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Concentrations of CTX I, CTX II, DPD, and PYD in the urine as a biomarker for the diagnosis of temporomandibular joint osteoarthritis: A preliminary study

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Abstract

Objectives—The aim of this study was to identify a marker for temporomandibular joint (TMJ) osteoarthritis (OA) diagnosis by comparing the concentrations of urinary pyridinoline (PYD), deoxypyridinoline (DPD), and C-terminal telopeptides type I collagen (CTX-I), and CTX-II of TMJ OA patients with those of a non-symptomatic group.

Methods—PYD, DPD, CTX-I, and CTX-II concentrations in the urine of 36 non-symptomatic subjects and 31 TMJ OA subjects were analyzed.

Results—The differences for only PYD and DPD were significant. In ROC analysis, PYD and DPD showed higher sensitivity and specificity than CTX-I and CTX-II. PYD and DPD concentrations in urine were significantly increased in TMJ OA patients and can therefore be used as a biomarker in the supplementary clinical diagnosis of TMJ OA.

Discussion—The findings suggest that measurement of their concentration can be a supplementary method for clinical diagnosis of TMJ OA.

Keywords

Temporomandibular joint; osteoarthritis; erosion; urine; biomarker

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Conflict of interest

The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.

Introduction

Temporomandibular joint osteoarthritis (TMJ OA) is a disease characterized by deformation of the articular surface between the condylar head and mandibular fossa caused by increased joint loading [1–5]. In the initial stages of TMJ OA, chondromalacia occurs on the articular surface. This is then followed by subchondral bone resorption [6], and if progressive bone change continues, the subchondral cortical layer eventually disappears and is accompanied by erosion of the bone [2]. Radiographic examination, along with clinical tests, is essential for TMJ OA diagnosis. Recently, cone-beam computed tomography (CBCT) has been widely used for investigating the changes in bones in the TMJ region [7]. However, detection of bony changes in the TMJ upon radiographic examination indicates that the pathological changes have already occurred to some degree. It is difficult to identify cartilage loss or other initial bone changes only through radiographic findings. Moreover, it is even more difficult to evaluate whether the progress of bone change is ongoing [2,8–10].

With regard to this aspect, a biomarker can play an important role in evaluating the severity and activity of the degeneration in cartilage and bone for diagnostic as well as prognostic decisions. The identification of a biomarker will provide a measurable indicator for TMJ OA diagnosis in the early stages and will help monitor the treatment process. Previously, Vos et al. [11] used carboxyterminal telopeptides of collagen type I and II (CTX-I and CTX-II), cartilage oligomeric matrix protein (COMP), and prostaglandin E2 (PGE2) from the synovial fluid as a diagnostic marker to identify degradation. They found that CTX-II was abundant in the synovial fluid of the TMJ OA patients [12]. In another study, Tanimoto et al. [13] suggested that the pyridinoline (PYD)/deoxypyridinoline (DPD) ratio could be used as a useful indicator for initial differential diagnosis of TMJ OA. The levels of PYD, DPD, CTX-I, and CTX-II in urine can be measured using minimally invasive techniques and can be used for TMJ OA diagnosis and for assessing the severity of the disease [14,15]. Therefore, the purpose of this study was to identify a valuable marker for TMJ OA diagnosis by comparing the concentrations of PYD, DPD, CTX-I, and CTX-II in the urine of TMJ OA patients with those of non-symptomatic controls.

Materials and methods

Subjects

Thirty-one subjects (8 males: mean 29.3 ± 6.9 years, 23 females: mean 29.9 ± 9.0 years) diagnosed with osteoarthritis (OA) who visited Pusan National University Dental Hospital with temporomandibular joint disorder (TMD) as the chief complaint from January 2014 to December 2014 and 36 healthy subjects (21 males, mean age: 32.3 ± 4.4 years; 15 females, mean age: 27.8 ± 0.6 years) were enrolled for this study. The screening of the OA patients was conducted by a trained specialist, based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) [5]. The exclusion criteria were systemic osteoarthritis, rheumatoid arthritis, any bone metabolic disease, endocrine disorders, and medications for inflammation. Before the study, informed consent was obtained from all subjects. This study was approved by the institutional review board of Pusan National University Dental Hospital (PNUDH-2013–038).

TMJ OA diagnosis

Patients who showed the symptoms listed in Table 1 were selected as the TMJ OA group, based on RDC/TMD [5]. In particular, patients who showed clear erosion in the radiological images were selected as the OA group in this study.

Urine sample collection and storage

First morning urine samples were collected from all subjects and stored at -70°C until analysis. The samples were centrifuged at 2000 g for 10 min at 4°C prior to analysis, and the supernatant was used for the analysis. The levels of PYD, DPD, CTX-I, and CTX-II concentrations were corrected for urinary creatinine.

Measurement of urinary CTX-I and CTX-II concentrations

Urine CrossLaps[®] EIA and Urine CartiLaps[®] EIA kits (IDS Nordic Bioscience, Herlev, Denmark), respectively, were used for the quantification of the degradation products of C-terminal telopeptides of Type I (CTX-I) and II collagen (CTX-II) in the urine samples. CTX-I and CTX-II levels were measured at 450 nm using absorbance at 650 nm as the reference on a spectrophotometer (Perkin Elmer, Santa Clara, CA, USA), according to the manufacturer's protocol. The concentrations of CTX-I and CTX-II were corrected for urinary creatinine, which was measured enzymatically using a kit purchased from Cayman Chemical Company (Ann Arbor, MI, USA). All the assays were performed in duplicate. The formula for calculating the concentrations were as follows: corrected CTX-I value (ng/mmol) = CrossLaps ($\mu\text{g/liter}$)/creatinine (mmol/liter), and corrected CTX-II value (ng/mmol) = $1000 \times \text{CartiLaps} (\mu\text{g/liter})/\text{creatinine (mmol/liter)}$. The intra assay and inter assay coefficients of variations (CVs) for CTX-I and II were less than 6 and 10%, respectively.

