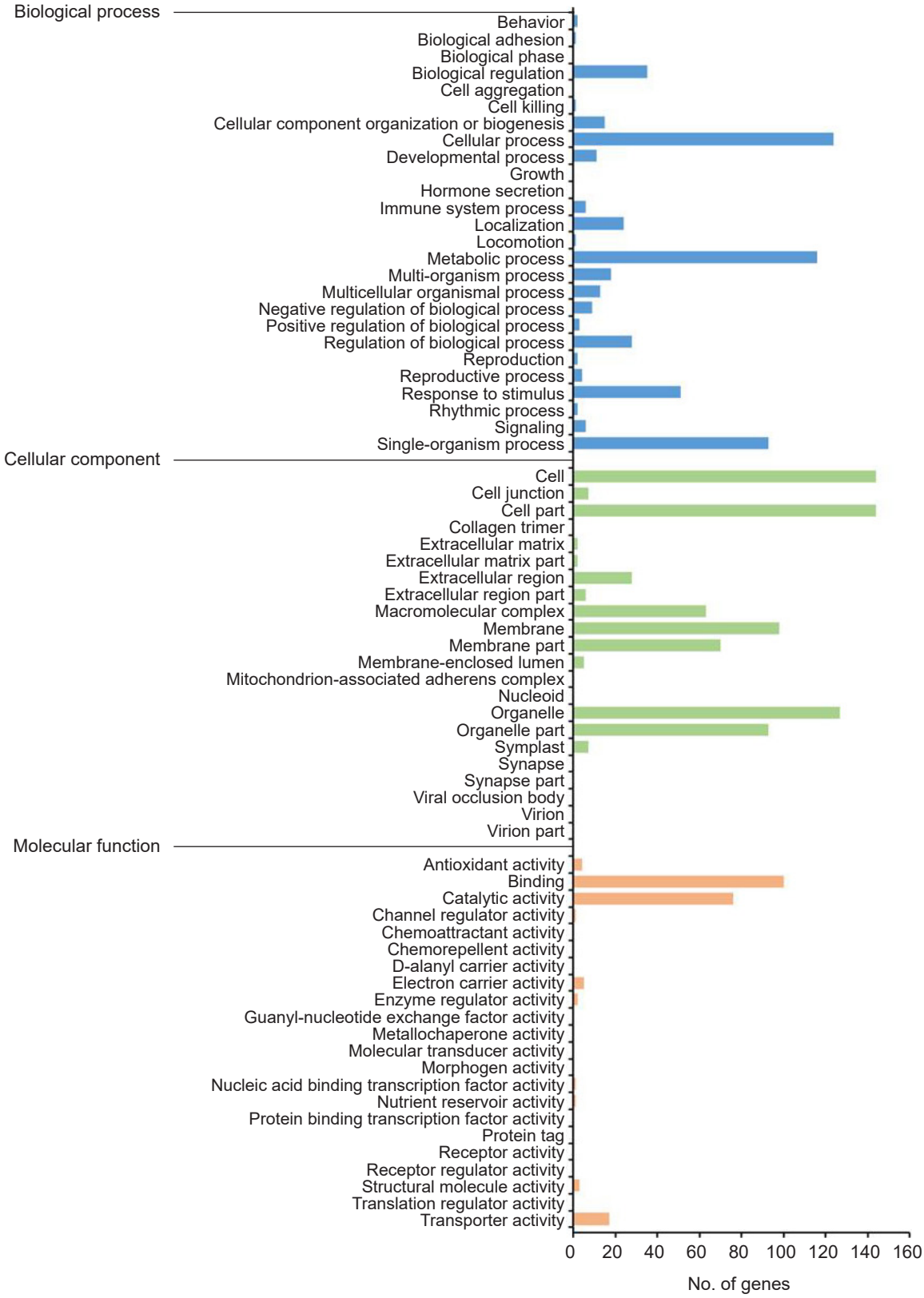

Fig. 3. Gene ontology (GO) annotation of differentially expressed genes. The GO results were summarized in three main GO categories: biological process, cellular component, and molecular function.

the mode of action of metronidazole. The mode of action of benzimidazole derivatives involves the selective binding of helminths tubulin, thus inhibiting microtubule formation. Therefore, the effect of this drug may be slower than that of anthelmintics, which act as neurotransmitter agonists [27]. The expression levels of stress-related genes, such as catalase, were significantly decreased, and those of superoxide dismutase (Cu-Zn) genes, which serve antioxidants were also decreased but not significantly.

Taken together, our results show that the intermittent use of mebendazole can be effective against *M. avidus* infection *in vitro*, although it can be different in salt waters. The gene expression profile after treatment with mebendazole revealed that most of the upregulated genes were related to membrane transport. The downregulated genes consisted of three main categories: tubulin, metabolism and transport-related groups. A couple of stress-related and glucose transporter-related genes were also downregulated but their expression was not statistically significant. These results suggest that the successful killing effect of mebendazole against *M. avidus* is due to changes in the membrane transporters and permeability. In addition, inhibition of tubulin polymerization and decreased metabolism have also been shown to play a role in its killing effect. Transcriptome analysis of mebendazole treatment against *M. avidus* will provide valuable genetic knowledge to explore the possibility of using mebendazole for scutica control.

Article information

Conflicts of interest

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

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Effectiveness of prophylactic calcium and vitamin D supplementation for preventing post-thyroidectomy hypocalcemia: a meta-analysis

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Background: Postsurgical hypocalcemia is the most common and troublesome consequence of thyroidectomy. We investigated the potential role of routine calcium or vitamin D supplementation in preventing postsurgical hypocalcemia.
Methods: We searched MEDLINE and Embase for English-language publications using the keywords "calcium," "vitamin D," and "thyroid cancer." The primary outcome was any postoperative hypocalcemia, and the secondary outcome was symptomatic hypocalcemia.
Results: Four studies that included 381 patients were eligible for this meta-analysis. A random-effects model showed no significant difference in the occurrence of hypocalcemia between calcium/vitamin D treatment and placebo/no treatment. However, the occurrence of symptomatic hypocalcemia was lower in patients with calcium/vitamin D treatment. In the combined results, preoperative calcium and vitamin D supplementation were associated with a reduced incidence of symptomatic hypocalcemia.
Conclusions: Our findings support the use of preoperative calcium and vitamin D supplementation in conjunction with routine postsurgical supplementation for patients after total thyroidectomy.

Keywords: Calcium; Hypocalcemia; Thyroid neoplasms; Vitamin D

Introduction

Thyroid cancer is the most common type of malignant endocrine cancer, and its incidence is continuing to rise worldwide [1]. Total thyroidectomy followed by radioactive iodine treatment together with life-long administration

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of thyroid hormone is the treatment strategy for most patients with thyroid cancer [2]. Total thyroidectomy is one of the most common endocrine-gland operations and most patients recover fully without any adverse events [3]. Hypoparathyroidism (hypoPTH) and hypocalcemia are the most common and troublesome long-term consequence of bilateral and reoperative thyroid operations [4,5]. Large epidemiologic studies have estimated that the overall incidence of hypoPTH after anterior neck surgery is approximately 8%, with 75% of cases resolving within 6 months and the remaining 25% resulting in permanent hypoPTH [6]. Postsurgical hypocalcemia impacts negatively of patient's quality of life due to the need for lifetime medication, regular visits and significant long-term costs. Most transient hypocalcemias may not warrant long-term calcium and vitamin D supplementation [3]. The most common early symptoms of postsurgical hypocalcemia are paresthesias, or numbness and tingling, of the perioral region and the fingertips. More sustained muscle contraction may lead to laryngospasm, and more severe neural excitability may lead to seizures. Moreover, severe hypocalcemia can cause life-threatening complications, such as laryngospasm and cardiac arrhythmias [4,7]. Therefore, close monitoring of calcium and parathyroid hormone (PTH) levels is indicated in order to identify hypoPTH before the development of severe, symptomatic hypocalcemia after surgery [6].

An empirical prophylactic approach for managing potential post-thyroidectomy hypocalcemia is to prescribe oral calcium routinely with or without oral calcitriol, without testing PTH or calcium levels [8,9]. This approach is cost-effective, is not labor intensive, is expeditious, and can hasten hospital discharge after thyroidectomy [4]. Previous studies have shown the efficacy of routine postsurgical calcium and vitamin D supplementation as a prophylactic strategy to prevent hypocalcemia in patients undergoing total thyroidectomy [10-12], but the effectiveness of preoperative supplementation is limited. Moreover, there is no consensus on the role of routine calcium and/or vitamin D before thyroid surgery [11].

Our objectives in this meta-analysis are to evaluate the potential role of routine calcium and vitamin D supplementation for the prevention of postsurgical hypocalcemia and to draw therapy guidelines that may prevent this common complication.

Methods

1. Data search and study selection

We did a systematic search for studies from MEDLINE (inception to March 2019) and Embase (inception to March 2019) using the keywords "calcium" and "thyroid cancer" or "vitamin D" and "thyroid cancer." The primary analysis included studies comparing the occurrence of postsurgical hypocalcemia in subjects with thyroid cancer who had preoperative calcium or vitamin D treatment. Manual searches included scanning of reference lists for relevant studies and eligible articles. We included articles written in English only in this study. Two of us conducted the search of our own, and disagreements were settled by discussion.

2. Data extraction

We extracted data from the publications and recorded the following information independently: first author, year of publication, country, data source, the number of patients and centers enrolled in the study, dosage of calcium or vitamin D, and definition of hypocalcemia. For the outcome data, we calculated the odds ratios (ORs) from the number of cases in which hypocalcemia occurred in each group.

3. Statistical analysis

The primary outcome was any postoperative hypocalcemia, and the secondary outcome was symptomatic hypocalcemia. We analyzed data from each study using Review Manager (RevMan, version 5.2, Copenhagen, Denmark: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012). We reported outcome measures as ORs and 95% confidence intervals (CIs), using a fixed-effect or random-effects model according to Mantel and Haenszel, and presented the results as forest plots. An OR greater than 1 implied more hypocalcemia for preoperative calcium or vitamin D users, whereas an OR less than 1 implied less hypocalcemia for such users. Heterogeneity of the studies were assessed using the chi-square test of heterogeneity; I^2>50% was considered significant heterogeneity, as described by Higgins et al. [13]. A p<0.05 was considered to indicate statistical significance.

Results

1. Study characteristics

We identified 607 articles through the database. After excluding conference abstracts (n=135), non-human studies (n=108), and non-English studies (n=49), we assessed 315 abstracts for eligibility. After reviewing full-text articles, four studies that included 381 patients were eligible for this analysis [14-17]. The details of the study selection process are depicted as a flowchart (Fig. 1). The summary of studies included is presented in Table 1.

2. Hypocalcemia

1) Any postoperative hypocalcemia

Three studies covering 306 patients among four studies were included in analyzing postoperative hypocalcemia. The random-effects model showed no significant difference in the occurrence of hypocalcemia between calcium/

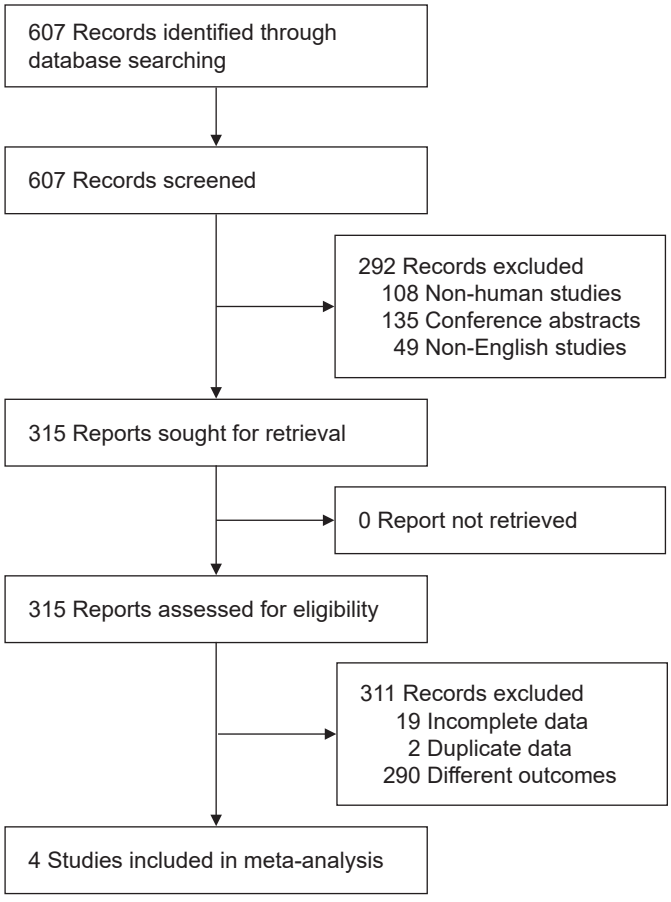

Fig. 1. Flowchart.

Table 1. Study characteristics

Author (year)	Country	No. of subjects		Dosage			Definition of hypocalcemia	
		Patient	Control	Calcium	Vitamin D	Placebo/no treatment	Any postoperative	Symptomatic
Rowe et al. (2018) [16]	Australia	72	78	-	6 Gelatin capsules of cholecalciferol (6×50,000 IU)	6 Capsules of rice flour	Corrected serum calcium <2.10 mmol/L) at any time point during the first 180 postoperative days	Any one of: perioral paresthesia, tetany, positive Chvostek's sign or prolonged QT–interval on ECG associated with biochemical hypocalcemia
Maxwell et al. (2017) [14]	USA	33	32	5 Days of preoperative oral calcium carbonate, 1,000–1,500 mg, 3 times daily and calcitriol, 0.25–0.5 μg twice daily supplementation	-	NA	Postoperative calcium levels and the development of postoperative hypocalcemia (calcium levels <8.0 mg/dL) at 24 hours	Calcium level <8 mg/dL with symptoms
Yu et al. (2017) [17]	USA	41	50	NA	-	NA	Calcium <8.0 mg/dL or ionized calcium <1.0 mmol/L	-
Jaan et al. (2017) [15]	India	30	30	Oral calcium 500 mg every 6 hours and calcitriol 0.25 μg every 6 hours (Shelcal CT) starting 7 days before surgery and continued for 7 days postoperatively	-	-	-	Paresthesia of fingertips and perioral area, tetany, neuropsychiatric manifestations, Chvostek and Trousseau signs, and electrocardiogram evidence of prolonged corrected QT interval by Bazett's formula

NA, not available; CT, computed tomography; ECG, electrocardiogram.

vitamin D treatment and placebo/no treatment (OR, 0.82; 95% CI, 0.36–1.86; I^2=60%, p=0.63) (Fig. 2).

2) Symptomatic hypocalcemia

Three studies covering 275 patients among four studies were included in analyzing postoperative hypocalcemia. The occurrence of symptomatic hypocalcemia was lower in patients with calcium/vitamin D treatment than in those with placebo/no treatment (OR, 0.44; 95% CI, 0.22–0.88; I^2=41%, p=0.02) (Fig. 3).

Discussion

The mainstay of therapy for differentiated thyroid cancer is thyroidectomy [2]. Postsurgical hypocalcemia following thyroidectomy is a common complication because of damage to the parathyroid glands. It also increases healthcare-associated expenditure because of increased monitoring requirements, pharmacotherapy, and prolonged hospitalization in addition to patient morbidity, Interventions that minimize postsurgical hypocalcemia are needed in order to improve patient care and waste of resources. In combined results, we found that preoperative calcium and vitamin D supplementation was associated with a reduced incidence of symptomatic hypocalcemia after total thyroidectomy.

The mechanisms of postsurgical hypoPTH are related to disruption of parathyroid arterial supply or venous drainage, mechanical, thermal or electrical injury, and partial or complete removal [18]. Therefore, the most straightforward way to avoid hypoPTH is to limit the extent of thyroidectomy to a unilateral approach [4]. The best prophylaxis to avoid postsurgical hypocalcemia after total thyroidectomy is parathyroid gland preservation during operation to preserve the blood supply to the parathyroid glands [19]. Even when these glands are thought to be well preserved during surgery, normal postsurgical parathyroid function is not guaranteed [20].

Several interventions to reduce the incidence of postsurgical hypocalcemia have been suggested because patients with symptomatic hypocalcemia undergoes physical and mental suffering [12]. Therefore, routine calcium and vitamin D supplementation is advocated in many clinical centers [4,19]. These prophylactic approaches to prevent postsurgical hypocalcemia is to routine prescription of oral calcium with or without calcitriol [8,9]. Typically, oral calcium carbonate is the most widely available and inexpensive preparation and is given as 500–625 mg to 1,000–1,250 mg two to three times a day. This routine administration of oral calcium is known to reduce postsurgical hypocalcemia

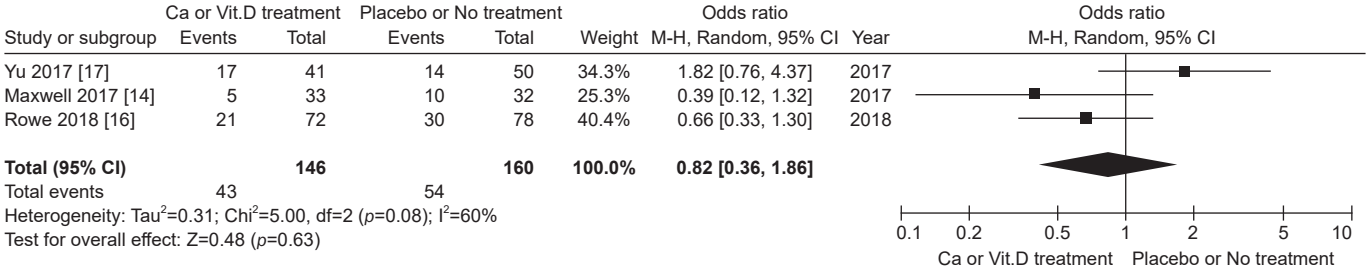

Fig. 2. Forest plot of any postoperative hypocalcemia. Ca, calcium; Vit.D, vitamin D; CI, confidence interval; M-H, Mantel and Haenszel.

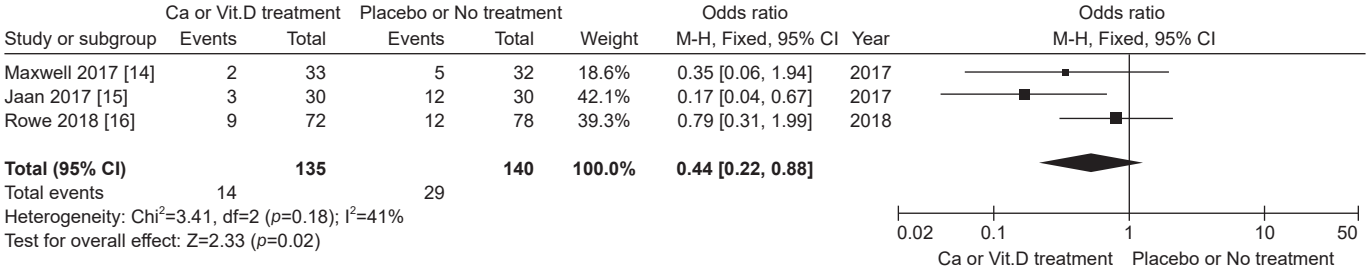

Fig. 3. Forest plot of symptomatic hypocalcemia. Ca, calcium; Vit.D, vitamin D; CI, confidence interval; M-H, Mantel and Haenszel.

to approximately 10% [21]. Because of low cost and ease of dosing for patients at risk for hypoPTH, we also recommend universal calcium prophylaxis like others [19].

