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Original Article

Seasonal variance of serum biomarker levels in osteoarthritis patients: a cross-sectional study of 104 patients

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Abstract: *Objective:* Prior studies have noted the importance of serum biomarkers in the pathogenesis of diseases. However, few studies have demonstrated the variance of inflammatory factors, bone metabolism factors, and hormones in OA (osteoarthritis) patients recruited from different seasons. This cross-sectional study was aimed to explore the seasonal influential factors of OA in patients recruited from different seasons. *Methods:* We retrospected 182 OA patients (from Feb 2014 to Jan 2015) hospitalized in the Department of Sports Medicine & Adult Reconstructive Surgery at Nanjing Drum Tower Hospital (the Affiliated Hospital of Nanjing University Medical School) and tested their preoperative blood samples for serum IL-6 (Interleukin-6), IL-1β, Tumor necrosis factor-α (TNF-α), Dickkopf1 (DKK1), Sclerostin (SOST), Osteopontin (OPN), Osteoprotegerin (OPG), Osteocalcin (OC), leptin, and Parathyroid hormone (PTH). *Results:* A total of 104 patients were included in this study (81 females, 23 males). The number of patients in each group was 24 in winter, 30 in spring, 26 in summer, and 24 in fall, respectively. Levels of serum TNF-α and IL-6 in the winter group were significantly higher than the other groups. IL-1β, however, showed the highest level in the fall group. Serum DKK1 topped in the winter group, while SOST peaked in the fall group. OPN level was higher in the spring and the summer group, which was the opposite of OPG. The fall group also presented higher levels of OC and PTH. Leptin level, on the other hand, topped in the summer group. *Conclusions:* Our findings showed that inflammatory factor (TNF-α), bone metabolism factors (DKK1, SOST, OPN, OPG), and bone metabolism hormones (OCN, PTH, leptin) in OA patients were associated with seasonal changes, which might provide a new perspective for identifying the pathogenesis of OA.

Keywords: Seasonal variance, serum biomarker, osteoarthritis

Introduction

Osteoarthritis (OA) is one of the most common types of arthritis, affecting more than 112 million middle-aged and elderly Chinese, especially the elderly [1]. Abnormal joint tissue metabolism is often considered as one of the initial stages of OA, followed by cartilage degradation, bone proliferation, and the development of osteophytes and inflammation, eventually resulting in the loss of mobility [2, 3].

In recent years, there has been a growing interest in the role of serum biomarkers during the pathogenesis of OA, and a variety of biomarkers have been used to diagnose and monitor OA progression in clinical settings [4]. Researchers have reported that TNF-α played a significant role in the pathogenesis of pain resulting from hip OA, and that it was negatively correlated with bone metabolism [5]. It has also been suggested that IL-1β participated in the progression of OA, and there was an ascent of

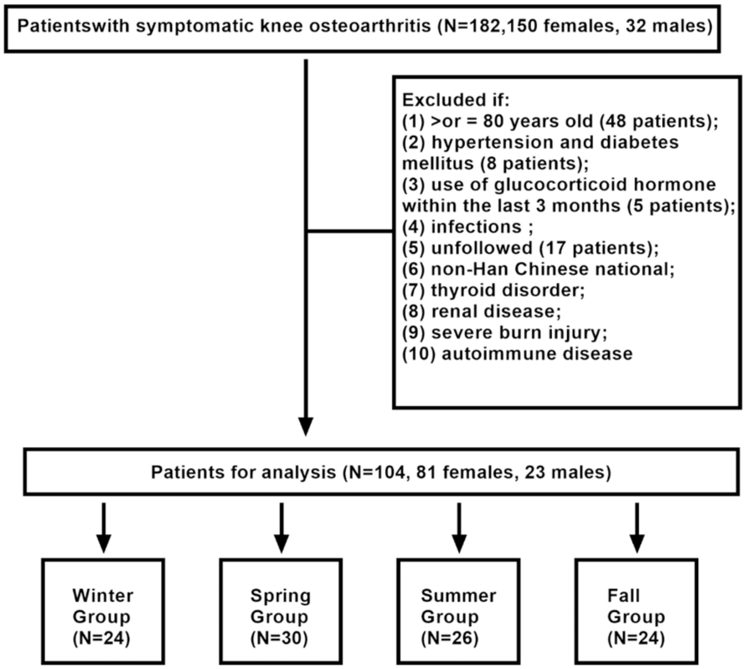

Figure1. Flowchart of participant distribution in the study. (Data were adjusted for age and gender and were compared across seasons with a one-way ANOVA test. *Means a significant difference, p<0.05).

serum IL-6 level in OA patients as well [6]. Besides, previous studies have reported that a reduction of DKK1 level would lead to the degradation of joint cartilage, [7] and an increment of SOST would result in the prevention of arthritis-related cartilage degradation [8]. In addition, OPN was found to be involved in the development of a wound boundary between new bone and preexisting bone [9], and serum OPG was higher in OA patients [10]. Furthermore, studies have also demonstrated that OCN, PTH, and leptin all took part in bone homeostasis [11-13].