Vitamin D deficiency is an independent risk factor of postsurgical hypocalcemia [5]. Moreover, the severity of hypocalcemia seems to be remarkably higher in those with lower than normal preoperative vitamin D levels [22,23]. Therefore, prophylactic treatment of hypocalcemia with vitamin D and calcium is a reasonable strategy with synergy [24]. Impaired PTH secretion with inhibition of bone resorption, reduction of vitamin D synthesis by the kidneys, and reduced intestinal absorption of calcium results in postoperative hypocalcemia [25]. Adding calcitriol ($1,25\text{-}(OH)_2\text{-}D_3$) adds to the cost but increases the effectiveness of oral calcium. It also increases calcium absorption and increasing intestinal calcium transport into the blood. This effect on calcium absorption usually takes few days [14]. Therefore, preoperative supplementation in patients undergoing thyroidectomy with calcitriol are expected to increase the efficacy of routine calcium supplementation in the immediate postsurgical period, thereby decreasing the duration of transient hypocalcemia [4].

PTH level has also been suggested as a reliable marker of postsurgical permanent hypoPTH [26]. However, development of acute hypocalcemia after thyroid surgery lags behind the decline in the serum PTH level, and the patient may have been from the hospital before their serum calcium having reaches a nadir, which may occur 24 to 72 hours after thyroidectomy [4]. Therefore, it is important to anticipate the possibility of progressive hypocalcemia, to educate patients about its possible development and steps they should take to avoid and treat it, and to institute preemptive measures that both prevent and correct hypocalcemia in the presurgical period.

Postoperative calcium plus vitamin D is known to be effective in preventing postoperative hypocalcemia and decreasing the demand for intravenous calcium supplementation [12]. This meta-analysis found the role of preoperative oral calcium and vitamin D supplementation in avoiding postsurgical symptomatic hypocalcemia. This prophylactic approach may cause uncommon but serious risks of overshooting and causing hypercalcemia and potential renal injury. Therefore, biochemical monitoring for medication tapering is mandatory [4]. However, the half-life of calcitriol is relatively short (5–8 hours), and toxicity from excessive calcitriol ingestion may be reversed quickly (within days) [4].

Our study has some limitations. First, the studies included in the meta-analysis were heterogeneous. There was a high heterogeneity especially among the studies including those at a young age. There is no universal agreement on standardized definitions for postsurgical hypocalcemia and hypoPTH after total thyroidectomy [11]. The reported incidence of postsurgical hypoPTH varies differs greatly, and previous research also suggested that the definition of hypoPTH is not universal throughout the literature [11]. Second, attempting to compare data from the surgical series is difficult and may be inaccurate. Analysis was even more difficult by the diversity of postsurgical electrolyte supplementation protocols used by different doctors. Third, we cannot define the adequate dosage and duration of calcium and vitamin D intake before surgery, because the regimens of each study studies were quite variable very different. Lastly, small numbers of studies included in this meta-analysis might be exposed to publication bias.

Postsurgical hypocalcemia is the most common complication of total thyroidectomy. Increased recognition and early implementation of various strategies can improve clinical outcomes and quality of life. We support the use of preoperative calcium and vitamin D supplementation in conjunction with routine postsurgical supplementation for patients after total thyroidectomy.

Article information

Conflicts of interest

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

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Author contributions

Conceptualization: KP, SS. Data curation: JWS, KK, IJK, KP. Formal analysis: KK, KP. Investigation: HM, HYK, MKP, KP, SS. Methodology: KP, SS. Visualization: KK, KP, SS. Writing - review & editing: HM, HYK, MKP, KP, SS. Approval of final manuscript: all authors.

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Clinical significance of copeptin as an early predictor of renal graft dysfunction in renal transplant recipients

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Background: Copeptin is the carboxyl-terminal part of the vasopressin precursor protein, and its concentration is an independent predictor of the onset of chronic kidney disease and a rapid decline in the glomerular filtration rate. The glomerular filtration rate is regarded as the best indicator of kidney transplant function and is a predictor of graft and patient survival. We investigated the clinical significance of copeptin as an early predictor of renal graft dysfunction in renal transplant recipients.

Methods: We measured serum creatinine, cystatin C, and copeptin concentrations in renal transplant recipients on the day of their operation, as well as on postoperative days 3, 7, 30, and 365. Acute rejection was defined as a sudden decrease in renal function accompanied by histological changes.

Results: Eight renal transplant recipients were enrolled in the study from July 2018 to December 2019. Four patients experienced histologically confirmed transplant rejection. All four cases involved acute T-cell rejection. No significant correlation was found between the copeptin level and the presence or absence of rejection at any time point. In subgroup analyses, changes in creatinine, the estimated glomerular filtration rate, cystatin, and copeptin did not show statistical significance.

Conclusions: We anticipated that copeptin would be useful to identify individuals at high risk of transplant rejection; however, our study failed to show an association. Further research will be needed to overcome the limitations of this study.

Keywords: Copeptin; Graft rejection; Kidney transplantation

Introduction

Renal transplant patients can experience graft dysfunction and rejection. Predicting which patients are at higher risk and why for this outcome may enable early and effective interventions. Copeptin, the C-terminal fragment of pro-arginine vasopressin precursor, was first discovered by Holwerda and his colleagues in 1972 as a Leucine-rich glycopeptide of relatively low molecular mass (about 3,248 Da) in the posterior pituitary of pigs, and its 39 amino acids sequence was determined in 1981 [1,2]. In a study using rats, continuous infusion of desmopressin, a vasopressin V2 receptor agonist, was associated with proteinuria and decreased renal function [3,4]. It has also been confirmed

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that renal function improves when water absorption is increased or when vasopressin levels are lowered using V1a/V2-receptor antagonists [5,6]. This is because it suppresses tubuloglomerular feedback by enhancing urea recycling and sodium chloride reabsorption. Vasopressin might be a suitable tool for predicting renal graft dysfunction and rejection. However, the study using desmopressin in kidney transplantation was mainly conducted on donors before transplantation. To the best of our knowledge, no studies have been reported on the association between desmopressin or copeptin and acute kidney transplant rejection in post-transplant recipients. Because vasopressin is difficult to measure due to technical complications, copeptin might be a better candidate. Copeptin is the carboxyl-terminal portion of the vasopressin precursor protein and it has been correlated with plasma vasopressin concentration [7]. Furthermore, copeptin independently predicts the onset of chronic kidney disease and a rapid decline in estimated glomerular filtration rate (eGFR) [8]. eGFR is generally regarded as the best indicator of renal graft function and is a predictor of both transplant and patient survival. Based on the results of previous studies, we theorize copeptin may be useful for predicting which patients are at higher risk of renal graft dysfunction and rejection.

This study assessed the correlation between changes in copeptin level with the function and prognosis of renal transplants by serially measuring the concentration of copeptin after transplantation.

Methods

Ethical statements: Our research protocol received the Institutional Review Board of Inje University Haeundae Paik Hospital's approval and was compliant with the Helsinki Declaration (IRB number: 2019-08-026-004). All study participants provided informed consent.

1. Patient population and classification

This study was conducted on patients undergoing renal transplantation at the Haeundae Paik Hospital from July 2018 to December 2019. Surgery time, amount of bleeding during surgery, cold ischemic time, warm ischemic time, human leukocyte antigen matching results, panel-reactive antibody screening, and immunosuppressants administered after surgery were all confirmed. In cases of renal function change in which clinically acute rejection was suspected during a 1-year follow-up period, confirmatory examination was performed via biopsy of the transplanted kidney.

2. Definitions

Acute rejection was defined as a case diagnosed pathologically through renal biopsy. Warm ischemic time was the time from clamping of the aorta to cold perfusion. Cold ischemic time was the time from cold perfusion of the kidney to the venous anastomosis.

3. Experimental design

Examinations were performed the day before renal transplantation as well as on postoperative days 3, 7, 30, and 365. Examinations included serum copeptin, creatinine, sodium, and cystatin C. Serum osmolality was added in a calculated way. eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation.

Copeptin testing was performed with a commercial enzyme-linked immunosorbent assay kit (CSB-E12130h; Cusabio Biotech Co., Ltd., Houston, TX, USA) using a serum sample stored at –20°C. The lo wer limit of detection was 19.53 pg/mL; the detection range was 78–5,000 pg/mL. Creatinine and cystatin C were measured on a Cobas c702 (Roche Diagnostics, Basel, Switzerland) using the following Roche reagents: CREJ2 (Creatinine Jaffe Gen.2) and CYSC2 (Tina-quant Cystatin C Gen.2).

The serum osmolality excluding ethanol was calculated by using the following formula for calculated osmolality excluding ethanol: 2 Na (mEq/L)+(urea [mg/dL])/2.8+(glucose [mg/dL])/18.

4. Statistical analysis

The study data were presented as frequencies with percentages for categorical variables and means±standard deviations for continuous variables. Mann-Whitney *U* test was performed to verify whether there was a significant difference in baseline, postoperative days 3, 7, 30, and 365. copeptin according to the presence or absence of kidney rejection. The correlation between changes in eGFR or cystatin C and copeptin was analyzed by Spearman correlation test. Spearman correlation analysis was performed using the median value of each test result from baseline to post-

operative days 365. All statistical analyses were carried out using SPSS 25.0 version software (IBM Corp., Armonk, NY, USA); *p*-values less than 0.05 were considered significant.

Results

Patient characteristics are presented in Table 1. Eight patients who underwent living or deceased donor kidney transplantation were enrolled in the study. Two of them were lost during follow-up. One patient stopped collecting samples after the baseline examination, and the other failed to perform additional examinations other than the baseline examination due to a thrombus and re-anastomosis that occurred after transplantation. Seven patients received a deceased donor kidney transplant and one had a living donor kidney transplant. There were five male and three female patients with an average age of 50.6 years. The average operation time was 239.4 minutes and the average amount of bleeding during surgery was 525.0 mL. All eight patients received tacrolimus, mycophenolate mofetil, prednisolone, and basiliximab as immunosuppressants after surgery. Cold ischemic time was 264.8 minutes, and warm ischemic time was 48.8 minutes.

Four patients underwent histological examination and transplantation rejection was confirmed. All were acute T-cell rejection. Of the six patients who had been followed for 1 year, three experienced renal rejection. The timing of occurrence of acute rejection in three patients is as follows; one patient 10 months after surgery, the other 8 months after surgery, and the other 2 months after surgery. Additional results were summarized for these six patients. Median copeptin concentration for the whole group at baseline was 122.25 pg/mL. Median copeptin concentration was 102.2 pg/mL for men and 138.35 pg/mL for women. The distribution of copeptin values ranged from 85.3 to 322.8 pg/mL. The median copeptin concentration in patients with rejection was 191.4 pg/mL at baseline and the median copeptin concentration in patients without rejection was 102.2 pg/mL at baseline. In both groups, copeptin level increased during the first 30 days, and decreased after 1 year of follow-up (Fig. 1). The baseline copeptin did not show any significant difference according to the presence or absence of rejection (p=0.686). There was no significant correlation in copeptin level for each period according to the presence or absence of rejection (day 3 copeptin, p=1.000, day 7 copeptin, p=0.800, day 30 copeptin, p=0.667, and day 365 copeptin, p=0.200).

Fig. 2 shows the results of clinical examinations during 1 year of follow-up. Patients 1 to 3 were patients with rejection and 4 to 6 were patients with no rejection. Fig. 2A shows the change in eGFR over time for each patient, Fig. 2B shows the change in copeptin, Fig. 2C shows the change in creatinine, and Fig. 2D shows the change in cystatin C. Most patients showed improvement of renal function compared to baseline, and renal function was maintained during 1-year follow-up. After transplantation, all six patients remained without dialysis.

As the baseline creatinine level increased, baseline copeptin concentration tended to increase, but there was no statistically significant correlation (rho=0.405, p=0.320). As the baseline eGFR level increases, the baseline copeptin concentration tends to increase, but there was no statistical significance (rho=0.405, p=0.320). The correlation between eGFR change and copeptin was calculated to be 0.300, but it did not show statistical significance (p=0.624). The correlation between cystatin C and copeptin was calculated as a correlation coefficient of –0.700 and did not show statistical significance (p=0.188). Subgroup analysis was performed as a group with rejection. The correlation coefficient between eGFR change and copeptin was calculated to be –0.200, but it did not show statistical significance (p=0.747). The correlation between cystatin C and copeptin was calculated as a correlation coefficient of –0.400 and did not show statistical significance (p=0.600).

Discussion

Our study investigated whether changes in the concentration of copeptin after transplantation could predict transplant function decline. Other well-known tests for predicting renal function include creatinine and cystatin C. However, due to many factors such as chronic illness, malnutrition, and muscle wasting, serum creatinine is not reliable as a marker of renal function in transplant patients. There is also a method for measuring renal function using inulin, ^{99m}Tc-DTPA, or iohexol, but these processes are complicated and expensive to use in routine clinical practice. Previous studies of renal function measurement that have been useful in kidney transplant patients have been conducted. According to White et al. [9], the cystatin C-based

Table 1. Characteristics of the study population

Characteristics	Patient number							
	1	2	3	4	5	6	7	8
Sex	Female	Female	Female	Male	Male	Male	Male	Male
Age (yr)	51	51	41	55	47	54	45	61
Rejection	Yes	Yes	Yes	No	No	No	Yes	No
DM	No	No	Yes	No	Yes	Yes	No	Yes
HTN	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Liver disease	No	No	No	No	No	No	No	No
HBsAg/HBsAb	–/+	– /–	–/+	–/+	–/+	–/–	–/+	–/–
Pre-transplant dialysis modality	HD	HD	HD	PD	HD	HD	PD	HD
Duration of dialysis (mo)	77	1	9	65	15	26	78	10
Cause of end-stage renal disease	DM	Polycystic kidney disease	DM	HTN	DM	DM	HTN	DM
Left ventricular ejection fraction (%)	55	63	70	63	63	57	46	66
Donor type	Deceased donor	Living donor	Deceased donor	Deceased donor	Deceased donor	Deceased donor	Deceased donor	Deceased donor
Donor age (yr)	48	54	44	47	68	54	47	53
Donor sex	Male	Male	Male	Male	Female	Female	Male	Female
Donor serum creatinine (mg/dL)	1.32	0.93	1.40	2.32	0.70	1.07	1.23	1.31
Operation time (min)	215	200	215	245	235	210	285	310
Cold ischemic time (min)	191	156	253	197	620	212	56	433
Warm ischemic time (min)	40	43	50	55	50	74	39	39
HLA mismatching	1	2	1	2	1	2	3	4
Panel-reactive antibody (calculated, %)	–	+ (33)	+ (87)	–	–	–	–	+ (12)
Baseline copeptin (pg/mL)	85.3	299.8	191.4	99.9	322.8	102.2	94.6	142.3
Baseline sodium (mmol/L)	138	142	136	138	134	133	134	132
Baseline serum osmolality (mOsm/kg)	310.9	315.8	298.9	309.3	298.7	306.9	292.6	284.3
Input on postoperative day 1 (mL)	2,900	3,100	2,100	2,300	5,700	4,000	4,900	3,500
Output on postoperative day 1 (mL)	2,500	4,900	4,600	1,500	3,600	4,100	4,000	200
Tacrolimus trough level on postoperative day 1 (ng/mL)	6.9	12.1	9.0	9.6	5.8	5.3	7.6	6.8

DM, diabetes mellitus; HTN, hypertension; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HLA, human leukocyte antigen; HD, hemodialysis; PD, peritoneal dialysis.

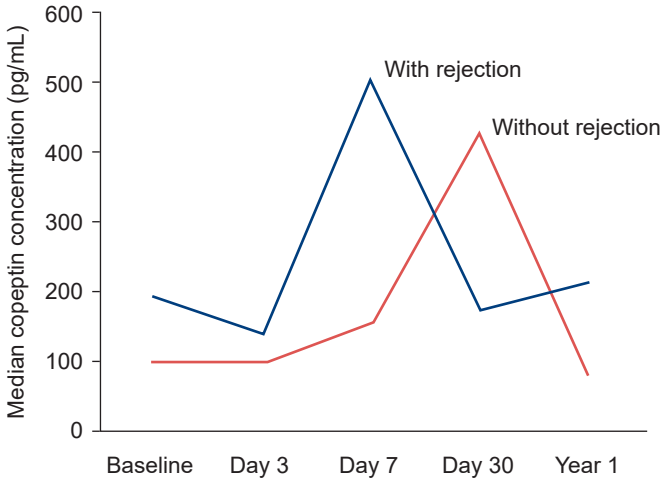

Fig. 1. Changes in median copeptin concentrations in patients with and without rejection.

equation was found to be more accurate in predicting eGFR in kidney transplant recipients than the existing creatinine-based equation. Meanwhile, according to a study by Luis-Lima et al. [10], both creatinine-based formulas and cystatin C-based formulas show low precision and accuracy to reflect actual renal function in kidney transplant recipients. As such, the test results for estimating renal function in transplant patients are considered to be different compared to the general population, and these results are related to events such as medications taken by patients, physiologic changes, infection, or rejection of the transplant. We believe this study is meaningful because accurate renal function tracking in kidney transplant patients is very important in predicting which patients are at higher risk of renal graft dysfunction and rejection.

Many risk factors for rejection are known, such as age, human leukocyte antigen mismatching, and the number of transplants. According to a study by Han et al. [11], hypo-

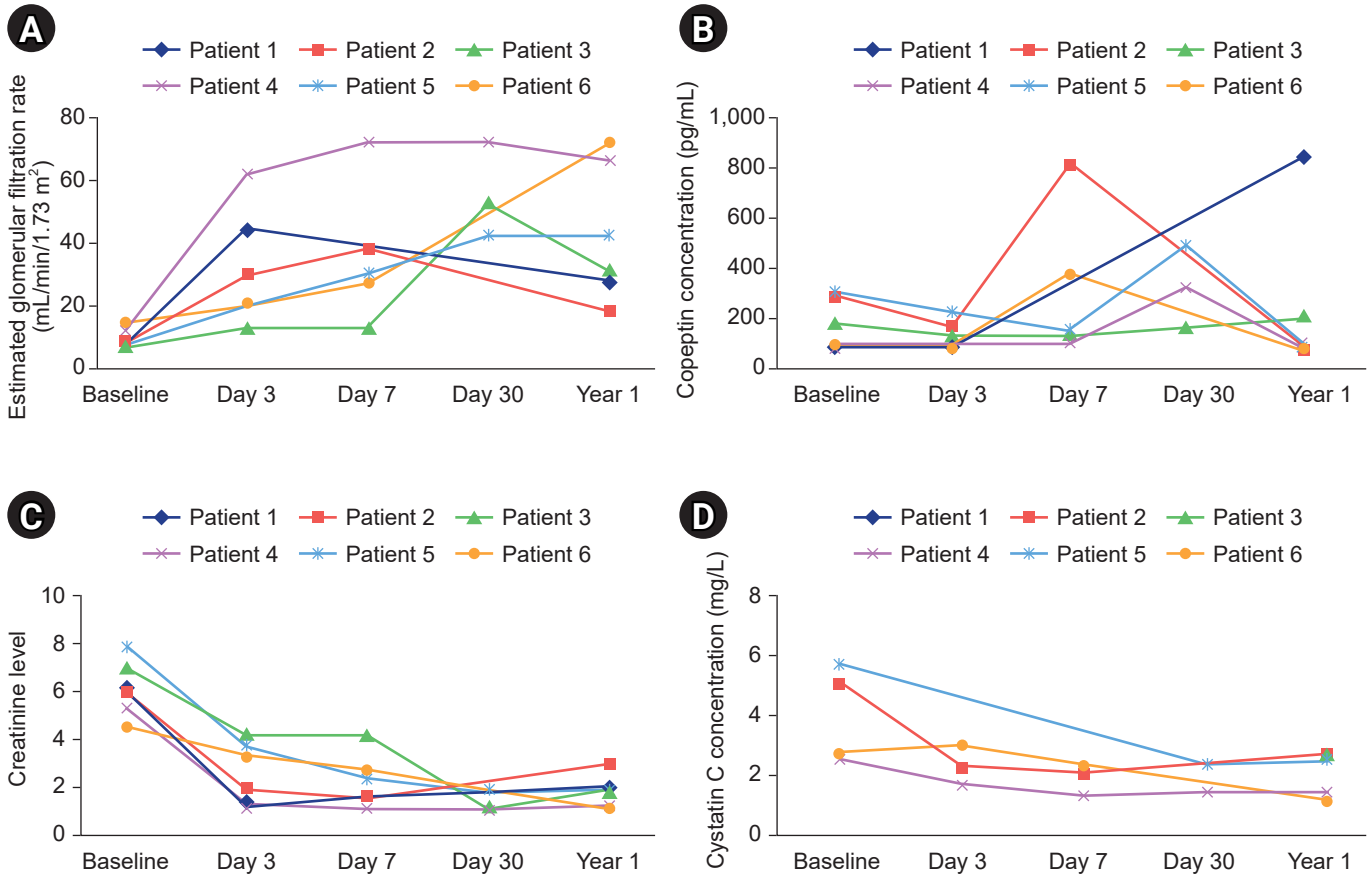

Fig. 2. Changes in parameters over time for each patient. Changes in (A) the estimated glomerular filtration rate, (B) copeptin concentrations, (C) creatinine levels, and (D) cystatin C concentrations.

natremia after kidney transplantation was associated with graft failure and mortality. After confirming that copeptin is related to osmoregulation, the study was expected to predict graft failure after kidney transplantation. However, in our study, copeptin level was not significantly different depending on the presence or absence of rejection. It should be considered that previous studies have shown hyponatremia is associated with graft failure, but the mechanism and direct causality have not been verified. If hyponatremia was a companion event to the outcome of the transplant, there was no significant difference in our study considering that all patients were in a dialysis-free state.

According to a study by Mazloum et al. [12], osmoregulation affects transplant results. Chronically elevated vasopressin is associated with worsening renal function. Copeptin, a vasopressin precursor, has been identified in previous cohort studies as an independent predictor of the development of new chronic kidney disease and rapid decrease in eGFR [8]. eGFR is generally regarded as the best indicator of graft function and a predictor of patient survival. Meanwhile, according to a study by Meijer et al. [13], since baseline copeptin level correlated with changes in the eGFR during the follow-up of 3.2 years, vasopressin may play a role in renal function changes in kidney transplant patients. However, this study confirmed the relationship with subsequent changes in renal function by measuring baseline copeptin. Therefore, considering that copeptin is associated with kidney and urinary tract diseases, infections of the upper respiratory tract, and some physiological states such as fasting or thirsting, it is difficult to control all other patient conditions at the time of copeptin measurement, which limits the study [7,14,15]. In addition, since copeptin levels are also associated with diabetic macrovascular and microvascular complications, it should be considered that renal function deteriorated due to complications from diabetes, which may affect the outcome of transplanted patients [16-19]. With these limitations in mind, we tried to confirm the association between serial changes in copeptin and renal function, but our study did not provide meaningful results. In addition, although there was improvement in eGFR and all patients were stable without dialysis, the copeptin concentration did not show a statistically significant relationship with changes in eGFR, creatinine, or cystatin C.

The reason for not finding statistically meaningful results is considered to be the limitation of our research. No statistical significance was found, but similar trends in copeptin concentration and renal function were confirmed. It is expected that statistical significance can be secured by collecting a large number of patients and clinical results over a longer period. This is a study conducted by a single institution and the relatively small sample size is a limitation. Since they were all T-cell rejection, it may have been more difficult to reveal the correlation, so it would be a limitation. It is also expected that a longer follow-up period will be required. Previous studies have shown that copeptin in the heart but not the kidney transplant population was independently associated with kidney and heart function. It may also predict the outcome of orthotopic heart transplants. It may be worthwhile to establish copeptin ranges in relation to kidney function among heart and kidney allograft recipients [20]. In our study, the left ventricular ejection fraction was presented as data reflecting the patient's heart function. We did not find an association between copeptin and renal function changes. Therefore, follow-up studies including additional data such as New York Heart Association Class or ProBNP or Soluble ST2 are expected. Copeptin levels can be affected by a variety of different physiologic and pathologic conditions, and additional corrections may be needed [7,14,15]. It is unlikely that the copeptin level will provide more information to predict renal function changes compared with creatinine and cystatin C, which are already in use. However, if we consider the relationship between copeptin and vasopressin, we should consider the possibility of benefiting from treatment with V2-receptor antagonists in patients with high copeptin levels or those at high risk of decreased renal function. To study these new treatments, it will be necessary to confirm the correlation between changes in copeptin levels and changes in renal function in certain patient groups, such as patients with renal transplantation.

Copeptin level was expected to identify individuals with a high risk of declining graft function or with a high likelihood of rejection; however, we found no statistically significant association. Further research is needed to overcome the limitations of our study, and it is expected that this will ensure a longer graft survival and increase the patient's survival.

Article information

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

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How does quiz activity affect summative assessment outcomes? An analysis of three consecutive years' data on self-directed learning

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Background: We investigated how quiz activities can improve summative assessment outcomes by analyzing the relationship between them.

Methods: We used 217 first-year medical students' medical informatics data from 3 consecutive years. We analyzed summative assessment outcomes between quiz completion and incompletion groups, one-time and multiple-time quiz learning groups, and three combined comparisons between subgroups of quiz learning activity frequencies: 1 versus 2, 3, 4, and 6 (group 1), 1 and 2 versus 3, 4, and 6 (group 2), and 1, 2, and 3 versus 4 and 6 (group 3). We then analyzed correlations between the final quiz scores and summative assessment outcomes.

Results: The summative assessment means for students who completed quizzes and those who did not were 87.16±8.73 and 83.22±8.31, respectively (p=0.001). The means for the one-time and multiple-time quiz learning groups were 86.54±8.94 and 88.71±8.10, respectively (p=0.223). The means for combined subgroups were not significantly different between groups (p>0.05), although a statistically significant increasing trend was found from groups 1 to 3 (0.223>0.203>0.075 using the t-test and 0.225>0.150>0.067 using the Mann-Whitney test, respectively). Summative assessment scores were not significantly correlated with quiz scores (r=0.115, p=0.213).

Conclusions: Quizzes helped students who used self-directed learning obtain better summative assessment outcomes. Formative quizzes presumably did not provide students with direct knowledge, but showed them their weak points and motivated them to work on areas where their knowledge was insufficient.

Keywords: Correlation of data; Formative assessment; Quiz; Self-directed learning; Summative assessment

Introduction

Doctors are required to continuously update and improve their medical skills and knowledge based on changes in the field that lead to better practices in medicine, and to voluntarily learn what is necessary to meet the requirements for medical professionals [1,2]. Therefore, individuals have to decide their own learning needs to plan and implement these processes for their successful lifelong professional development [1]. From this perspective, self-directed learning has always been a cornerstone of the ideal student learning methods [3].

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Students need to be able to accurately evaluate their competency of curricular topics and modulate learning goals accordingly to be successful self-directed learners [4]. Formative assessment is very important as an instructional tool in this regard [5], because it can be considered not only as an assessment resource but also a guide for students to recognize areas where they experience difficulties in their acquisition process by tracing their own progress in self-directed learning [6]. In general, the term "formative assessment" includes any activities that happen between trainers and trainees after an assessment [7]. Therefore, formative assessments are designed to help students improve learning by providing summative assessment familiarization and feedback that guides student learning [8].

Although there are various kinds of formative assessment tools, quizzes are used most often [9-11]. This is because they promote motivational completion by increasing medical student and teacher interactions [12]. Repeated testing enhances long-term information retention compared to repeated studies [13]. This implies that testing is not only an assessment tool, but also plays a significant role in student learning. Furthermore, information retrieval demonstrated by taking tests is a key to effective long-term information retention [14]. As a result, when quizzes are implemented as a method of test-enhanced learning, they can be useful for students' learning complex sets of medical facts [15].

Quizzes where students are expected to strengthen their learning by completing the quiz activity are called formative quizzes. Formative quizzes have been reported to improve summative assessment outcomes [16-18]. Although many studies have reported the usefulness of quizzes for improving learning achievements, they have not evaluated various quiz activity conditions with respect to the quiz's relationship to the summative assessment outcome. In other words, critical points within the quizzes should be included when determining positive learning effects. Currently, only quiz scores or quiz activity completion are used when analyzing the relationship between the quizzes and summative assessment outcome.

Much information about the relationship between formative assessments and summative assessments has already been reported in previous studies. However, the association between various quiz activities such as quiz activity frequency and sequential quiz activity with learning accomplishments has not been sufficiently validated. Therefore, we evaluated the effects of various analytical quiz activities on summative assessment outcomes in this study [19]. Several critical quiz activity points, such as quiz activity frequency and score trends for sequential quiz activities, were analyzed to determine the factors that played a role in improving summative assessment outcomes. We attempted to explore how quiz activity affected summative assessment outcomes by analyzing the data from 3 consecutive years to compare the average and final quiz scores, quiz activity frequency, and summative assessment outcomes.

Methods

Ethical statements: This study was approved by the Institutional Review Board of Kosin University Gospel Hospital (KUGH 2021-11-004). Informed written consent was exempted. Data remained confidential throughout this study.

1. Enrolled students

We provided a web-based instruction (WBI) platform during a "medical informatics" course for students in their first year of medical school. Three consecutive data from the course could be retrieved and we analyzed the data retrospectively for the analyses of this study.

2. Quiz

Learning goals provided before class time were established based on the ultimate achievements required for medical students after completion of the medical informatics course. Formative quizzes were provided for students to test and review what they learned during their medical informatics class time. Each quiz consisted of 16 questions based on the learning goals given to students in advance. Written feedback was not provided in any of the formative question results. This was intended for students to recognize their weak points in learning and implement further study voluntarily. Instead, a forum site was provided for students to ask any questions during their self-directed learning.

We analyzed 217 first-year medical students' records from a medical informatics class taken from 2019 to 2021. Students were asked voluntarily to complete quizzes created using Moodle version 3.0 software (Martin Dougiamas, Perth, Australia; http://www.moodle.org/) (Fig. 1). Stu-

dents either installed the Moodle app, a WBI platform, on their smartphone or used the WBI website online through a computer [20].

3. Summative assessment

After completing the medical informatics course, students used a summative assessment as their final examination. The assessment's level of difficulty differed each year, which could introduce analytical bias. To account for this, we raised the top score to 100 and adjusted other scores accordingly. The summative assessments consisted of 20 to 25 questions.

4. Summative assessment outcome for quiz completion versus quiz incompletion groups

We divided students into quiz completion and quiz incompletion groups and calculated the summative assessment outcome means for each group. We then compared the statistical differences between the two groups.

5. Quiz activity frequency and summative assessment outcome

We divided students into six subgroups according to quiz completion frequency, from 0 to 6. Once students answered 16 quiz questions in each attempt, they were considered to have completed one round of quiz activity. We analyzed the quiz completion frequency for each student and investigated whether it caused a better summative assessment outcome. We compared quiz final score means with summative assessment outcomes for each quiz frequency group.

6. Score trends for sequential quiz activity

We combined subgroups of various quiz learning activity frequencies to create three comparison combinations: 1 versus 2, 3, 4, and 6 (group 1), 1 and 2 versus 3, 4, and 6 (group 2), and 1, 2, and 3 versus 4 and 6 (group 3). We then compared the summative assessment outcome with the three groups.

7. Correlation between the summative assessment outcome and final quiz scores

We compared the final quiz scores with the summative assessment outcomes. When students performed more than one quiz learning activity, the last activity's score was used for this analysis.

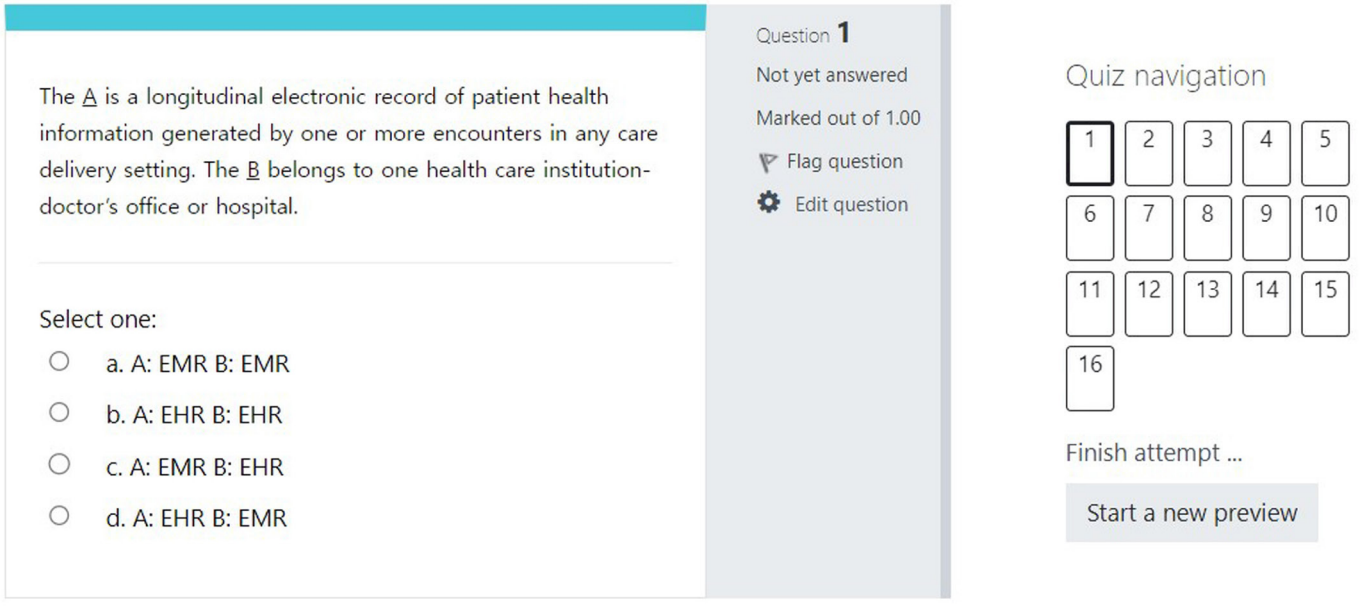

Fig. 1. Formative quizzes provided for students' self-directed learning. Formative quizzes were made using the "Quiz" function on the Moodle platform. Students could select questions and were allowed unlimited attempts to solve them. The highest grade was applied for a question with multiple attempts. EMR, electronic medical record; EHR, electronic health record.

8. Statistical analysis

We used the *t*-test to analyze the mean differences between quiz completion and incompletion groups and between one-time quiz completion and multiple-time quiz completion groups. We used the Mann-Whitney test for non-parametric mean difference analyses. Parametric and non-parametric analyses of the mean differences depending on quiz activity frequency were analyzed using the one-way analysis of variance (ANOVA) test and Kruskal-Wallis test, respectively. We used Pearson correlation to evaluate any summative assessment outcome correlation with final quiz scores. Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $p<0.05$.

Results

A total of 217 students' data were obtained for 3 consecutive years and used for analyses. The number of students enrolled for this study in 2019, 2020, and 2021 were 74, 72, and 71, respectively.

1. Summative assessment outcome for quiz completion versus quiz incompletion groups

The quiz completion group (n=119) and quiz incompletion group (n=98) summative assessment results, including standard deviations (SDs), were 87.16±8.73 and 83.22±8.31, respectively. They were significantly different ($p=0.001$) (Fig. 2).

2. Quiz activity frequency and summative assessment outcome

We divided the quiz completion group into two quiz frequency groups, the one-time quiz learning group and multiple-time (2, 3, 4, and 6 times) quiz learning group. The groups consisted of 85 and 34 students, and the means±SD were 86.54±8.94 and 88.71±8.10, respectively. These results are not statistically different ($p=0.223$) (Table 1). The summative assessment scores among groups were not significantly different ($p=0.376$ on one-way ANOVA, $p=0.335$ on Kruskal-Wallis test).

3. Score trends for sequential quiz activity

The number of combined subgroups is not large enough for parametric analysis, so we used a non-parametric method (Mann-Whitney test). The mean in the combined subgroups was not significantly different between groups ($r>0.05$) (Table 2). Although we did not calculate a statistically significant *p*-value in either the parametric or non-parametric statistical analyses, the *p*-values showed decreasing trend from groups 1 to 3 (0.223>0.203>0.075 on *t*-test and 0.225>0.150>0.067 on Mann-Whitney test).

4. Correlation between the summative assessment outcome and final quiz scores

Each student's final quiz score was compared to their sum-

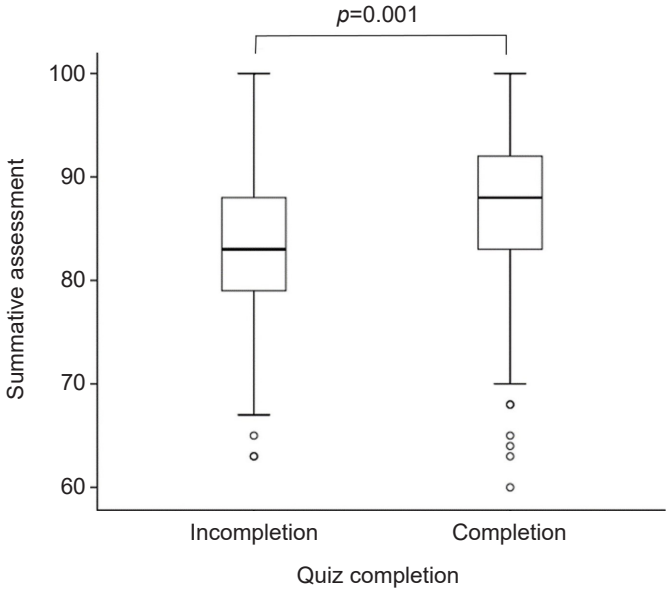

Fig. 2. Summative assessment outcomes for quiz completion and incompletion groups. The thick horizontal line in the middle of the box is the mean for each group's summative assessment score. The mean scores were significantly different using the *t*-test ($t=-3.377$, $p=0.001$).

Table 1. Summative assessment outcomes depending on quiz learning activity frequency

Frequency of quiz learning activity	No. of students	Mean±SD	95% CI for mean
0	98	83.22±8.31	81.56–84.89
1	85	86.54±8.94	84.61–88.47
2	21	87.86±7.76	84.32–91.39
3	7	87.29±10.44	77.63–96.94
4	5	92.00±4.90	85.92–98.08
6	1	100.00	-

SD, standard deviation; CI, confidence interval.

Table 2. Mean differences in summative assessments for combined subgroups based on quiz activity frequency

Group	Frequency of quiz learning activity	No. of students	Mean±SD	*p*-value	
				t-test	Mann-Whitney test
Group 1	1	85	86.54±8.94	0.223	0.225
	2, 3, 4, 6	34	88.71±8.10		
Group 2	1, 2	106	86.80±8.70	0.203	0.150
	3, 4, 6	13	90.08±8.76		
Group 3	1, 2, 3	113	86.83±8.77	0.075	0.067
	4, 6	6	93.33±5.47		

SD, standard deviation.

mative assessment score. Results show the summative assessment scores were not significantly correlated with the last quiz scores (r=0.115, p=0.213) (Fig. 3).

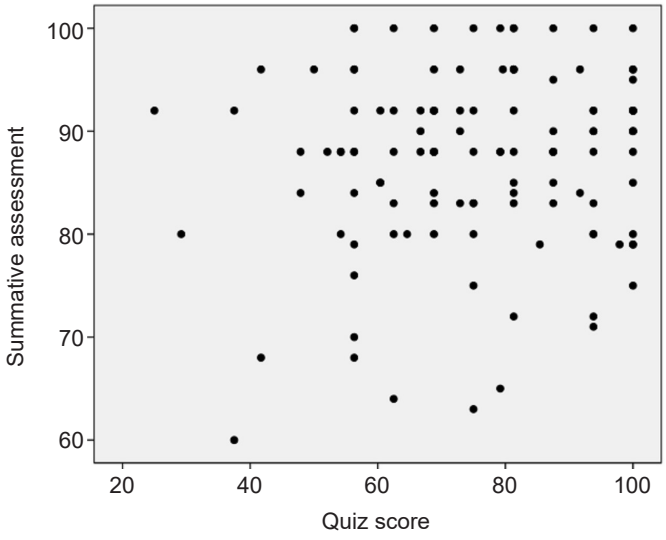

Fig. 3. Correlation between the final quiz score and the summative assessment outcome. We plotted a total of 119 students' summative assessment and quiz scores. No significant correlation is visually observed. Pearson correlation analysis resulted in the same results (r=0.115, p=0.213).