The internal environment of the human body is in a status of dynamic equilibrium, which is not only followed by the 24-hour rhythms, but also abided by the circannual rhythm. For example, Yu HJ *et al.* claimed that serum vitamin D level was associated with seasonal changes [14]. And Dawson-Hughes B *et al.* illustrated a seasonal variance of SOST in normal people [15]. To our knowledge, no study has looked into the difference in serum biomarkers of OA patients hospitalized in different seasons. The purpose of the present study was to determine the potential seasonal influential factors of OA patients from different admission seasons.

Methods

Study design

The study was designed as a cross-sectional, observational, and monocentric study.

Blood samples of 104 patients in total were collected for further analysis in order to determine potential seasonal variance of representative serum biomarkers, including inflammatory factors, bone metabolism factors, and hormones.

Setting

We studied the patients hospitalized from February 2014 to January 2015 in the Department of Sports Medicine & Adult Reconstructive Surgery at Nanjing Drum Tower Hospital (the Affiliated Hospital of Nanjing University Medical School).

Participants

All patients had been suffering from symptomatic knee OA. As shown in **Figure 1**, a total of 104 patients (84 females, 23 males) were enrolled in this study. The mean age was 67.312±0.57 (min. 41, max. 80). Exclusion criteria were as follows: (1) over eighty years of age; (2) diagnosed with hypertension or diabetes mellitus; (3) use of glucocorticoid hormone within the last 3 months; (4) suffering from infection; (5) patients lost to follow-up; (6) non-Han Chinese national; (7) diagnosed with thyroid disorder; (8) diagnosed with renal disease; (9) suffering from severe burn injury in the last 3 months; (10) diagnosed with autoimmune disease. Patients who underwent bilateral reconstructive surgery were only included on their first visit. The study was approved by the ethics committee of Drum Tower Hospital, and all patients provided written informed consent. All patients signed the consent form and agreed to donating their blood samples for scientific studies.

Table 1. Demographic characters of OA patients in this study

	Winter	Spring	Summer	Fall	Total
NO.	24	30	26	24	104
Female/Male	18/6	23/7	21/5	19/5	81/23
Age	64.79±11.53	70.65±9.37	64.75±8.05	71.76±7.03	68.8±9.78
THA/TKA	10/14	12/18	11/15	10/14	43/61
KL 2	6	7	9	7	29
KL 3	10	14	11	9	44
KL 4	8	9	6	8	31

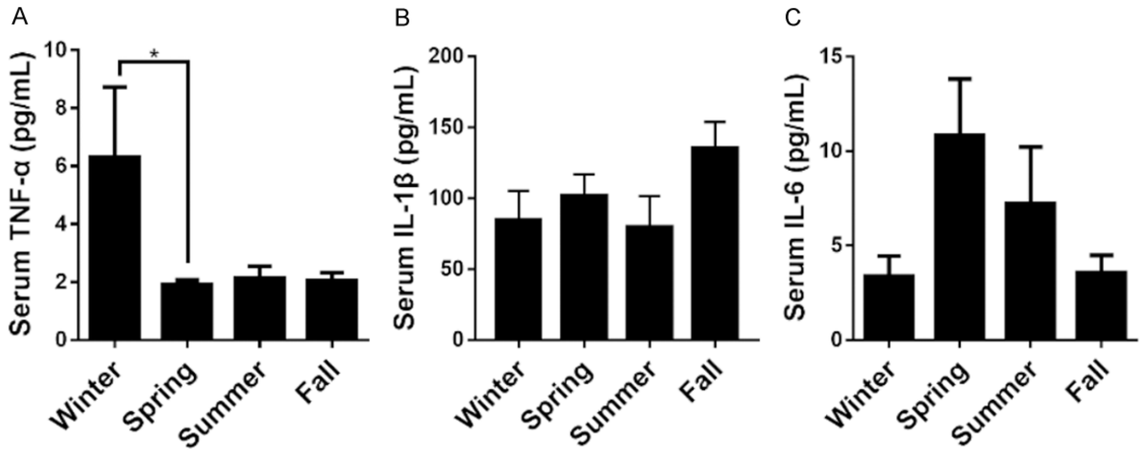

Figure 2. A. Serum TNF-α level of patients from different seasons. B. Serum IL-1β level of patients from different seasons. C. Serum IL-6 level of patients from different seasons. (Data were adjusted for age and gender and were compared across seasons with a one-way ANOVA test. *Means a significant difference, $p<0.05$).