Discussion

Formative quizzes were implemented for self-directed learning and formative assessment, which are usually expected to accompany feedback from trainers. The purpose of formative quizzes in this study was not the same as those in traditional learning environments as described in the methods section. The formative quizzes were devised for students to recognize what they needed to improve upon for further studying. This was the main intention of the formative quizzes. It is assumed that any students who participated in supplementary study to compensate for a lack in knowledge during the formative quiz activities could obtain better scores on summative assessments.

Given that students were informed during the first-class period of the course that the formative quiz scores would not be included in their final grades, it is believed that the formative quizzes were mainly utilized by students as a measuring tool for their status of learning. We recognize the possibility that there were some students who had previous knowledge of the content before answering the formative quizzes. However, the formative quiz could still guide students' learning via formative questions regardless of any prior exposure to the quiz content.

There was a significant summative assessment outcome difference between students who completed their quizzes and those who did not. This is concordant with previous studies [17,18]. The summative assessment measures the extent of learning while the formative assessments are a tool to help guide students toward their learning goals. There is a plethora of evidence that formative assessments are associated with positive learning outcomes [6,21,22]. Feedback during formative assessments was assumed to be a core component for positive summative assessment outcomes [21]. Medical students want feedback in a timely manner, either verbally, aurally, through video, or via self-assessment [23-26]. Feedback should be different depending on the information or skills the student needs. With the intent to enable self-directed learning, we focused on students using quizzes to identify their knowledge gaps. The quiz questions focused on key medical informatics concepts and knowledge.

In general, formative assessments are low stress and stu-

dents do not feel threatened and judged when taking them [27]. The formative quizzes did not have a completion time and scores were not included in the final grades. Since the quizzes were not mandatory, it is likely that they did not overwhelm the students; it is plausible that the students who completed them were more motivated to learn the material than those who did not since there were no other included incentives regarding their participation [28]. We assumed the students who completed the quizzes were more apt to voluntarily and vigorously reflect upon their learning goals during the formative assessment portions of the course.

Repeated exposure to testing enhances self-efficacy on tests [29]. Information provided repeatedly over time is more easily retained than when all information is offered at once [30]. Information retention is more enhanced on delayed tests rather than repeated studying [31]. As the quiz frequency increased, the summative assessment scores increased (Table 1). Although the summative assessment outcome differences in each group were not statistically significant and the number of students in the high frequency groups was not high enough for statistical analysis, we still observed an increasing trend in the summative assessment score. The time intervals between quizzes spanned up to 100 days (data not shown). We created several combinations of quiz frequency groups to reinforce analytical power related to the low student numbers in high frequency groups. Although not statistically significant, the summative outcome scores increased as the quiz frequency combinations increased to the highest frequency combination group (group 3) (p=0.075 and p=0.067 in t-test and Mann-Whitney test, respectively) (Table 2). This increase might be caused by the repeated testing over time, which likely facilitated information retention and helped students better prepare for subsequent testing.

Taking quizzes repeatedly was self-directed because there was no other participation incentive. When incentives were introduced, the number of students who scored well on the quizzes did not correspond to the number of students who scored well on the summative assessment in the previous study [10]. This result may be explained by the assumption that self-directed learning is strengthened when it is implemented voluntarily, without any external pressure.

As repeated tests have shown over time, we assumed that the final quiz score would correspond to the summative assessment outcome. Interestingly, there was no statistically significant correlation between the final quiz score and the summative assessment outcome (Fig. 3). This strongly implies that the formative quiz scores are not a direct predictor of summative assessment outcomes. Instead, the main role of formative quizzes is not just to provide knowledge, but to also enable students to understand in which areas improvement was needed. Although positive correlations between quiz scores and summative assessment outcomes have been reported in previous studies [32-36], the educational conditions in other studies were not fully comparable to those of the current one, which could explain the differences in the correlational results. In our study, students were not concerned about quiz scores because they were not included in the final grades nor did the students feel judged by their tutors, two factors that appeared to act positively on the students' self-directed learning.

This study had some limitations. First, it focused specifically on the quiz activity's relationship to the summative assessment outcome. It did not consider other factors such as individual tutor feedback or other available resources. Second, students took the quizzes voluntarily, which does not always mean that students who abstained from taking the quizzes did not participate in another form of self-directed learning. We didn't analyze other possible self-directed learning actions unrelated to formative quizzes. Third, since this study was retrospectively analyzed with data recorded during a medical informatics course over 3 consecutive years, it does not include any information asking for direct responses of students regarding the degree of compliance to the intention of the formative quizzes. Fourth, we showed a significant difference in summative assessment outcomes between the quiz completion and quiz incompletion groups. This result could be reinforced through the further analysis of the difference in summative assessments depending on the students' academic performances of all learning activities. It could not be determined whether the significant difference of summative assessments between the two groups was caused solely by the quiz activity or if it was affected by the excellence in the students' learning abilities in total learning activities.

In conclusion, self-directed learning using quizzes is thought to be useful for a better summative assessment outcome regardless of frequency and the final score ob-

tained. Students who performed better on their summative assessments are assumed to have improved in their weaker areas through the quiz learning activities. From this perspective, students used the quiz learning activities to overcome their lack of knowledge. The quizzes themselves did not provide direct knowledge, but instead revealed their weaker points, and were believed to have motivated them to voluntarily make up for those insufficiencies. More devices for self-directed learning need to be developed and recommended to students to help them voluntarily improve their performance. Furthermore, it is suggested that additional studies be conducted in order to analyze the difference in the summative assessment outcome according to the level of students' excellence in their academic performance.

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A novel technique for transurethral vesicovaginal fistula tract resection followed by transvaginal fistula repair: a two-step procedure

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Background: The principle of treatment for a vesicovaginal fistula (VVF) tract is complete removal of the fistula tract and surrounding scar tissue, followed by anastomosis without tension from surrounding healthy tissue. We present our novel two-step procedure for VVF repair.

Methods: We retrospectively analyzed 12 women, aged 14 to 67 years, who were treated between 2011 and December 2018. Conservative treatments failed, as these patients had complex VVFs. This technique consisted of two steps: first, transurethral resection of the fistula tract and surrounding scar tissue; second, transvaginal repair of the bladder mucosa, bladder muscle, and vaginal mucosa with tensionless anastomosis. If an interposition flap was needed, we used a Martius flap.

Results: The mean operation time was 186.3 minutes (range, 145–320 minutes), and the mean urethral catheter indwelling time was 10 days. Ten patients successfully underwent surgery through a transvaginal approach with no intraoperative or postoperative complications. However, one patient developed peritoneal perforation during transurethral resection of the fistula due to severe granulation tissue formation around the fistula, which prompted conversion to an abdominal approach. In two cases, we used a Martius flap because of the poor tissue condition due to previous radiation therapy and an inflammatory reaction. At a mean follow-up of 37 months (range, 16–51 months), no recurrence of VVF was observed in any patients.

Conclusions: This novel technique for transurethral VVF tract resection followed by transvaginal fistula repair was very safe and effective technique, and this straightforward technique is expected to reduce surgeons' burden.

Keywords: Endoscopic surgical procedure; Gynecologic surgical procedures; Vesicovaginal fistula

Introduction

Vesicovaginal fistula (VVF) is an abnormal passage between the vaginal and bladder that results in uncontrolled urinary leakage from the vagina. Although VVF is not a life-threatening condition, VVF has a significantly negative impact on the quality of life. Etiologic factors and prevalence rates of this condition vary from one country to another.

In developed countries, 90% of VVFs raised from previous pelvic surgery and of these, 70% were due to abdominal hysterectomy for benign disease [1]. The remaining 10% of VVFs occurred because of radiation, infection, foreign bodies, and pelvic malignancies.

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In underdeveloped countries, the main cause of VVFs is poor obstetrics and gynecological conditions, so obstetrical injuries are the most common [2].

Gynecologic operation-related VVFs usually appear approximately 10 days after operation, while radiation-induced fistulas frequently occur many years after treatment [3,4].

The principle of VVF repair is complete excision of fistula and scar tissue, followed by tensionless anastomosis of well-vascularized clean tissue. This principle is very important for reducing the recurrence rate. Traditionally, for VVF repair, transvaginal approach or transabdominal approach techniques have been used. However, in general, the VVF is located deep in the vagina, making the transvaginal approach difficult to keep this principle. Furthermore, this principle is not simple to follow with the transabdominal approach due to access to the fistula site (e.g., fibrotic tissue condition by radiation, postoperative adhesion) and a higher rate of postoperative complications. In this study, we present our novel two-step procedure of transurethral VVF tract resection followed by the transvaginal fistula repair technique.

Methods

Ethical statements: This study was approved by the Institutional Review Board of Dong-A University Hospital (No. 15-008). The informed consent was waived because this design is a retrospective study.

1. Patients and study design

We analyzed 12 women, aged 14 to 67 years, treated between 2011 and December 2018, retrospectively. The data were collected using hospital medical documentation. The study was conducted after the approval of the institutional review board.

For assessing of characteristics of the fistula (localization, number, and size), we evaluated surgical history, vaginal examination, cystoscopy, and computed tomography, preoperatively [5]. Fistulas found at sites other than the bladder (ureterovaginal fistula or urethrovaginal) were excluded.

Classically, VVFs were classified as simple and complex [6]. Simple VVFs were defined as small (≤0.5 cm) and single non-radiated fistulas. Complex VVFs were classified as medium (0.5–2.5 cm), large (≥2.5 cm), multiple, and recurrent fistulas. All patients were conservatively managed with continuous drainage through a Foley catheter for 2 months in anticipation of spontaneous healing. These conservative treatments failed as every patient had complex VVF. Clinical success was defined as postoperative removal of the Foley catheter, no further urine leakage, and no recurrence during the follow-up period.

2. Surgical technique

The surgical technique consists of two steps. The first step involves complete transurethral resection of the fistula tract and surrounding scar tissue. All patients underwent general anesthesia or spinal anesthesia. The patients were operated in the dorsal lithotomy position. Firstly, fistula resection is performed using a 24-Fr resectoscope under continuous flow. We used a 30° lens and wire cutting loop. To achieve a clear resection field, a continuous flow sheath is very important (Fig. 1). During resection with a cutting loop should be performed in a systematic, piecemeal manner, aiming at the complete resection of the granulation tissue around the fistula. Practically, resection should begin bladder mucosa toward the deeper layers of vagina. If bleeding occurs, electrocautery should be used minimally to save vascularity. After endoscopic surgery, we placed a Foley catheter and changed to conventional transvaginal surgical approach. The second step involves transvaginal tensionless repair of bladder mucosa followed by bladder muscle and vaginal mucosa repaired layered closure. If an interposition flap is needed due to poor tissue condition, the Martius flap could be used.

After fat pad was harvested from the labia major, pulled up to the suture line through deep tunneled vaginal mucosa.

Results

A total of 12 patients underwent this novel transurethral VVF tract resection followed by transvaginal fistula repair technique. The mean age of patients was 47.1 years (range, 14–67 years) and the mean follow-up period was 37 months (range, 16–51 months). Based on the surgical history, four patients had a radical hysterectomy due to cervical cancer, six had a laparoscopic hysterectomy due to uterine myoma, and one underwent a hypogastric artery

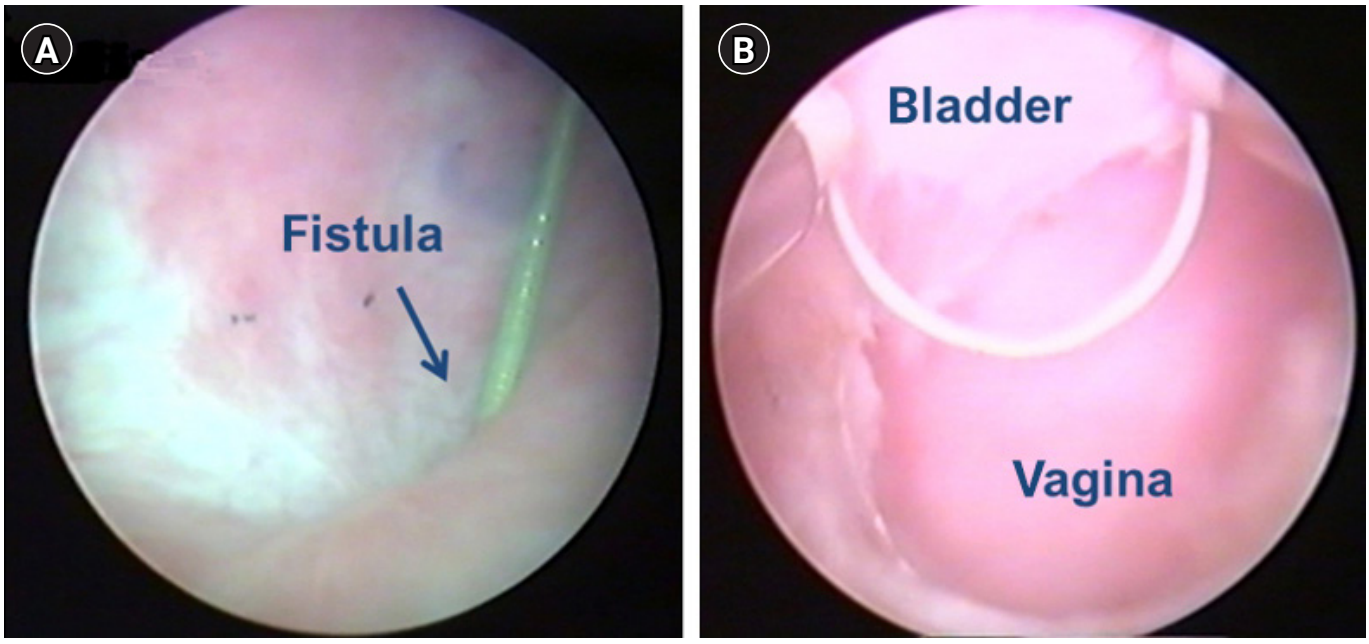

Fig. 1. Cystoscopic view of fistula before and after resection. (A) Cystoscopic image of a vesicovaginal fistula and an inserted guidewire. (B) Resection of the bladder fistula for margin clearance through the transurethral approach.

Table 1. Patients' information

Patient no.	Age (yr)	Fistula etiology	Previous surgical history	Time to operation (mo)	VVF size (cm)	Note
1	47	Cervical cancer	RTVH	2	0.8	Second operation
2	67	Cervical cancer	RTVH+RTX	60	0.7	Percutaneous nephrostomy
3	57	Cervical cancer	RTVH+CTX	6	1.5	Martius flap
4	62	Cervical cancer	RTVH+RTX	3	1.6	-
5	44	Uterine myoma	LAVH	8	1.0	-
6	52	Uterine myoma	LAVH	2	0.7	Second operation
7	47	Uterine myoma	LAVH	2	0.8	-
8	48	Uterine myoma	LAVH	6	0.8	Second operation Martius flap
9	50	Uterine myoma	LAVH	5	1.5	-
10	42	Uterine myoma	LAVH	4	1.2	-
11	35	Vaginal bleeding after cervical biopsy	Hypogastric artery ligation	6	1.4	Systemic lupus erythematosus
12	14	Foreign body reaction	None	2	1.0	Sexual abuse

VVF, vesicovaginal fistula; RTVH, radical transvaginal hysterectomy; RTX, radiotherapy; CTX, chemotherapy; LAVH, laparoscopy-assisted vaginal hysterectomy.

ligation due to vaginal bleeding following a cervical biopsy. The last patient had no surgical history, but she inserted a foreign body into the vagina (Table 1). Three patients were second trial of VVF repair.

The VVF was located high supra-trigonal in all patients, and its size ranged from 0.7 to 1.6 cm (mean, 1.1 cm), indicating complex VVFs.

The mean operation time was 186.3 minutes (range, 145–320 minutes), and the mean urethral catheter indwelling time was 10 days. In 10 patients, the operation was conducted successfully through a transvaginal approach with no intraoperative or postoperative complications.

However, one patient developed a peritoneal perforation during transurethral resection of the fistula due to severe granulation tissue formation around the fistula and was converted to an abdominal approach.

In two cases, we used an interposition flap by Martius flap for VVF repair because of poor tissue condition due to previous radiation therapy and inflammatory reaction. At a mean follow-up of 37 months (range, 16–51 months), there was no recurrence observed in all 12 patients.

Discussion

The treatment of VVF is remained a challenge to the surgeon because there were several controversies still exist. The most cited controversies are about the operation timing, ideal surgical approach, and need for adjuvant measures. A trial of conservative therapy was conducted with proper and undisturbed bladder drainage for the small fistulas. The success rate of conservative therapy was limited success (7%–12.5%) in only selected cases [7,8]. After conservative therapy, there is persistent urinary leakage in the vagina, surgical correction treatment is necessary.

Several operation techniques exist, such as endoscopy, laparoscopy, robot-assisted, and conventional open surgery through the vagina or abdomen [9-11]. However, the ideal approach for VVF repair remains controversial. The transabdominal approach is adopted for the repair of supra-trigonal vesicovaginal, ureterovaginal, or vesicouterine fistulas, and if bladder augmentation is required; the omentum is used as a flap. The transvaginal approach is preferred for repair wherever possible, and a Martius flap is usually used from the subcutaneous fat of the labium [12,13]. The success rate has varied between 75% and 95% with these various techniques [9-12,14-17]. In this study, we achieved clinical success in all patients with no recurrence. In addition, two patients needed an interposition flap (Martius flap) due to a previous radiation treatment history and poor tissue condition due to an inflammatory reaction.

The first attempt to treat VVF using an endoscopic procedure demonstrated transurethral pointed electrode VVF repair. It was feasible in patients with multiple, small VVFs [18].

For a successful operation, several principles must be satisfied as follows. First step involves adequately mobilizing the bladder from the vagina, exposing the fistula tract. And complete remove surrounding scar tissues while revealing healthy tissue edges. Second, closing the bladder and vagina in separate layers in water-tight manner without tension with healthy tissues. If proper healthy tissues were not secured, interposition flap could be used between the bladder and vagina suture lines. Third, urine natural drainage should be maintained, postoperatively [15,19,20].

In this study, before repairing the fistula, we effectively removed the VVF and scar tissues on the bladder and vagina by endoscopic resection. Then, we closed the bladder and vagina with viable clean tissue through transvaginal approach. We consider anastomosis with viable clean tissue a crucial step for a VVF repair.

The operation times reported in the literature ranged from 70 to 280 minutes [21-23]. Our mean operative time was 186.3 minutes which seems consistent with the most reported cases. Operation time is affected by several factors. Representative factors are fistula location, onset time, previous surgical history, failed fistula repair history and experience and skill of the surgeon. In our case, three patients had a previous history of failed fistula repair. In one of the cases, peritoneal perforation occurred during transurethral resection of the VVF. Therefore, the patient converted to open surgery, and VVF was completely repaired.

The time of surgical repair of VVF is still controversial. Classically, there should be 3 to 6 months following the onset of the VVF before surgical repair to allow the surgical inflammatory reaction may subside. The overall success rate of VVF repair, including those for whom repairs were done within 3 months post-injury, and those for whom the repairs were intentionally delayed, ranged from 86% to 100% [10,24-27]. In our study, transvaginal VVF was repaired at least 2 months after the onset of symptom or the initial surgery to allow subsiding of the infection or inflammation at the fistula site. According to the literature, urethral catheter is removed at 10 to 28 days [21,23]. In our case, the urethral catheter was kept for 10 days in all patients. Before catheter removal, all patients were checked cystography to confirm a complete closure of the VVF. No leakage was observed in all patients, and they were dry after catheter removal for follow-up periods.

A limitation of this study includes a single-center, retrospective trial, which may decrease the quality of evidence. Another limitation is the small sample size of patients. Despite this limitation, transurethral resection of VVF and surrounding scar tissue is simple under clear vision. As a result, the success rate of the VVF repair may be improved.

In conclusion, this novel transurethral VVF tract resec-

tion followed by transvaginal fistula repair technique was a very safe and effective technique. And this easy technique is expected to reduce the burden of surgeons.

Article information

Conflicts of interest

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The clinical significance of circulating microRNA-21 in patients with IgA nephropathy

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Background: Urinary microRNA-21 (miR-21) has been reported to correlate with the histologic lesions of IgA nephropathy (IgAN). We investigated whether urinary or circulating miR-21 could serve as a biomarker for detecting the renal progression of IgAN.

Methods: Forty patients with biopsy-proven IgAN were enrolled in this study. Serum and urinary sediment miRs were extracted, and the expression of miR-21 was quantified by real-time quantitative polymerase chain reaction. Renal progression was defined as end-stage renal disease, a sustained doubling of serum creatinine, or a 50% decrease in estimated glomerular filtration rate (eGFR) from baseline.

Results: Six patients experienced renal progression during the follow-up period. The baseline eGFR was lower in the progression group (49±11 mL/min/1.73 m^2 vs. 90±23 mL/min/1.73 m^2, $p<0.05$) than in the non-progression group. The level of circulating miR-21 on kidney biopsy was higher in the progression group than in the non-progression group (40.0±0.6 vs. 38.2±1.1 ΔCt value of miR-21, $p<0.01$), whereas there was no significant difference in urinary miR-21 (38.1±2.1 vs. 37.8±1.4 ΔCt value of miR-21, $p=0.687$) between the two groups. Receiver operating characteristic curve analysis demonstrated that circulating miR-21 had good discriminative power for diagnosing renal progression of IgAN, with an area under the curve of 0.975.

Conclusions: The level of circulating miR-21 was higher in the progression group than in the non-progression group at the time of kidney biopsy. Therefore, circulating miR-21 could be a surrogate marker of renal progression in patients with IgAN.

Keywords: Glomerulonephritis; Immunoglobulin A; MicroRNAs

Introduction

IgA nephropathy (IgAN) is a mesangial proliferative glomerulonephritis with a variable clinical course ranging from asymptomatic urinary abnormalities to rapidly progressive kidney failure [1]. A key issue in the field is prediction of patient risk of rapid renal progression. The clinical predictors of renal outcome in IgAN, such as reduced renal function, proteinuria, and blood pressure during diagnosis, are acknowledged [2]. The histologic finding of IgAN is associated with development of end-stage renal disease [3]. However, recurrent renal biopsy is not an appropriate approach for evaluation of disease severity due to its invasive nature. Therefore, novel biomarkers of IgAN, which can differentiate individuals with disease from healthy people prior to renal biopsy, are needed to evaluate and administer timely and relevant management.

MicroRNAs (miRs) are non-coding, single-stranded RNA

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molecules that regulate pathological and physiological processes by a posttranscriptional mechanism [4]. Many miRs reveal organ-specific patterns of expression, with dysregulation being linked with diverse diseases [5]. Several studies have reported the relationship between miRs and glomerulonephritis [6-8]. Among all miRs, miR-21 has been researched extensively in nephrology including glomerulonephritis [9-11]. In IgAN, intra-renal expression of miR-21 was reported to reflect renal fibrosis and renal survival [7]. However, there are few studies that investigate the role of circulating miR-21 in progression of IgAN. Therefore, we performed this study to determine if a circulating miR-21 could serve as a marker of progression of IgAN.

Methods

Ethical statements: This study was approved by the Institutional Review Board of the Presbyterian Medical Center, Jeonju, South Korea (approval number: 2014-07-032). Written informed consent from participants was obtained prior to sample collection. This research abided by the principles of the Declaration of Helsinki.

1. Study design and participants

From 2014 to 2020, a total of 40 patients with biopsy-proven IgAN were enrolled in this study. The inclusion criteria were patients ≥18 years who had provided informed consent. The exclusion criteria are as follows: patients with secondary IgAN; patients who had received immunosuppressants including corticosteroids before enrollment of this study, patients with systemic diseases such as diabetes, patients who underwent renal replacement therapy including renal transplantation or dialysis, pregnant patients, and ≤8 glomeruli in renal biopsy samples. Following renal biopsy, the patients received angiotensin-converting enzyme inhibitors/angiotensin receptor blockers or corticosteroids. In this study, the enrolled patients were followed for more than 1 year.

All data including clinical history were obtained through retrospective chart review during renal biopsy. The clinical end-point was defined as a composite of any of the following events over the study duration: 50% decline of estimated glomerular filtration rate (eGFR) from baseline, persistent doubling of serum creatinine, or initiation of renal replacement therapy. Patients who developed the clinical end-point during follow-up were included in the progression group. The eGFR was evaluated using the abbreviated Modification of Diet in Renal Disease equation [12]. A pathologist diagnosed with IgAN based on three microscopic analyses of the specimens and categorized the observed lesion into one of five classes consistent with the Oxford classification [13]. The degree of mesangial proliferation, glomerular sclerosis, inflammation, and interstitial fibrosis, as well as tubular atrophy were assessed from the Masson's trichrome-stained sections of each biopsy utilizing a semi quantitative method. The percentage of cortical area involved in interstitial fibrosis or tubular atrophy was quantitated. A score of T0, T1, or T2 was conferred if the percentage of involved cortical area was 0%–25%, 26%–50%, or >50%, respectively. Herein, T0-T1 and T2 findings were defined as mild and severe renal fibrosis, respectively.

2. Measurement of miR-21

Total RNA was isolated from urinary exosomes employing the mirVana PARIS total RNA isolation kit (Life Technologies, Carlsbad, CA, USA; Cat. #AM1556) as per the manufacturer's protocol. For the endogenous small RNA control, we incorporated cel-mir-39 (25 fmol, Life Technologies Cat. #4464066) to each sample, as previously described [14]. Using the TaqMan MicroRNA reverse transcription kit (Life Technologies, Cat. #4366596), a fixed RNA content of 4.8 ng from RNA elute was reverse transcribed. For quantitative real-time polymerase chain reactions (qRT-PCRs), 1.33 μL of the reverse transcription product was incorporated with 10 μL of TaqMan universal master mix (Cat. #4440038), 7.67 μL of H_2O, and 1 μL of primers including miR-21 (Life Technologies, Cat. #4440887, assay ID:000397) in a 20 μL final reaction volume. The qRT-PCR was conducted on an Applied Biosystems (Waltham, MA, USA) 7500 Real-Time PCR system at 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute [15,16]. The values of the threshold cycle were computed using SDS 1.4.1 software (Applied Biosystems). All qRT-PCR reactions were performed in triplicate. The average expression levels of miR-21 were normalized utilizing cel-mir-39 (Applied Biosystems) and subsequently analyzed by the two (median cel-mir-39 Ct value–average Ct value of the given sample) method, as previously described [16-18]. All data were visualized using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). *p*-values <0.05

were considered statistically significant.

3. Statistical analyses

All data are presented as mean±standard deviation unless otherwise specified. The baseline characteristics of patients in the non-progression and progression groups were compared using the chi-square test, *t*-tests, or Fisher exact test, as appropriate. Clinically, the relevant that were significantly associated with renal progression on univariate analysis were subjected to multivariate analysis using binary logistic regression. Statistical significance was noted at a *p*-value <0.05. All statistical analyses were performed using the SPSS software, version 22.0 (IBM Corp., Armonk, NY, USA).

Results

1. Comparison of clinical characteristics between progression and non-progression groups

Among 40 patients with IgAN, the mean age was 39±14 years, 40% were male, and the mean follow-up duration was 43 months (range, 6–84 months). The value of mean eGFR and proteinuria were 85±25 mL/min/1.73 m^2 and 1,584±2,338 mg/dL, respectively. There were no histologic differences except baseline renal function and fibrosis. In the Oxford classification, patients with progression had remarkably higher T2 scores than those without progression. Patients with progression had a poorer renal function (49±11 mL/min/1.73 m^2 vs. 90±23 mL/min/1.73 m^2, p<0.01) on admission and more frequent severe renal fibrosis (50% vs. 0%, p=0.002) than that observed in the non-progression group. The level of circulating miR-21 (40.0±0.6 vs. 38.2±1.1 miR-21 ΔCt value, p<0.01) was higher in the progression group than in the non-progression group, while the level of urinary miR-21 did not differ between the two groups (38.1±2.1 vs. 37.8±1.4 miR-21 ΔCt value, p=0.687) (Table 1).

2. Correlation between circulating miR-21 level and clinical parameters

Circulating miR-21 levels were directly correlated with proteinuria (Pearson's correlation=0.337, p=0.033). However, we detected a significant inverse correlation between circulating miR-21 level and eGFR (Pearson's correlation=–0.330, p=0.037) (Fig. 1). The area under the receiver operator characteristic (ROC) curve was 0.975 for circulating miR-21 (Fig. 2).

Additionally, the level of circulating miR-21 was higher (40.2±0.6 vs. 38.3±1.2 ΔCt value of miR-21, p=0.013) in pa-

Table 1. Comparison of baseline characteristics according to whether patients experienced progression

Characteristics	Total (n=40)	Progression (n=6)	Non-progression (n=34)	*p*-value
Age (yr)	39.0±14.0	49.0±11.0	37.0±14.0	0.066
Male sex	16 (40.0)	3 (50.0)	13 (38.2)	0.456
Follow-up duration (mo)	43.0±24.0	33.0±19.0	45.0±25.0	0.284
Oxford classification				
M1	10 (25.0)	3 (50.0)	8 (23.5)	0.245
E1	9 (22.5)	2 (33.3)	7 (20.5)	0.378
S1	25 (62.5)	4 (66.6)	21 (61.7)	0.460
T1	16 (40.0)	3 (50.0)	13 (38.2)	0.178
T2	3 (7.5)	3 (50.0)	0	<0.01
Baseline proteinuria (mg/dL)	1,584±2,338	4,897±4,769	1,000±819	0.102
Hemoglobin (g/dL)	12.7±1.9	11.8±3.0	12.9±1.7	0.231
Creatinine (mg/dL)	1.1±0.3	1.5±0.5	1.0±0.1	0.08
eGFR (mL/min/1.73 m^2)	85.0±25.0	49.0±11.0	90.0±23.0	<0.01
Albumin (mg/dL)	3.9±0.7	3.5±0.8	3.9±0.6	0.231
Urinary miR-21, relative expression level of miR-21	37.9±1.5	38.1±2.1	37.8±1.4	0.687
Circulating miR-21, relative expression level of miR-21	38.4±1.3	40.0±0.6	38.2±1.1	<0.01

Values are represented as mean±standard deviation or number (%).
eGFR, estimated glomerular filtration rate; miR-21, microRNA-21.

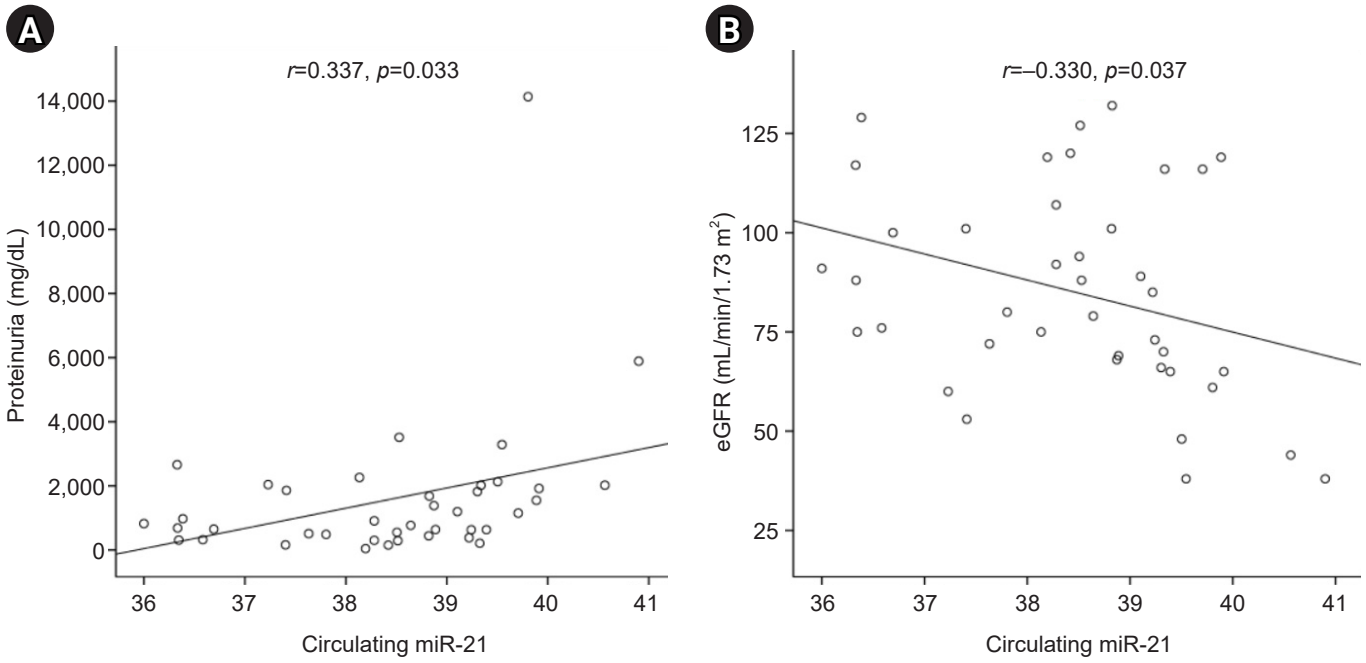

Fig. 1. Correlation of circulating microRNA-21 (miR-21) with clinical parameters. Circulating miR-21 levels correlated directly with proteinuria (A) and inversely with the estimated glomerular filtration rate (eGFR) (B).

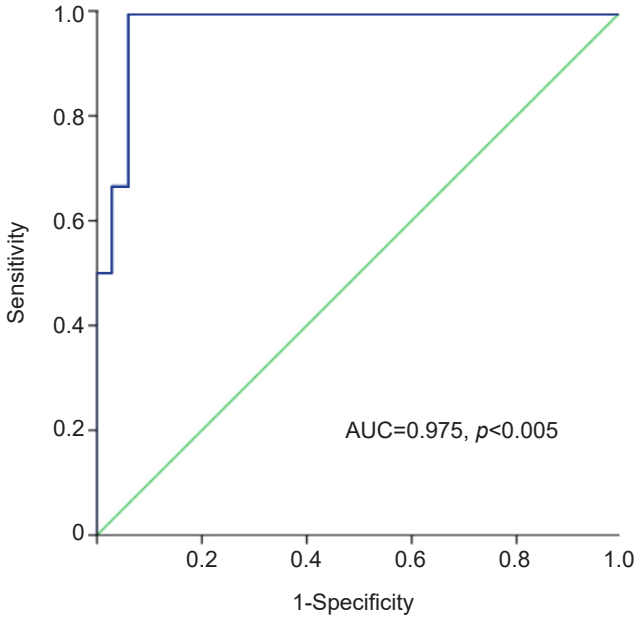

Fig. 2. Receiver operating characteristic curve and performance for circulating microRNA-21 (miR-21) at the time of kidney biopsy. The area under the receiver operating characteristic curve (AUC) was 0.975 for circulating miRNA-21.

tients with severe renal fibrosis (Fig. 3).

Discussion

Herein, we revealed that circulating miR-21 level was higher in patients with IgAN with renal progression than in those without renal progression and correlated directly with proteinuria on kidney biopsy. Additionally, the ROC curve analysis for circulating miR-21 depicted good discriminative ability for predicting IgAN renal progression. Therefore, circulating miR-21 could be a surrogate marker for renal progression of IgAN. Our results offer a rationale for employing circulating miR-21 as a biomarker for prediction of clinical outcome of IgAN.

The clinical course of IgAN is extremely diverse [1]. Therefore, the examination of non-invasive and more reliable biomarkers to evaluate disease progression is imperative for a clinician. Recently, miR has been in the spotlight as a disease biomarker since multiple miRs exhibit differential expression in diverse organs [5]. Previous studies demonstrated that miR-21 plays a central role in inflammation, stress response, and apoptosis [15,19]. Up-regulation of miR-21 expression has been reported to be correlated

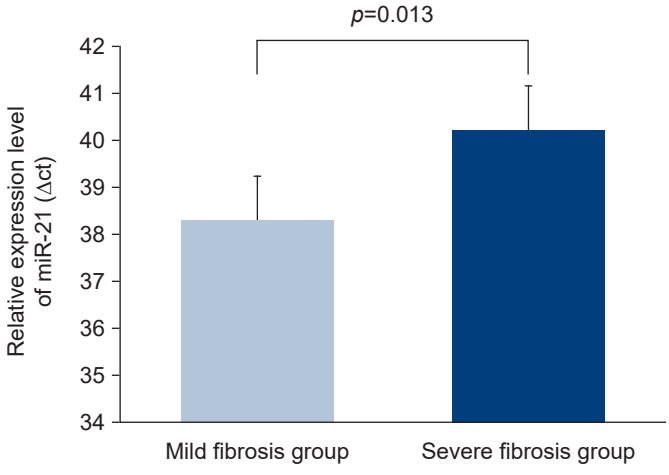

Fig. 3. Circulating microRNA-21 (miR-21) levels were higher in patients with severe fibrosis than in those with mild fibrosis. After reference gene normalization, the relative expression intensity of circulating miR-21 was higher in patients with severe fibrosis than in patients with mild fibrosis.

with several renal disorders including diabetic nephropathy and acute kidney injury [20,21]. Liang et al. [7] reported that the level of miR-21 in urinary sediment and intra-renal expression of miR-21 were related to histologic findings of IgAN. In our study, the urinary miR-21 level was higher in the progression group than that in the non-progression group. However, the difference was not statistically significant. We believe that this might be due to the small sample size in this study.

Previous miR studies in renal disorders including glomerulonephritis have focused on urinary miRs [5]. Urinary levels of miR-146a and miR-155 are increased in patients with IgAN and associated with proteinuria. Additionally, urinary miR-30d and miR-10a levels in focal segmental glomerulosclerosis (FSGS) are high in mouse and human and may signify a novel biomarker of kidney injury [22]. Recently, understanding of pathological or physiological roles was increased by the discovery of circulating miRs, although their exact role is obscure. Thus, circulating miRs are regarded as fascinating biomarker candidates and may reflect kidney disease [17]. Differentially expressed circulating miRs have been discovered in patients with diverse glomerular diseases including IgAN [23]. A previous study explored the specific profile of circulating miRs of nephrotic syndrome such as FSGS, proposing a possibility of biomarker [24]. In addition, Sun et al. [25] recently reported that circulating miRs in extracellular vesicles can be beneficial in identifying idiopathic membranous nephropathy among nephrotic syndrome and predicting the treatment response in patients.

Some researchers also imply the roles of diagnostic, prognostic, or therapeutic biomarker for miRs in patients with IgAN [26,27]. Hu et al. [26] demonstrated that plasma miR-29a could be a marker for reflecting renal damage and predicting IgAN progression. Li et al. [27] suggested that miR-23b may be an interesting future therapeutic target for treatment of IgAN. Interestingly, in this study, the level of circulating miR-21 was higher in the progression group than in the non-progression group. Furthermore, those levels correlated directly with proteinuria and inversely with eGFR during kidney biopsy, which was already known as an IgAN predictor [2]. The level of circulating miR-21 was also associated with renal fibrosis in this study. Therefore, we propose that circulating miR-21 could be useful to predict IgAN progression. However, the level of urinary miR-21 was not related to clinical parameters. Therefore, to illuminate the role of circulating and urinary miR-21 in IgAN; to confirm our results, larger, prospective, randomized, and controlled trials are required.