Variables

OA was diagnosed according to the clinical and radiological criteria revised by the American Rheumatism Association (ARA) [16]. The outcome of the blood samples was defined by levels of serum biomarkers of OA patients included in this study. The participants were divided into four groups according to their seasons of recruitment. The four seasons were divided according to the climate of China, namely: winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Age, gender, physical activity, severity of OA, and percentage of body fat were considered as potential confounders. Also, given the possible existence of observational bias, we adjusted our data according to the age and sexual ratio to obtain a more efficient statistical method.

Data measurement

Participants were required to fast overnight, then blood samples were taken to test for serum TNF-α, IL-6, IL-1β, DKK1, SOST, OPN, OPG, OC, PTH, and leptin. The preoperative blood samples were obtained between 07:00 and 09:00 using disposable ethylenediaminetetraacetic acid (EDTA) vacuum blood collection tubes. After eight hours of standing, the samples were centrifuged at 1800× g for 10 min to separate the serum, which was then stored in PE tubes at -80°C until analyzed. Measurements were performed using MILLIPLEX® MAP Human High Sensitivity Cytokine/Chemokine Panel Kits (EMD Millipore, Billerica, MA, and Cat. No. HBNMAG-51K) according to manufacturer's instructions.

Statistical analysis

Results are expressed as mean ± standard error of mean. Statistical analysis was performed using SPSS v22.0 (SPSS Inc., Chicago, IL, USA) and graphs were done with GraphPad Prism v7.0 (GraphPad Software Inc., La Jolla, CA, USA). Data were adjusted for age and gender and were compared across seasons with a one-way ANOVA test. A *p* value less than 0.05

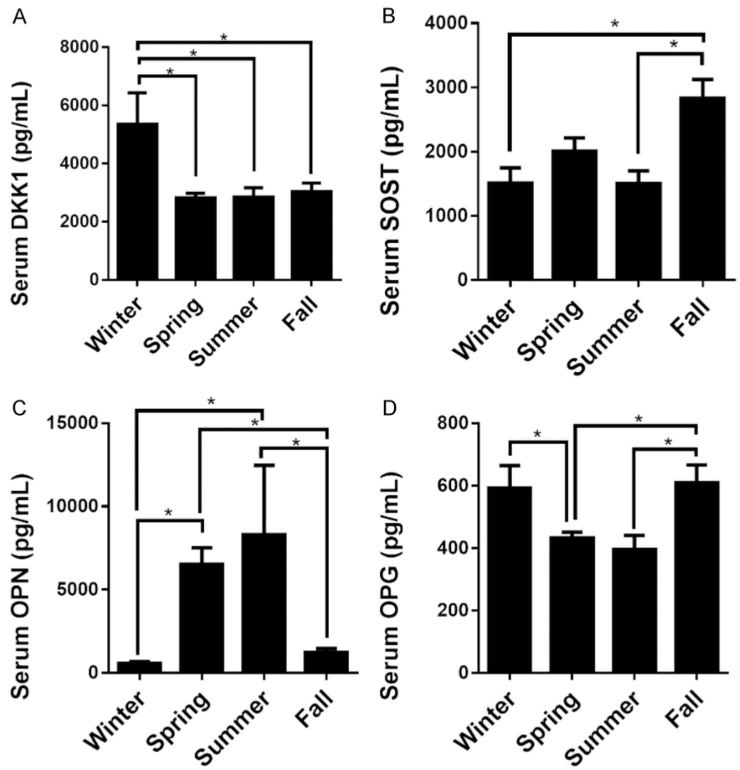

Figure 3. A. Serum DKK1 level of patients from different seasons. B. Serum SOST level of patients from different seasons. C. Serum OPN level of patients from different seasons. D. Serum OPG level of patients from different seasons. (Data were adjusted for age and gender and were compared across seasons with a one-way ANOVA test. *Means a significant difference, $p<0.05$).

was considered statistically significant. LSD post hoc analysis was used if the ANOVA was significant.

Results

Clinical characteristics of participants

As shown in **Table 1**, this study included a total of 104 patients: 24 in winter, 30 in spring, 26 in summer and 24 in fall. The majority of the participants were female, accounting for 81, and 23 were male. The mean age of the participants was 68.8 years old. There were 43 patients requiring total hip arthroplasty (THA) and 61 patients requiring total knee arthroplasty (TKA). According to the Kellgren and Lawrence (KL) classification, plain radiography of the affected knee was used to assess the severity of OA. There was no significant deviation of the severity of arthritis in different groups.

Preoperative serum inflammatory factors of patients recruited in different seasons

In order to identify the differences in inflammatory factors in the participants before total joint arthroplasty (TJA), classic inflammatory markers (IL-6, IL-1β, and TNF-α) were detected. Interestingly, serum TNF-α level of the winter group was significantly higher ($p=0.026$) than that of the other groups, and the same trend was noticed in the other three seasons (**Figure 2A**). Serum IL-6 of the spring group (10.83 pg/ml) was the highest, while the winter group showed the lowest IL-6 level of 3.399 pg/ml (**Figure 2B**). However, serum IL-1β level was of no significant difference in patients recruited from different seasons (84.67 pg/ml in winter, 102 pg/ml in spring, 79.55 pg/ml in summer and 135.21 pg/ml in fall) (**Figure 2C**). Taken together, IL-6, IL-1β, and TNF-α demonstrated season variance in osteoarthritis patients.

Preoperative serum bone metabolism factors of patients recruited in different seasons

To determine the variance of bone metabolism factors in the participants before TJA, two inhibitors of the Wnt signaling pathway (DKK1 and SOST) and two critical metabolic proteins (OPN and OPG) were investigated. Changes in bone metabolism factors appeared to be more seasonal.

Serum DKK1 level of the winter group was significantly higher than other groups (winter vs. spring, $p=0.0006$; winter vs. summer, $p=0.0279$; winter vs. fall, $p=0.0156$), which were analogous to the trend of serum TNF-α level (**Figure 3A**). As for serum SOST, patients recruited from fall held the highest level, then went the spring group and the winter group (winter vs. fall, $p=0.0133$), while the summer group showed a relatively lower level (summer

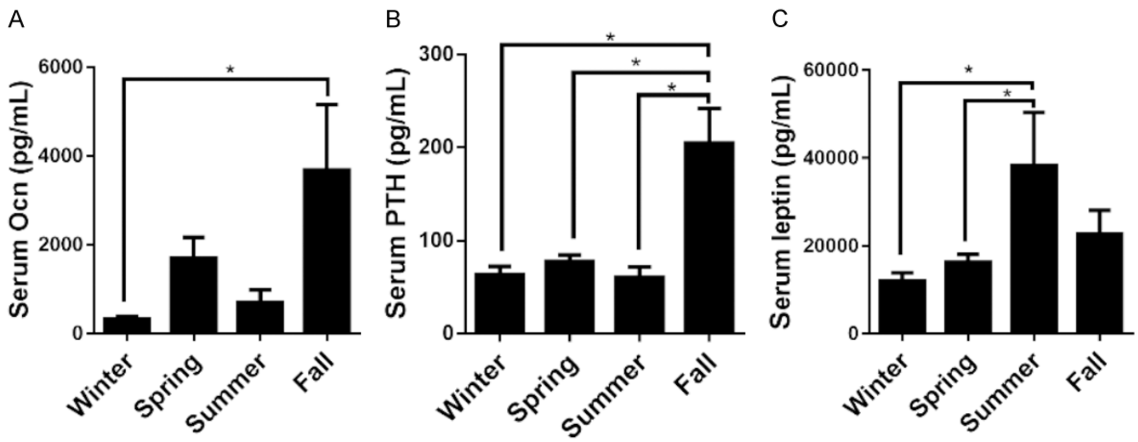

Figure 4. A. Serum Ocn level of patients from different seasons. B. Serum PTH level of patients from different seasons. C. Serum leptin level of patients from different seasons. (Data were adjusted for age and gender and were compared across seasons with a one-way ANOVA test. *Means a significant difference, p<0.05).

vs. fall, p=0.0424) (**Figure 3B**). It has been reported that OPN regulates expression of various inflammatory factors associated with the pathogenesis of OA, including matrix metalloprotease 13, interlukine-6 and 8 [17]. In our study, serum OPN level of the summer group peaked in all groups, while the winter group presented the lowest level (winter vs. spring, p=0.0109; winter vs. summer, p=0.0130; spring vs. fall, p=0.0349; summer vs. fall, p=0.0305) (**Figure 3C**). In contrast with serum OPN, serum OPG levels of the winter group and the fall group turned out higher than that of the spring group and summer group (winter vs. spring, p=0.0227; spring vs. fall, p=0.0164; summer vs. fall, p=0.0432) (**Figure 3D**).