Apart from miR-21, several small studies have reported miR expression in IgAN as a diagnostic or prognostic biomarker [6-8,22,28-31]. Serino et al. [30] showed that a miR-148b was involved in the pathogenesis of IgAN, explaining the aberrant glycosylation of IgA1. Subsequently, circulating miR-148b and miR-let-7b were demonstrated to be helpful in discriminating patients with IgAN from healthy controls and patients with other types of primary glomerulonephritis [31]. However, there are some questions when miRs are used as biomarkers for renal diseases including IgAN. Since most miRs target multiple proteins, one miR can be associated with diverse diseases, reducing the unique role in differentiation from pathology and physiology [5]. Consequently, finding causes for changes in this level is challenging. Another hurdle to overcome for being a biomarker in renal disorders is the method of identification of miRs. To inspect circulating miRs, several approaches have emerged including qRT-PCR, microarrays, and next-generation sequencing. Every method has its pros and cons such as quantification, simplicity, and validity [32]. The sensitivity and specificity of these techniques are often

dependent on the sample type and volumes of plasma or serum. Furthermore, the methods are not standardized or validated among research laboratories [32]. Therefore, discreet validation and standardization are required before the miRs are translated to clinical decision-making. Of these, qRT-PCR is the most advantageous for analyzing diverse specific miR due to its simplicity and speed. However, controversy on selection of the most appropriate endogenous reference genes for circulating miRs expression level normalization has been ongoing [33]. Therefore, to broaden the clinical utility for miRs, such methodologic issues should be resolved.

Our study has some limitations. First, this was a single-center study with a small number of participants. Therefore, larger, prospective, randomized, and controlled trials are needed. Second, we did not enroll a healthy control in the present study. Third, we evaluated the urinary miR expression in urinary sediments, which are composed of varying cell types, but did not evaluate their cellular source. Finally, we did not evaluate the miR-21 expression in kidney biopsy specimen.

In conclusion, we found increased circulating miR-21 level in IgAN patient with renal progression compared to that in patients without renal progression. Furthermore, circulating miR-21 level on kidney biopsy was associated with proteinuria and baseline renal function. Therefore, circulating miR-21 could be utilized as a biomarker for IgAN prognosis. To confirm our results, larger, prospective, randomized, and controlled studies are required in the future.

Article information

Conflicts of interest

This research was supported by Daewon Pharmaceutical Company. Except for that, no potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: AYC, IOS. Data curation: JHO, KYL. Formal analysis: AYC, IOS. Funding acquisition: IOS. Investigation: IOS. Methodology: AYC, IOS. Project administration: AYC, IOS. Resources: IOS. Software: IOS. Supervision: IOS. Validation: IOS. Visualization: AYC, IOS. Writing - original draft: AYC, IOS. Writing - review & editing: AYC, IOS. Approval of final manuscript: all authors.

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Case report

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A rare case of pure-type embryonal carcinoma in a 75-year-old woman mimicking epithelial ovarian carcinoma

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Embryonal carcinoma, a very rare ovarian germ cell tumor, involves pure and mixed phenotypes. Pure-type embryonal carcinoma has never been reported in postmenopausal women. The current case was, thus, misdiagnosed as an epithelial ovarian carcinoma based on radiologic findings. Herein, we describe the case of ovarian embryonal carcinoma in a 75-year-old woman along with a literature review. Magnetic resonance imaging findings were suggestive of epithelial ovarian malignancy associated with endometrioma, including ureteral invasion. The patient underwent complete surgical staging, and a pathologic diagnosis of pure-type embryonal carcinoma was made. The patient's postoperative course was uneventful, and adjuvant chemotherapy was administered. Embryonal carcinoma in the postmenopausal woman is a clinical challenge owing to the possibility of its misdiagnosis as epithelial ovarian carcinoma. To the best of our knowledge, this is the first report of pure-type ovarian embryonal carcinoma in a postmenopausal woman, with a description of the clinicopathologic characteristics and review of the relevant literature.

Keywords: Case reports; Embryonal carcinoma; Neoplasms, germ cell and embryonal; Postmenopause

Introduction

Ovarian germ cell tumors (OGCTs) are considered to be derived from primitive germ cells of the embryonic gonad, making them the most common malignancy of the ovary (1%–2% of all cases) in young women and adolescents [1]. Embryonal carcinoma, a very rare OGCT, most commonly appear as one or more other germ cell tumor types. Cases of pure-type ovarian embryonal carcinoma are very rare [2]. Unilateral oophorectomy is the most common fertility-sparing treatment, followed by combination BEP (bleomycin + etoposide + cisplatin) chemotherapy [1].

Ovarian embryonal carcinoma in postmenopausal women has been reported, and occurs more commonly as mixed germ cell tumor and rarely as pure tumor [3]. The knowledge concerning the development, treatment, and outcomes of postmenopausal embryonal carcinoma is scarce, and the characteristics and prognosis might differ between

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postmenopausal and premenopausal patients. Herein, we describe the first case of pure-type ovarian embryonal carcinoma in a postmenopausal woman and review the relevant literature.

Case

Ethical statements: This study was approved by the Institutional Ethics Committee of Pusan National University Hospital (No. 2203-039-113). Informed consent was obtained from the patient agreed to join in the research.

A 75-year-old postmenopausal woman presented with a 1-month history of a palpable mass in the lower abdomen. Magnetic resonance imaging (MRI) revealed an 11-cm solid cystic mass with heterogeneous signal intensity in the left adnexa, and left hydronephroureterosis showing abrupt narrowing at the ovarian mass level, suggestive of ureteral invasion and epithelial ovarian cancer (Ovarian-Adnexal Reporting & Data System [ORADS] score=5) associated with an endometrioma, such as clear cell or endometrioid carcinoma (Fig. 1). No enlarged lymph nodes or distant metastases were noted in the upper abdomen.

Laboratory findings included elevated levels of serum lac-

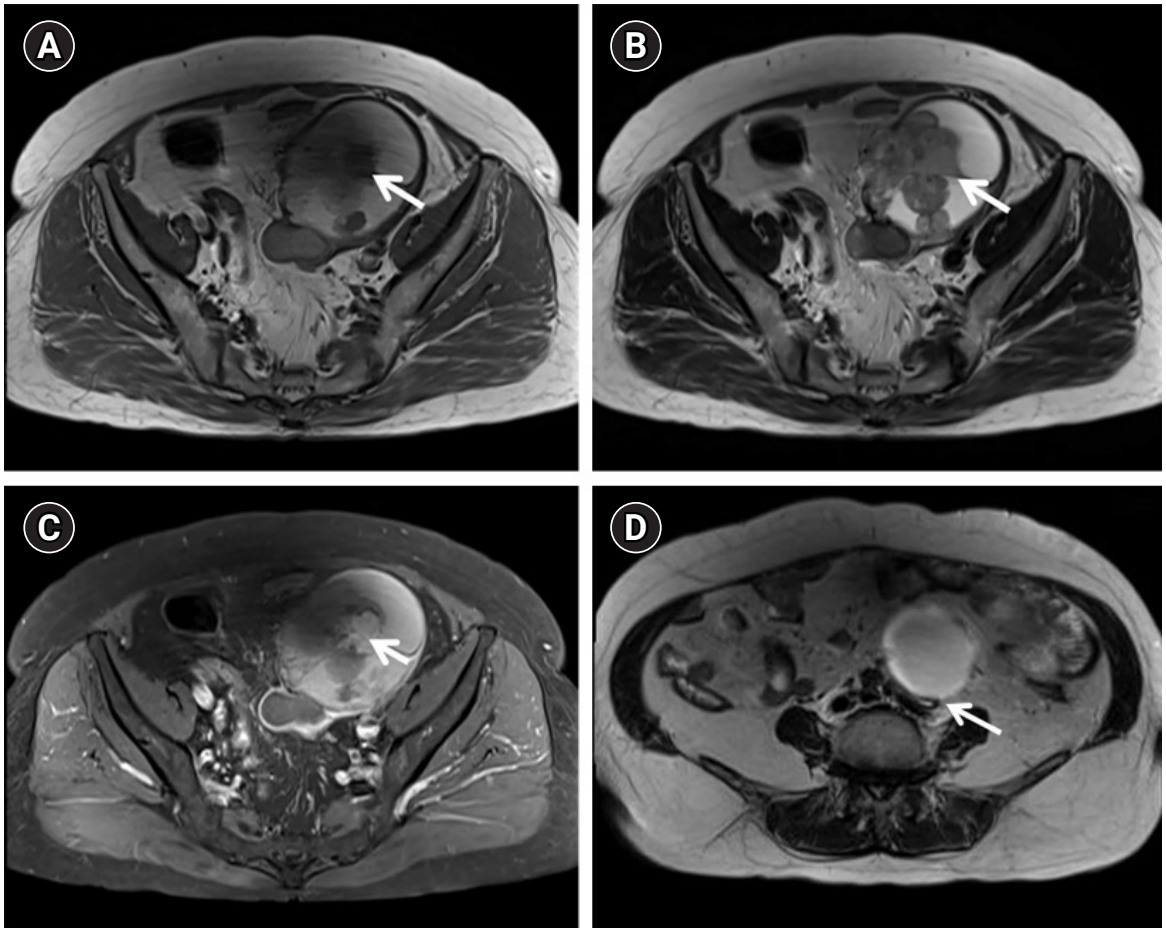

Fig. 1. Magnetic resonance images. Axial T1-weighted (A) and T2-weighted (B) images showing a septated hemorrhagic cystic mass with papillary projections (arrow) of the left ovary (ORADS 5). High T1 signal intensity with the T2 dark spot sign, suggestive of epithelial ovarian cancer associated with endometrioma (e.g., clear cell carcinoma or endometrioid carcinoma). (C) Contrast-enhanced axial T-weighted image shows enhancement (arrow) of the papillary projections in the left ovarian tumor. (D) Axial T2-weighted imaging revealed left hydronephrosis (arrow), showing abrupt narrowing at the ovarian mass level suggestive of ureteral invasion. ORADS, Ovarian-Adnexal Reporting & Data System.

tate dehydrogenase (LDH; 372 IU/L [range, 135–225 IU/L]), cancer antigen 125 (17.6 U/mL [range, 0–35 U/mL]) and C-reactive protein (0.68 mg/dL [range, 0–0.5 mg/dL]).

An exploratory laparotomy was performed for staging given the patient's age. A 12- to 15-cm cystic and solid mass showed an infiltrative growth pattern with adhesions to the adjacent tissue and organs including the bowel, mesentery, ureter, and retroperitoneum. The retroperitoneal mass from the left adnexa was subjected to frozen section biopsy, which revealed malignancy. To confirm oncologic certainty, further dissection of the ureter, extra-fascial total abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy, appendectomy, pelvic adhesiolysis, and multiple resections of the mesenteric mass, pelvic wall mass, and lymph nodes was performed. The permanent biopsy of the left adnexa and the mass revealed a malignant tumor consistent with embryonal carcinoma confined to the ovary with an intact capsule and no extraovarian spread. The uterus, right ovary, omentum, appendix, mesentery, and pelvic lymph nodes were free of tumor cells. Peritoneal washings were also negative for malignant cells, confirming stage IA disease.

Microscopically, the tumor had a predominantly solid pattern of highly anaplastic cells and numerous mitotic figures. The excised mass showed immunoreactivity for CD30, p53, and WT1, and focal positivity for panCK (Fig. 2).

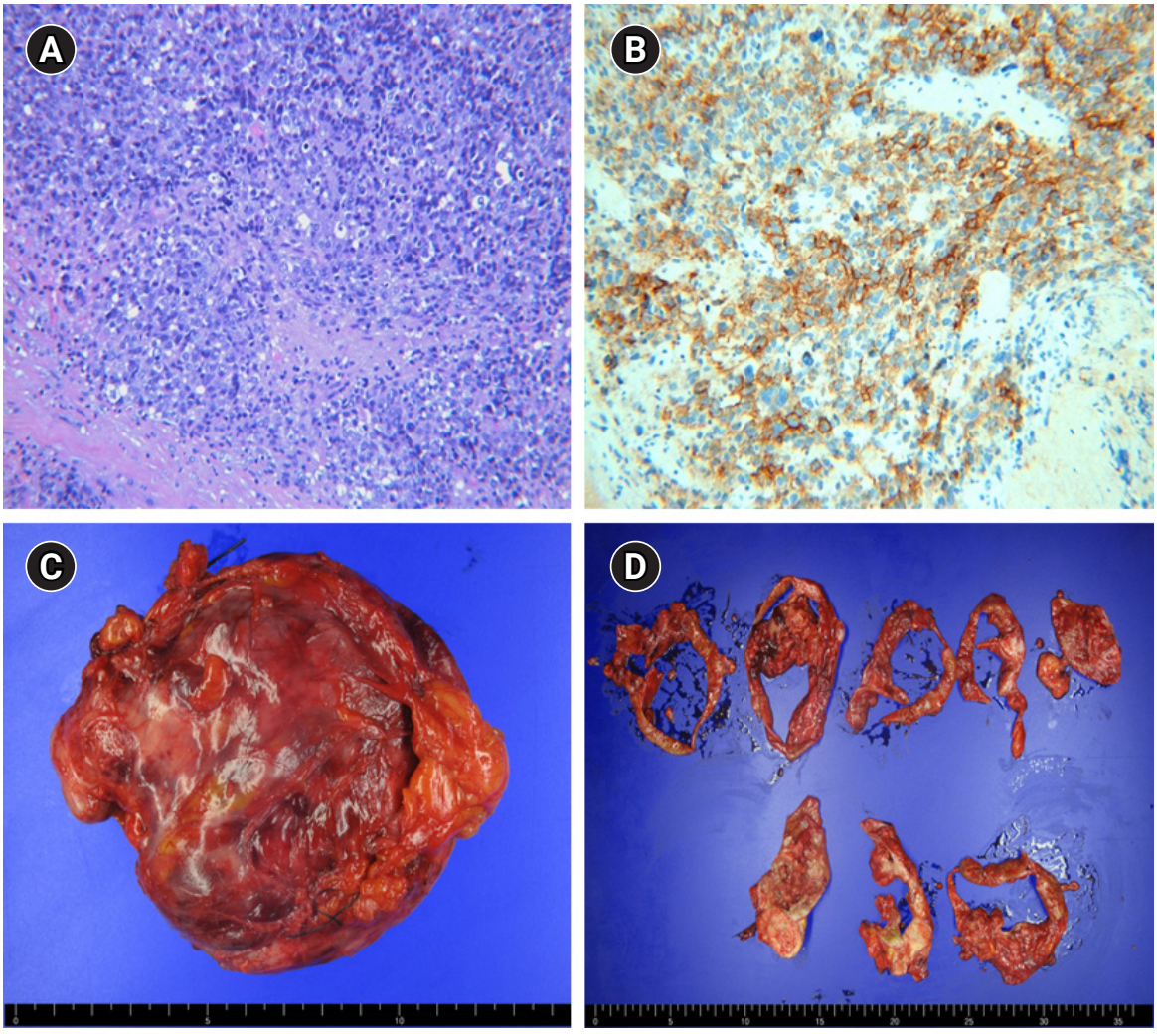

Fig. 2. Pathologic findings. (A) Tumor showing a predominantly solid pattern of highly anaplastic tumor cells and numerous mitotic figures (H&E, ×200). (B) The tumor cells tested positive for CD30, a specific marker of embryonal carcinoma (×200). (C) Gross findings. A 12- to 15-cm cystic and solid mass was confined to the ovary with an intact capsule. (D) Cutting sections of the tumor.

Postoperative laboratory tests for alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (β-hCG) showed normal serum levels: 2.1 IU/mL (range, 0–10.0 IU/mL) and 1.08 mIU/mL (range, 0–5 mIU/mL), respectively. The LDH level decreased to 200 IU/L (range, 135–225 IU/L). The patient's postoperative care was uneventful. Subsequently, the patient underwent 4 courses of BEP. She has been recurrence-free for 12 months.

Discussion

Embryonal carcinoma was first described in 1976 by Kurman and Norris [4]. Patients with ovarian embryonal carcinoma may present with abdominal pain, a palpable mass, and abdominal distension; irregular heavy bleeding may occur due to abnormal hormonal secretion. Our patient had no symptoms other than a palpable mass. Most of these tumors are stage I and confined to one ovary [4]. The overall 5-year survival rates for embryonal carcinoma in the first reported series of 15 patients were reportedly 39% and 50% for all stages and stage I, respectively [4]. However, BEP chemotherapy for adjuvant therapy improved mean survival rates exceeding 90% [5].

Immunohistochemistry is important to the diagnosis of embryonal carcinoma. CD30 is consistently positive in most embryonal carcinoma cases. The tumor cells can produce AFP and β-hCG and contain giant or syncytiotrophoblastic cells with necrosis and hemorrhage that stain positive for cytokeratin and hyaline bodies in premenopausal patients [2]. In the current case, the excised mass tested positive for CD30, p53, and WT1 and focal positivity for panCK. The tumor showed a predominantly solid pattern of highly anaplastic tumor cells and numerous mitotic figures without necrosis and hemorrhage and other histological types could not be seen from pathological findings; thus, it could be diagnosed as pure-type.

The treatment of embryonal carcinoma in younger women is unilateral oophorectomy and combination chemotherapy with BEP. In the case of embryonal carcinoma in young women, similar to other germ cell tumors, the

Table 1. The current patient versus the most recent case of a postmenopausal embryonal carcinoma patient and the previously published case of pure-type embryonal carcinoma in a premenopausal woman

Variable		Postmenopausal woman		Premenopausal woman [6]
		Previous case [3]	Present case	
Age (yr)		53	75	13
Type		Mixed type (EST with EC)	Pure type	Pure type
Figo stage		1A	1A	1A
Tumor marker	AFP	+	NA	-
	β-hCG	+	NA	-
	CA-125	-	-	-
	LDH	-	+	+
Radiologic findings		26×25×15-cm multicystic pelvic/abdominal mass appearing to arise from the left adnexa (USG)	11 cm, cystic and solid mass with a mural nodule–highly suspicious for epithelial-type ovarian carcinoma (ORADS=5) (MRI)	8.3×16×16 cm, multicystic septated solid abdomino-pelvic mass maintaining fat planes (USG, CT)
Operative field		Infiltrative growth and adhesion to the adjacent organs and tissues were noted intraoperatively. Co-operation with other general surgery and urology teams would have been prudent.	Adhered to the cul-de-sac and sigmoid colon serosal surface	No adhesion and no infiltrative growth
Pathologic findings		No necrosis and hemorrhage CD30, p53, and WT1 and focal positivity for panCK	NA	Necrosis and hemorrhage Cytokeratin and hyaline bodies
Treatment		Complete surgical staging (3 courses of BEP)	Complete surgical staging (4 courses of BEP)	Fertility-sparing surgery USO, omental biopsy, appendectomy (3 courses of BEP)

AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; CA-125, cancer antigen 125; LDH, lactate dehydrogenase; EST, endodermal sinus tumor; EC, embryonal carcinoma; NA, not available; USG, ultrasonography; ORADS, Ovarian-Adnexal Reporting & Data System; MRI, magnetic resonance imaging; CT, computed tomography; BEP, bleomycin + etoposide + cisplatin; USO, unilateral salpingo-oophorectomy.

tumor did not show an infiltrative growth pattern or form adhesions with adjacent organs, making it less difficult to perform debulking surgery and sensitive to chemotherapy [7,8]. In contrast, it showed a more infiltrative growth pattern in postmenopausal patients; therefore, the survival rate of germ cell tumors in postmenopausal women is reportedly lower than that of women of reproductive age or adolescents [9]. Therefore, ovarian preservation is not recommended for postmenopausal OGCT patients and a thorough staging operation is necessary [10]. On radiologic imaging, in younger patients, malignant germ cell tumors are generally large and nonspecific with a complex but predominantly solid form and ascites with hemorrhage and necrosis on imaging, while the invasion of other pelvic organs is more likely to occur in postmenopausal patients [11]. On MRI, an incorrect initial diagnosis was made of epithelial ovarian malignancy (ORADS score 5) associated with endometrioma such as clear cell or endometrioid carcinoma. Moreover, other findings included hydronephrosis showing abrupt narrowing at the ovarian mass level suggestive of ureteral invasion. This is the first report of the MRI findings of pure-type embryonal carcinoma in a postmenopausal woman.

Laboratory tests for AFP, β-hCG, LDH, and other tumor markers may contribute to the preoperative diagnosis and choice of therapeutic effects. The disease may be associated with high AFP and β-hCG levels, but such a pattern is not absolute and different associations could be observed, especially when the embryonal carcinoma is pure-type (Table 1) [6].

The present case is the first of pure-type embryonal carcinoma of the ovary. Table 1 compares the current case versus the previously published postmenopausal embryonal carcinoma patient and the most recent case of pure-type in a premenopausal woman. As OGCTs including ovarian embryonal carcinoma in postmenopausal women might have different characteristics and prognoses from those of premenopausal women, more research and case reports are needed to better understand this rare entity.

Article information

Conflicts of interest

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

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Author contributions

Conceptualization: DSS. Data curation: KUC, NKL. Formal analysis: EJK, YJS. Funding acquisition: DSS. Investigation: HBJ. Supervision: KHK. Validation: KHK. Writing - original draft: HBJ. Writing - review & editing: ETK. Approval of final manuscript: all authors.

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Case report

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Secondary hyperparathyroidism due to multiple parathyroid carcinomas in a patient with chronic hemodialysis: a case report

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Parathyroid carcinoma (PC) in cases of secondary or tertiary hyperparathyroidism is relatively uncommon, and only a few case reports have described this entity. Although some papers have reported patients with one or two parathyroid malignancies, multiple PC–especially three or more–have been even more rarely reported. Herein, we report a case of secondary hyperparathyroidism due to multiple PCs in a chronic hemodialysis patient. A 54-year-old man with end-stage kidney disease was referred for hyperparathyroidism. He had been diagnosed with chronic kidney disease in 2001 and had begun hemodialysis in 2009. In laboratory tests, intact parathyroid hormone (iPTH) was markedly elevated to 1,144.1 pg/mL (normal range: 15.0–68.3 pg/mL) and serum calcium was mildly elevated to 10.56 mg/dL (normal range: 8.5–10.3 mg/dL). Ultrasonography showed hypoechoic nodules in the posterior part of both thyroid glands. All three nodules showed increased uptake on a ^{99m}Tc sestamibi scan. The patient underwent total parathyroidectomy with autotransplantation to the right forearm. Histopathology findings showed three PCs with capsular invasion and one parathyroid hyperplasia. In the immediate postoperative period, the iPTH level dropped from 1,446.8 to 82.4 pg/dL and, after 1 month, to 4.0 pg/dL. This patient needed oral calcium carbonate and active vitamin D to maintain appropriate serum calcium levels. Although multiple PCs are rare, they can cause secondary hyperparathyroidism. Therefore, clinicians should suspect multiple PCs when patients' serum iPTH levels are exceptionally high. Additionally, since PCs could occur in multiple glands, autotransplantation of the parathyroid gland after parathyroidectomy should be done carefully.

Keywords: Case reports; Parathyroid neoplasms; Renal insufficiency, chronic; Secondary hyperparathyroidism

Introduction

Parathyroid carcinoma (PC) is one of the rarest endocrine malignancies [1]. It is known to cause 0.5% to 5% of primary hyperparathyroidism (HPT) and usually develops in patients aged between 40 and 50 with no gender preference [2,3].

Secondary HPT is one of the common complications in patients with chronic kidney disease (CKD). In CKD patients, decreased activation of vitamin D causes hypocalcemia and hyperphosphatemia, which leads to overproduction of parathyroid hormone [4]. Most of the PC cases are associated with primary HPT, however, there are few cases

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of PC are combined with secondary or tertiary HPT. In this paper, we report a rare case of secondary HPT due to multiple PC in a patient with CKD.

Case

Ethical statements: This study was approved by the Ethics Committee of Pusan National University Hospital (No. 2204-008-113). Written informed consent from patient was obtained.

1. Medical history

A 54-year-old man with end-stage renal disease was referred to the endocrinology department due to markedly elevated serum intact parathyroid hormone (iPTH) level. He had been diagnosed with CKD in 2001 and started hemodialysis in 2009. He showed no symptoms related to HPT. A bone mineral density showed a T score of 0.6 in the spine, –1.7 in the femur neck and –0.9 for total hip, consistent with osteopenia (distal forearm was not evaluated at initial). As he had no history of parathyroid or bone disease, and was subsequently diagnosed with secondary HPT. The patient was taking several medications including sevelamer carbonate (phosphate binder), cinacalcet (calcimimetics) or paricalcitol (vitamin D receptor activator) injections. Though his serum calcium level was mildly elevated and there was no evidence of renal stone or severe bone disease, we recommended him to have parathyroid surgery because of large size of parathyroid glands and elevated iPTH which was not controlled by the medication. He was and underwent a total parathyroidectomy.

2. Laboratory and imaging studies

As a potential kidney transplantation recipient, blood tests and routine imaging studies were performed in the Nephrology department. Initial iPTH was markedly elevated to 1,144.1 pg/mL (reference range: 15.0–68.3 pg/mL) while there was a mild elevation of serum calcium level to 10.56 mg/dL (reference range: 8.5–10.3 mg/mL).

Other laboratory data (with reference ranges) were as follows: albumin 4.60 (3.3–5.2) mg/dL, phosphorous 3.66 (2.0–4.6) mg/dL, creatinine 11.59 (0.4–1.2) mg/dL, hemoglobin 11.4 (13.5–17.5) g/dL, thyroid stimulating hormone 0.69 (0.3–0.5) μIU/mL, 25-OH vitamin D_3 19.16 (30–150 ng/mL). Ultrasonography of the thyroid and parathyroid showed 0.62×0.77 cm hypoechoic nodule with well-defined margin at posterior of right thyroid gland, 1.20×1.94 cm and 1.91×1.79 cm hypoechoic nodules with irregular margin and inhomogeneous internal echo at posterior of left thyroid gland which were suspected to be parathyroid lesions (Fig. 1A-C). On ^{99m}Tc-sestamibi parathyroid single photon emission computed tomography, all of the three nodules persistently showed increased uptake after 2 hours of intravenous ^{99m}Tc sestamibi injection (Fig. 1D).

3. Surgery and postsurgical histopathology

The patient underwent total parathyroidectomy with auto-transplantation on his right forearm. Right superior, right inferior, left superior, left inferior parathyroid glands were all removed. Since intraoperative gross observation of right inferior parathyroid gland was fairly smaller than that of other masses, and frozen biopsy showed no malignancy, this tissue was auto-transplanted on the patient's right forearm.

The final histopathology report revealed three PC with capsular invasion (1.5×1.2×1.0 cm at right superior, 2.2×1.7×1.0 cm at left superior, and 2.2×1.7×1.5 cm at left inferior parathyroid glands). The right inferior parathyroid mass showed parathyroid hyperplasia (Fig. 2).

4. Postoperative course

Postoperative laboratory results showed noticeably dropped iPTH from 1,446.8 to 82.4 pg/dL and gently dropped serum calcium level from 10.49 to 9.20 mg/dL. Serum phosphorous level after parathyroidectomy was changed from 2.18 mg/dL to 4.88 mg/dL. During the postoperative period, the patient's biochemical results exhibited postsurgical hypocalcemia (iPTH 4.0 pg/dL and serum calcium 6.46 mg/dL) and the patient was discharged with calcium carbonate and oral active vitamin D supplements.

Discussion

In this paper, we presented a case of multiple PCs with secondary HPT. Secondary HPT is one of the most frequent complications in patients with chronic hemodialysis. However, we rarely encounter secondary HPT which is caused by PC, since majority of the cases are due to parathyroid adenoma or hyperplasia. Considering that clinical presentation of PC is similar to that of benign primary HPT,

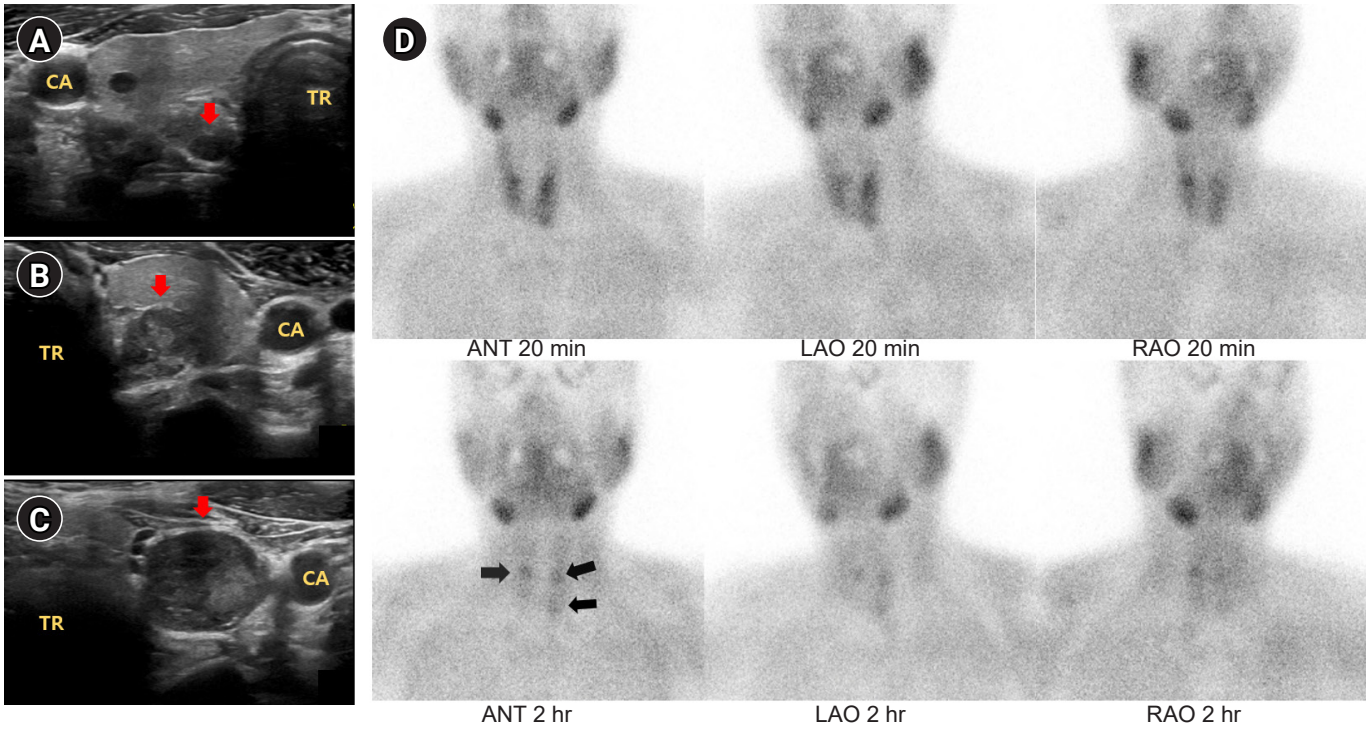

Fig. 1. Ultrasonography and ^{99m}Tc sestamibi single photon emission computed tomography (SPECT) of parathyroid glands (arrow). (A) Right superior parathyroid gland. (B) Left superior parathyroid gland. (C) Left inferior parathyroid gland. (D) Parathyroid SPECT images acquired 20 minutes and 2 hours after the intravenous ^{99m}Tc sestamibi injection. CA, carotid artery; TR, trachea, ANT, anterior; LAO, left anterior oblique; RAO, right anterior oblique.

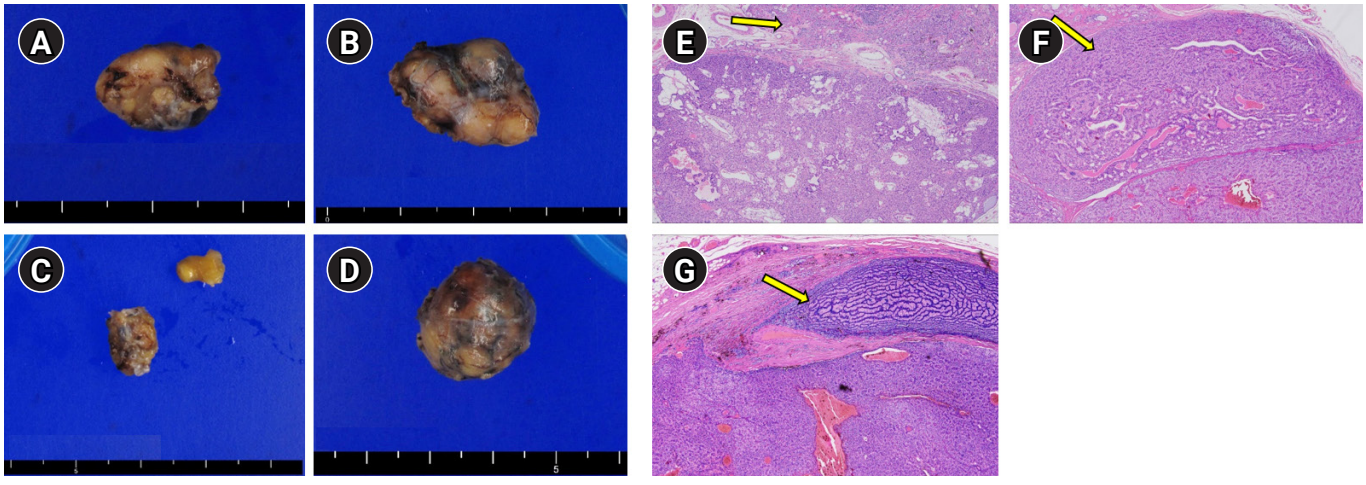

Fig. 2. Postoperative gross and microscopic histology of each parathyroid gland. Gross pathology images of (A) the right superior parathyroid gland, (B) the left superior parathyroid gland, (C) the right inferior parathyroid gland, and (D) the left inferior parathyroid gland. Microscopic examinations of three parathyroid carcinomas: (E) right superior parathyroid gland (H&E, ×40), (F) left superior parathyroid gland (H&E, ×40), (G) left inferior parathyroid gland (H&E, ×40). Capsular invasion is indicated by an arrow.

preoperative diagnosis of PC from other benign parathyroid diseases is rather challenging [5]. Even parathyroid gland ultrasonography and ^{99m}Tc sestamibi scan do not provide definitive differential diagnosis between benign and malig-

nant masses of parathyroid gland [6]. Low incidence of PC also makes it difficult to distinguish it from parathyroid adenoma or hyperplasia. In addition, for subjects who are on chronic hemodialysis, it is even more difficult to suspect PC before surgery than in patients with normal kidney disease. One of the reasons could be relatively low calcium level, due to hemodialysis and oral calcimimetics. Furthermore, continuous stimulating of four parathyroid glands owing to low serum vitamin D level can lead to high serum iPTH level, which can make the differential diagnosis of PC and benign diseases more complicated [7]. For these reasons, most PC cases are likely to be confirmed by histological examination after surgical removal of the tissue in patients with end-stage renal disease. Like benign parathyroid disease, signs and symptoms of PC can include hypercalcemia, bone and joint pain, osteoporosis, nephrolithiasis, renal and cardiac dysfunction [2]. However, PC tends to present with more severe course than benign parathyroid lesions. Referring to several literatures, severe hypercalcemia (>14 mg/dL), markedly elevated iPTH level, palpable neck mass (>3 cm) and significant renal or skeletal involvement may indicate features of PC [8,9]. In this case, initial serum calcium level was 10.56 mg/dL which was gently elevated. Possible causes could be hemodialysis, or oral cinacalcet which the patient had been taking.

Generally, postsurgical hypocalcemia occurs more in patients with secondary HPT than in primary HPT [10], postoperative hypocalcemia or hungry bone syndrome should be carefully examined. This patient is also taking calcium and vitamin D supplements, on close observation. He had autotransplantation parathyroid gland on his right arm, and generally it takes 2 to 3 months for transplanted parathyroid become viable, he might need to maintain taking oral supplements for some periods.

Multiple PC is a rare disease. Referring to the data from previous literature, multiple PC occurs infrequently, and there are just a few case reports [11,12]. In this case, the patient had three individual cancers developed from each parathyroid gland. Although we did not know those parathyroid lesions were malignant before the surgery, we readily decided to remove most of the sites of parathyroid glands because of the high iPTH level despite of maximal medical therapy. To some degree, it was an unexpected result that all of the three parathyroid lesions were cancerous. It is difficult to distinguish PC from benign diseases before surgery. Even fine-needle aspiration of the parathyroid mass is not recommended as it may cause tumor rupture and seeding [13]. Therefore, intraoperative findings are important to make the appropriate decision. The size, color (grayish-white), hardness, and soft tissue adherence could be the findings in high suspicion of carcinoma [5].

Surgical resection is the mainstay of the treatment for PC, and *en bloc* resection is generally recommended, to bring the optimal prognosis [14]. Complete exploration of all four parathyroid glands and watchful inspection for adjacent structures can minimize the recurrence rate. According to previous study, patients who had been diagnosed PC preoperatively and underwent *en bloc* resection had a recurrence rate of 33%, while those who were diagnosed PC postoperatively and underwent suboptimal resection had a recurrence rate of 50% [15]. However, recognition of malignancy before or even during the surgery is not simple; only 12% of PC patients are reported to have *en bloc* resection [16]. Our patient also had not been diagnosed malignancy preoperatively, and extensive manipulation was not routinely done during the surgery. Further surveillance might be crucial in this patient.

In this case, we presented an extraordinary case of secondary HPT with multiple PC in patient with chronic hemodialysis. Although it is rare, multiple PC can cause secondary HPT. Therefore, clinicians should suspect multiple PC when patient's serum iPTH level is exceptionally high. Additionally, since PC could occur in multiple glands, autotransplantation of parathyroid gland after parathyroidectomy should be done carefully.

Article information

Conflicts of interest

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

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Author contributions

Conceptualization: SR, WY, MK, BHK. Data curation: BJL, SHS. Methodology: MK. Project administration: BHK. Visualization: BJL. Writing-original draft: SR, WY. Writing-review

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Instructions to authors

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I. AIMS AND SCOPE

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

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

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

II. MANUSCRIPT SUBMISSION

Authors should submit manuscripts using the KMJ's electronic manuscript management system at https://submit.kosinmedj.org. Please log in first as a member of the system and follow the directions. **Manuscripts should be submitted by the corresponding author, who should supply the address, phone number, and email address for correspondence in the title page of the manuscript. If available, a fax number would be helpful.**

Document forms

Before you log into the online submission portal, it is helpful to prepare the following documents as you will be asked to upload them during the electronic submission process.

- **Author statement forms**
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- **A cover letter** that includes the address, telephone number, and email address of the corresponding author
- **English proofreading documentation**—authors whose native language is not English must show that their manuscript has been edited by an English-language proofreading or editing service

The revised manuscript should be submitted through the same web portal using the same identification numbers. Items pertaining to manuscripts submitted for publication, as well as letters or other forms of communication regarding the editorial management of the KMJ, should be sent to:

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III. CATEGORIES OF PUBLICATIONS

The KMJ publishes editorials, review articles, original articles, and case reports.

- **Editorials** are invited perspectives on an area of medical science that deal with active fields of research and current

medical interests and provide fresh insights and debate.
- **Review articles** provide a concise review of a subject of importance to medical researchers. They are usually solicited by the Editor, but we will also consider unsolicited material. Please send us a Presubmission Inquiry before writing a review article.
- **Original articles** are papers reporting the results of basic and clinical investigations that are sufficiently well documented to be acceptable to critical readers.
- **Case reports** deal with clinical cases of medical interest or innovation.

IV. ETHICAL CONSIDERATIONS

Authorship

The KMJ accepts authorship criteria recommended by the ICMJE (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html) and the Good Publication Practice Guidelines by the Korean Association of Medical Journal Editors (KAMJE, 2019, http://kamje.or.kr).

Each author is expected to have made a substantial contribution to the conception or design of the work; the acquisition, analysis, or interpretation of data; the creation of new software used in the work; or have drafted the work or substantively revised it; AND to have approved the submitted version (and any substantially modified version that involves the author's contributions to the study); AND to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, are appropriately investigated and resolved, and the resolution documented in the literature.

Those who do not meet the above criteria should be acknowledged as contributors rather than authors. The corresponding author is responsible for completing this information at submission, and it is expected that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. When submitting a manuscript authored by a group, the corresponding author should clearly indicate the preferred citation and identify all individual authors as well as the group name. Journals generally list other members of the group in the Acknowledgments. Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship.