Preoperative serum hormones of patients recruited in different seasons

To investigate the variance of serum hormones in the participants before TJA, three hormones (OC, leptin, and PTH) mostly closely linked with bone metabolism were chosen as the indicators. OC was an essential hormone related to bone turnover and glucose homeostasis [18]. In this study, serum OC levels topped in the fall group, while the summer group and winter group exhibited lower levels (winter *vs.* fall, p=0.0193) (**Figure 4A**). A similar pattern emerged in serum PTH level, which peaked in the fall group (winter *vs.* fall, p<0.0001; spring *vs.* fall, p<0.0001; summer *vs.* fall, p<0.0001= (**Figure 4B**). Serum leptin level of the summer group peaked distinctly among all groups, where the level of the fall group was higher than that of the spring group and winter group (winter *vs.* summer, p=0.0015; spring *vs.* summer, p=0.0039) (**Figure 4C**).

Discussion

In the present study, we found a noticeable seasonal variance of serum biomarkers in OA patients, namely that TNF-α and DKK1 levels were significantly higher in the winter group, IL-6 level peaked in the spring group, OPN and leptin levels reached the maximum in the summer group, and SOST, OCN, PTH levels were at their highest in the fall group.

Previous studies have looked into different inflammatory responses during different seasons [19]. It has also been reported that TNF-α is not only related to inflammatory pain, but also correlated with bone metabolism [5]. Our results showed that participants recruited in winter had the highest serum TNF-α level (**Figure 2A**), and those recruited in spring had a much higher IL-6 level, while IL-1β level showed no significant difference in OA patients from different seasons (**Figure 2B**, **2C**). This might suggest that OA inflammatory progression was affected by seasonal factors, and more attention should be paid to the pain management of OA patients during winter.

Numerous researches have indicated that DKK1 and SOST exert important effects on OA pathogenesis as a result of their role as antagonists in the Wnt signaling pathway. Scientists have reported that in hip arthritis patients with subchondral sclerosis, down-regulated serum DKK1 level is closely associated with an increased risk of joint damage [20], and that SOST participates in cartilage degradation [8]. Interestingly, it was found in our study that serum DKK1 level in winter was relatively higher compared with the other three seasons, while serum SOST level in winter was comparably lower. In summer, on the contrary, DKK1 level was relatively lower while SOST level was higher. This may indicate that DKK1 and SOST have a complementary biological effect on the metabolism of bone and cartilage through the Wnt pathway during seasonal transitions. Thus OA patients should reduce their activities in summer in case of arthrodial cartilage deteriorates.

On the homeostasis level, researchers have discovered that OCN acts as the link connecting bone homeostasis and energy metabolism [11], and that PTH participates in bone metabolism via various mechanisms [12]. Also, leptin has been known as both a regulator of energy homeostasis and a bone metabolism regulator [13]. Our study found a higher OCN and PTH level in the fall group. However, the highest leptin level occurred in the summer group while the second highest level occurred in the fall group, indicating that the season of fall might provide a better state for bone metabolism and energy homeostasis. This suggests that fall is likely to be the optimal time for a total joint replacement procedure, as the more active bone and energy metabolism in fall is better for recovery.

It should be noted that certain limitations exist in this study. Firstly, as the concentrations of some biomarkers are related to physical activities or the percentage of body fat, potential confounding variables may exist. Therefore, the data were adjusted according to participants' age and gender. As for physical activities, patients included in this study were those with unbearable pain or limited motion ranges who could barely do physical exercises, thus eliminating the factor. In addition, we believe that the influence of the confounding factors is not as much as it seems. Secondly, given the tension between patients and medical workers, collecting blood samples of the same patient from different seasons was not allowed. As a result, we only had access to data during the patient's stay in the hospital, and the study relied solely on patients' preoperative blood samples. For the next steps, we intend to continue with a long-term follow-up study focusing on the prognoses.

In conclusion, our study is the first to find potential seasonal changes in serum biomarkers in an Asian cohort of OA patients, which may provide new insights into the treatment of OA and help us gain a more extensive understanding of OA pathogenesis, highlighting the influence of seasonal changes on OA progression.

Acknowledgements

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Disclosure of conflict of interest

None.

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