Contributor Roles Taxonomy (CRediT)
- Conceptualization
- Data curation
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- Methodology
- Project administration
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- Software
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- Validation
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- Writing – review and editing

The KMJ accepts notice of equal contributions for the first or corresponding authors when the study was clearly performed by co-first or co-corresponding authors. The KMJ does not allow authorship corrections after publication unless the editorial staff made an obvious mistake. Before publication, authorship can be changed if all authors request the authorship correction.

Declaration of ethics including authorship

The corresponding author must agree to abide by research ethics guidelines, including those involving authorship, by including the following sentences in a cover letter.

"All of the bylined authors meet the ICMJE criteria for authorship. We well understand the privilege and responsibility of authorship of scientific publications. We declare that we are following global and/or local guidelines of research and publication ethics strictly including authorship."

All authors should provide details of their position in the affiliated institution on the online submission system (e.g., professor, college lecturer, graduate student, researcher, college student, post-doctoral fellow, middle or high school teacher, or high school student, etc.).

Redundant publication and plagiarism

Redundant publication is defined as "reporting (publishing

or attempting to publish) substantially the same work more than once, without attribution of the original source(s)." Characteristics of reports that are substantially similar include the following: (a) "at least one of the authors must be common to all reports (if there are no common authors, it is more likely plagiarism than redundant publication)," (b) "the subjects or study populations are the same or overlapped," (c) "the methodology is typically identical or nearly so," and (d) "the results and their interpretation generally vary little, if at all."

When submitting a manuscript, authors should include a letter informing the editor of any potential overlap with other already published material or material being evaluated for publication and should also state how the manuscript submitted to the KMJ differs substantially from other materials. If all or part of a patient population was previously reported, this should be mentioned in the Methods, with citation of the appropriate reference(s). The duplication will be checked through crosscheck (https://app.ithenticate.com) or eTBLAST (https://bio.tools/etblast) before submission. If duplicate publication related to the papers in this journal is detected, the manuscripts may be rejected, the authors will be announced in the journal, and their institutes will be informed. There will also be penalties for the authors.

Secondary publication

It is possible to republish manuscripts if the manuscripts satisfy the conditions for secondary publication in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals established by the ICMJE (http://www.icmje.org).

- The authors have received approval from the editors of both journals (the editor concerned with the secondary publication must have access to the primary version).
- The priority for the primary publication is respected in the form of a publication interval negotiated by the editors of both journals and the authors.
- The paper for secondary publication is intended for a different group of readers; an abbreviated version could be sufficient.
- The secondary version faithfully reflects the data and interpretations of the primary version.
- The secondary version informs readers, peers, and documenting agencies that the paper has been published in whole or in part elsewhere—for example, with a note reading, "This article is based on a study first reported in (journal title, with full reference)"—and the secondary version cites the primary reference.
- The title of the secondary publication should indicate that it is a secondary publication (complete or abridged republication or translation) of a primary publication. Of note, the US National Library of Medicine (NLM) does not consider translations to be "republications" and does not cite or index them when the original article was published in a journal indexed in MEDLINE.

Conflict of interest statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to pharmaceutical companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

Commercial sponsorships or funding

The corresponding author must inform the editor of any commercial sponsorships or fundings.

Registration of the clinical trial research

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

Data-sharing statement

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

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

Statement of informed consent and IRB approval

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

Statement on human and animal rights

Clinical research should be done in accordance with the Ethical Principles for Medical Research Involving Human Subjects, as outlined in the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). Clinical studies that do not meet the Helsinki Declaration will not be considered for publication. For publication, the human subjects' identifiable information, such as the patients' names, initials, hospital numbers, dates of birth, or other protected healthcare information should not be disclosed. For animal subjects, the research should be performed based on the National or Institutional Guide for the Care and Use of Laboratory Animals, and the ethical treatment of all experimental animals should be maintained.

Process to manage the research and publication misconduct

When the Journal faces suspected cases of research and publication misconduct, such as a redundant (duplicate) publication, plagiarism, fabricated data, changes in authorship, undisclosed conflicts of interest, an ethical problem with a submitted manuscript, a reviewer who has appropriated an author's idea or data, complaints against editors, and other issues, the resolution process will follow the flowchart provided by the Committee on Publication Ethics (http://publicationethics.org/resources/flowcharts). The Editorial Board of the KMJ will discuss the suspected cases and reach a decision. The KMJ will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

Errata

Errata provide a means of correcting errors that occur during the writing, typing, editing, or publication (e.g., a misspelling, dropped word or line, or mislabeling of a figure) of a published article. Errata can be submitted through the KMJ online manuscript management portal as a Microsoft Word file. In the Abstract section of the submission form (a required field), enter "Not Applicable" and upload the text of the proposed erratum. Please see a recent issue for correct formatting.

Author corrections

If the addition or deletion of authors or a change to the order of authorship is required, the corresponding author must complete the authorship change form and submit it through the KMJ online manuscript management portal with the signature of all existing authors and new authors. In the Abstract section of the submission form (a required field), enter "Not Applicable." The text of the proposed erratum should be uploaded as a Microsoft Word file. When there is a request for a change by the author, the editorial committee assembles an ethics committee to judge whether the change is appropriate. If a new author should be added or an author should be deleted after submission, it is the responsibility of the corresponding author to ensure that all authors are aware of and agree to the change in authorship. The KMJ assumes no responsibility for such changes. Please see a recent issue for correct formatting.

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Retractions should be submitted through the KMJ online manuscript management portal as a Microsoft Word file. In the Abstract section of the submission form (a required field), put "Not Applicable." Letters of agreement signed by all of the authors must be supplied as Miscellaneous Files Not for Publication (scanned PDF files). The retraction will be assigned to the journal's editor-in-chief, and the editor who handled the paper and the chair of the KMJ editorial board will be consulted. If all parties agree to the publication and content of the retraction, it will be sent

to the journals department for publication.

For the policies on research and publication ethics not stated in the Instructions, Guidelines on Good Publication (http://publicationethics.org) or Good Publication Practice Guidelines for Medical Journals (http://kamje.or.kr) can be applied.

V. MANUSCRIPT PREPARATION AND FORMAT

1. General requirements

- Every manuscript should be written in English.
- The main document, including manuscript text and tables, should be prepared in MS Word.
- The manuscript should be double-spaced on 21.6 × 27.9 cm (US letter) or 21.0 × 29.7 cm (A4) paper with top, bottom, right, and left margins of 3.0 cm.
- All manuscript pages are to be numbered consecutively, beginning with the abstract as page 1. Neither the authors' names nor their affiliations should appear on the manuscript pages.
- Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. A spelled-out term followed by the abbreviation in parentheses should be used on first mention unless the abbreviation is widely recognized (e.g., UN for United Nations).
- The names and locations (city, state, and country) of manufacturers of equipment and non-generic drugs should be given.
- When quoting from other sources, supply a reference number after the author's name or at the end of the quotation (see References section).
- Authors should express all measurements in conventional units, using the International System (SI) of units.
- To make papers more readable and informative, mark the followings in italics.
  - Biological names of organisms: *Saccharomyces cerevisiae*, *E. coli*
  - Restriction enzymes and some enzymes: *EcoRI, Taq polymerase*
  - Names of genes: *src, c-H-ras, myc*
  - Latin words: *in vivo, in vitro, in situ*
  - Centrifugal acceleration: *g* (e.g., 100,000 *g*)

2. Reporting guidelines for specific study designs

Research reports frequently omit important information. As such, reporting guidelines have been developed for a number of study designs, and some journals may ask authors to follow them. Authors are also encouraged to consult the reporting guidelines relevant to their specific research design. A good source for reporting guidelines is the EQUATOR Network (http://www.equator-network.org/home) and the United States National Library of Medicine (http://www.nlm.nih.gov/services/research_report_guide.html).

3. Original articles

Manuscript style

The manuscript should be prepared according to the "ICMJE Recommendations for the Conducts, Reporting, and Publication of Scholarly Work in Medical Journals" (2021) (http://www.icmje.org). In addition to the ICMJE recommendation, a number of reporting guidelines have been developed by experts to facilitate reporting of research studies or clinical trials (http://www.equator-network.org/library). For reporting of randomized controlled trials, the KMJ requires compliance with the CONSORT statement (http://www.consort-statement.org) and the ICMJE Statement on Data Sharing (http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html).

Manuscript components

The manuscript, including tables and their footnotes and figure legends, should use the 12-point Times New Roman font and line spacing should be set to "double." The manuscript should be in the following sequence: title page, Conflicts of interest, Sources of funding, Author contributions, ORCID iDs, Abstract and Keywords, Introduction, Methods, Results, Discussion, Acknowledgments, References, Tables (each table complete with a title and footnotes), and Figure Legends (figures should be uploaded separately, not embedded in text). All pages should be numbered consecutively starting from the title page. All numbers should be written in Arabic numerals throughout the manuscript except for the first word of a sentence. Tables and figures should not be inserted in the main text. A combined table file can be uploaded separately from the main manuscript file. A combined figure file or individual figure files can be uploaded separately from the main manuscript.

File formats

Drawings, photographs, graphs, or combined figures can be submitted as PDF files. We accept graphic files in the PDF, JPEG, or TIFF formats only. It is permissible to send low-resolution images for peer review, but we will ask for high-resolution files before publication. Acceptable file formats for supplemental video files are mov, wmv, mpg, mpeg, and mp4. The file size and running time of each video should be no more than 25 MB and 5 minutes, respectively.

Title page

Include the following items on the title page: (1) the title of the manuscript (the title should be short, informative, contain the major keywords, and be no more than 200 characters in length, including spaces between words; acronyms and abbreviations should be avoided, and the species of any experimental animal must be indicated in the title; (2) an author list (include ORCID*); (3) the names of each author's institutional affiliation; (4) the name, address, telephone number, and email address of the corresponding author; (5) the source of any research funding, if any, and a list of where and when the study has been presented in part elsewhere; and (6) a running title of no more than 50 characters.

*ORCID: We recommend that the open researcher and contributor ID (ORCID) of all authors be provided. Authors can obtain an ORCID at http://orcid.org. Registration is free to every researcher in the world.

Conflict of interest

State any potential conflict of interest that could influence the authors' interpretation of the data, such as financial support from or connections to pharmaceutical companies, political pressure from interest groups, or academically related issues.

Author contributions

What authors have contributed to the study should be described in this section. To qualify for authorship, all contributors must meet at least one of the 12 core contributions defined by CRediT (conceptualization, methodology, software, validation, formal analysis, investigation, data curation, funding acquisition, project administration, resources, supervision, and visualization), as well as at least one writing contribution (original draft preparation, review, and editing). Authors may also satisfy the other remaining contributions; however, these alone will not qualify them for authorship.

Contributions will be published with the article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and it is expected that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

An example:

Conceptualization: GDH. Data curation: YSJ, YC. Formal analysis: CSK. Methodology: YK, GDK.; Software: YK, YSJ. Validation:..... Investigation:..... Funding acquisition:..... Project administration:..... Resources:..... Supervision:..... Visualization:..... Writing - original draft: GDH, YSJ. Writing - review and editing: YC, YK, CSK.

Abstract and keywords

The abstract should briefly describe the content of the manuscript in a structured format within 250 words. The abstract should be structured as follows; Background, Methods, Results, and Conclusion. Initialisms, acronyms and informal abbreviations should be avoided where possible; if needed, they should be kept to an absolute minimum and accompanied by proper definitions. Three to five keywords should be listed below the Abstract on the Abstract page. For the selection of keywords, refer to Medical Subject Headings (MeSH) in PubMed, or http://www.nlm.nih.gov/mesh/MBrowser.html.

Main text

- Introduction

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

- Methods

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

experiments using laboratory animals or pathogens should mention approval of the studies by relevant committees in this section. The sources of special chemicals or preparations should be supplied, along with their location (name of company, city and state, and country). The method of statistical analyses and the criteria for determining significance levels should be described. An ethics statement should be placed in this section when the studies are performed using clinical samples or data, and animals. Examples are provided below.

(For clinical study)

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

(For animal study)

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

(For clinical trials)

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

Other international registration is acceptable. Manuscripts reporting interventional clinical trial should include a data-sharing plan following the ICMJE statement by referring to the ICMJE Statement on Data Sharing.

When describing participants, ensure correct use of the terms sex (for biological factors) and gender (identity, psychosocial, or cultural factors), and, unless inappropriate, report the sex or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population, (e.g., only one sex), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should describe how they determined race or ethnicity and justify their relevance.

• Results

This section should be presented logically and in a manner consistent with the Methods section. Tables and figures are recommended when they can present data more succinctly and clearly. Do not duplicate the content of tables or figures in the Results section. Briefly describe the core results related to the conclusion in the text when data are provided in tables or in figures. In the Results section, audio or video files are also welcomed. Supplementary results can be placed in an Appendix.

• Discussion

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

Acknowledgments

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

References

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

Authors are responsible for the accuracy and completeness of their references and correct text citations. Papers in press may be listed among the references with the journal name and tentative year of publication. Gray literature

sources are not allowed for references. Internet materials are acceptable with the correct URL and the date accessed.
Examples of reference style:

1. Journal
   Kim TS, Kang SH, Kang PM, Ha H, Kim SD, Yoon J, et al. Clinical significance of serum neutrophil gelatinase-associated lipocalin in the early diagnosis of renal function deterioration after radical nephrectomy. Kosin Med J 2018;33:20-8.
   Verbalis JG. Renal physiology of nocturia. Neurourol Urodyn 2014;33(Suppl 1):S6-9.
2. Book
   Hong GD, Kim C, Park J. KMJ reference style: a guide for authors. 5th ed. Seoul: Daehakro Press; 2017.
3. Chapter in a book
   Floch MH. Probiotics, probiotics and dietary fiber. In: Buchman A, editor. Clinical nutrition: a guide for gastroenterologists. Thorofare: SLAK Incorporated; 2005. p. 18-24.
4. Conference/meeting presentation
   Morrow GR, Ryan JL, Kohli S. Modafinil for persistent post-treatment fatigue: an open label study of 82 women with breast cancer. MASCC international symposia 2006, Abstract 11-070.
5. Internet or electronic resource
   Testa J. The Thomson Reuters journal selection process [Internet]. Philadelphia: Thomson Reuters; c2012 [cited 2013 Sep 30]. http://wokinfo.com/essays/journal-selection-process.

Tables and figures

Tables: Tables are to be numbered in the order in which they are cited in the text. A table title should concisely describe the content of the table so that a reader can understand the table without referring to the text. Each table must be simple and provided on a separate page with its title. Explanatory matter is placed in the footnotes below the table and not in the title. All non-standard abbreviations should be explained in the footnotes. The symbols a), b), c), d), should be used (in that order) for footnotes. Statistical measures such as standard deviation (SD) or standard error (SE) should be identified. No vertical or horizontal rules should appear between table cells.

Figures and legends for illustrations: Figures must be submitted as separate files in JPEG, TIFF or PDF format (do not embed the figures in a Microsoft Word file). To supply figures in other file formats, contact the editorial office. All images should the following image resolutions or greater: line art (an image composed of lines and text): 1,000 dpi; halftone (a continuous tone photograph, which contains no text): 300 dpi; or combination (line art and halftone): 600 dpi.

Photographs of recognizable persons should be accompanied by a signed release from the person in the photograph or an appropriate legal guardian authorizing reproduction. Written permission should be obtained for the use of all previously published illustrations (and copies of permission letters should be included).

Figures should be numbered, using Arabic numerals, in the order in which they are cited. In the case of multiple prints bearing the same number, use English letters immediately after the numerals to indicate the correct order. (e.g., Fig. 1A, Fig. 1B and C; Fig. 2A–D).

If any tables or figures are moved or modified from other papers, authors should obtain permission through the Copyright Clearance Center (https://www.copyright.com) or from the individual publisher if the materials are not from open access journals published under a Creative Commons license. If tables or figures are from open access journals, accurately describe the source of the journal in the footnote. Note that a free access journal is different from that of an open access journal, and it is necessary to obtain permission from the publisher of a free access journal when using tables or figures from these sources.

Description of *p* value: p is always italicized and in lower case. When p values are statistically significant, the corresponding data should be marked with superscripted asterisks ($p<0.05$, $p<0.01$, $p<0.001$).

Abbreviations: Except for units of measurement, abbreviations are strongly discouraged. Do not use abbreviations in the title or abstract and limit their use in the text. Define all abbreviations at first mention.

Units of measurement: Laboratory values are expressed using conventional units of measure, with relevant System International (SI) conversion factors, if necessary expressed secondarily (in parentheses) only at first mention. Figures and tables should use conventional units, with conversion factors given in legends or footnotes. The metric system is

preferred for the expression of length, area, mass, and volume.

Names of drugs, devices, and other products: Generic names of pharmaceuticals should be used. When proprietary brands are used, include the brand name and the name of the manufacturer in parentheses after the first mention of the generic name in the Methods section.

Gene names, symbols and accession number: Authors describing genes or related structures in a manuscript should include the names and official symbols provided by the US National Center for Biotechnology Information (NCBI) or the HUGO Gene Nomenclature Committee.

Supplementary materials

Authors can submit supplementary materials for online-only publication when there is insufficient space to include the materials in the main article. Supplementary materials should be original and important to the understanding and interpretation of the report. As supplementary materials will not be edited or formatted after publication, authors are responsible for the accuracy and presentation of this material.

Supplementary materials should be submitted in a single MS Word document or a single PDF file, which should include all materials (information, tables, figures, and references). Each element included in supplementary material should be cited in the text of the main manuscript (e.g., Supplementary Table 1, Supplementary Fig. 1, Supplementary Methods). The first page of the online-only document should list the number and title of each element included in the document.

4. Review articles

Reviews are comprehensive analyses of specific topics. They are typically solicited by the Editor, but we also consider unsolicited material. Please send us a Presubmission Inquiry before writing a review article. All review articles undergo the same review process as other types of articles prior to acceptance. Reviews are organized as follows: title page, abstract (a single unstructured paragraph of no more than 250 words) and three to five keywords (as in MeSH), introduction, main text, conclusion, acknowledgments, references, tables, and figure legends. Reviews have no restrictions on word count or the number of figures and tables. However, authors should eliminate redundancies, emphasize the central message, and provide only the data necessary to convey that message. A review article is limited to less than 6,000 words, with a maximum of 100 references, excluding tables or figure legends.

5. Case reports

Case reports will be published only in exceptional circumstances, if they illustrate a rare occurrence of clinical importance. The manuscript should be in the following sequence: title page, abstract (no more than 250 words) and three to five keywords (as in MeSH), introduction, case(s), discussion, acknowledgments, references (no more than 20), tables, and figure legends. The manuscript should have no more than six tables or figures in total. For case reports involving humans, whether informed consent for publication of clinical data was obtained from the study participants must be stated in the case description section.

6. Editorials

Editorials are invited by the Editor and should provide commentary on articles in the current issue. Editorial topics could include active areas of research, fresh insights, and debates in all fields of interest to KMJ readers. Editorials should not exceed 1,000 words, excluding references, tables, and figure legends. No more than 20 references should be cited. The manuscript should have no more than three tables or figures. Any article longer than these limits should be discussed with the editor.

Table 1. Specifications for publication types

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

NL, not limited; NR, not required.
[a]Maximum number of words is exclusive of the abstract, references, and figure legends.
[b]Except meta-analyses or systematic reviews.

VI. INSTRUCTIONS FOR SUBMISSION OF REVISED MANUSCRIPTS

To prepare a revised version of your manuscript, authors should carefully follow the instructions given in the Editor's

letter. Failure to do so will delay a decision on a revision. If references, tables, or figures are moved, added, or deleted during the revision process, all subsequent tables, references, and figures should be renumbered to ensure they are cited in order.

Revised manuscript submissions should include a point-by-point response to the reviewers' comments. Authors should describe how each comment was addressed or why it was not be addressed, and clearly indicate which paragraph was revised to address each comment. The response to reviewers will be shared with all reviewers. Authors may include data in support of their argument even if the data are not included in the manuscript.

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