Measurement of urinary PYD and DPD concentrations

Pyridinium cross-links and the PYD and DPD cross-links were measured using the MicroVue PYD[™] EIA and DPD[™] EIA kits (Quidel Corp., San Diego, CA, USA), respectively. All the assays were performed in duplicate. Urinary PYD and DPD levels were corrected for urinary creatinine. The formula for calculating the concentration was as follows: corrected PYD or DPD (nmol/mmol creatinine) = PYD or DPD (nmol/liter)/creatinine (mmol/liter). The intra assay and inter assay CVs for PYD were 9.9 and 11.2%, respectively, and those for DPD were 8.4 and 4.8%, respectively.

Measurement of urinary creatinine levels

The creatinine urinary colorimetric assay (Cayman Chemicals) was used to measure creatinine levels in the urine samples. All the assays were performed in duplicate. The samples were diluted 1:10 with HPLC-grade water. The creatinine levels were determined enzymatically at 500 nm on a spectrophotometer (Perkin Elmer). The assay relied on Jaffe's reaction. The results obtained were standardized to milligrams of urinary creatinine, and the final concentrations in the adjusted samples were determined using linear regression.

Statistical analysis

Differences in the levels of PYD, DPD, CTX-I, CTX-II, and PYD/DPD between the non-symptomatic group and the OA group were compared using independent *t*-test. Pearson correlation coefficients were calculated to analyze the correlation between PYD and DPD. Receiver operation characteristics (ROC) and the cutoff values were calculated to determine the sensitivity (true positive rate) and specificity (false positive rate) of PYD and DPD as OA biomarkers. All the statistical calculations were done using R-programming.

Results

In the independent *t*-test for each component, PYD levels in the non-symptomatic group (mean = 25.3 ± 10.0 mmoles/liter) showed a statistically significant difference ($p < 0.05$, Table 2) from those in the OA group (mean = 32.9 ± 17.0 mmoles/liter). The mean DPD level in the non-symptomatic and TMJ OA groups was 5.0 ± 2.0 mmoles/liter and 6.5 ± 2.8 mmoles/liter, respectively ($p < 0.05$, Table 2), showing a statistically significant difference (Figure 1). The mean PYD/DPD in the non-symptomatic and OA groups were 5.1 ± 1.4 and 5.1 ± 1.1 , respectively, but the difference was not significant ($p > 0.05$, Table 2) (Figure 1). The difference of CTX-I between the two groups (mean: non-symptomatic group = 148.7 ± 65.3 ng/mmol and OA group = 171.5 ± 94.3 ng/mmol) was not significant ($p > 0.05$, Table 2). CTX-II in both the non-symptom group (mean = 278.5 ± 156.2 ng/mmol) and the OA group (mean = 392.7 ± 365.0 ng/mmol) had no statistically significant difference either ($p > 0.05$) (Figure 1). AUC (area under the ROC curve) for PYD was 0.691 with a cut-off value of 23.0 mmoles/liter (Figure 2). AUC for DPD was 0.693 with a cut-off value of 5.0 mmoles/liter. For CTX-I and CTX-II, the AUCs were 0.561 and 0.532, respectively, with cut-off values of 195.53 and 286.57 ng/mmol, respectively. The correlation co-efficient between PYD and DPD was defined as $PYD = 5.35 + 4.05 \times DPD$, in which the two factors showed a correlation of $R^2 = 0.85$ (Figure 3).

Discussion

In this study, the urine concentrations of different biomarkers were evaluated to determine their diagnostic values for TMJ OA and to determine their efficacy in the evaluation of the recovery of patients with bone erosion induced by bone remodeling in OA of the TMD. Type II collagen exists in the condylar cartilage. CTX-II, the specific biomarker for the turnover of type II collagen, is useful for diagnosing cartilage changes [7,10]. Sarukawa et al. [16] reported that CTX-II was effective for the diagnosis and monitoring of OA in an animal study. Reijman et al. [15] also showed that CTX-II was associated with both radiographic OA results at the hip and knee. Similar to other joints in the body, the TMJ consists of a bone, cartilage, and synovial membrane. Vos et al. [11] showed that concentrations of CTX-II and CTX-I in the synovial fluid of OA patients were higher than those in non-symptomatic TMJs. The potential of CTX-II as an initial diagnostic marker of TMJ OA has been suggested previously.

However, in this study, the CTX-I concentration in the urine was higher in the OA group (mean = 171.5 ± 94.3 ng/mmol) than in the non-symptomatic group (mean = 148.7 ± 65.3 ng/mmol), but the difference was not statistically significant ($p > 0.05$). CTX-II

concentration in urine was also higher in the OA group (mean = 392.7 ± 365.0 ng/mmol) than in the non-symptom group (mean = 278.5 ± 156.2 ng/mmol), but the difference in this case was not significant either ($p > 0.05$). The reason for this may be that CTX-II is the main biomarker of cartilage, while the subjects in this study were patients with TMJ erosion who showed degradation and remodeling of the bone [17].

PYD and DPD are crosslinks between mature collagen fibers and can be detected in various tissues; these can be used as markers for bone metabolism. In addition, both PYD and DPD are not metabolized *in vivo* and are excreted in the form of urine. Urinary PYD and DPD levels have already been verified to be effective as a factor for the diagnosis of arthritis of the knee and hip joints; however, the association between these two factors and TMJ arthritis is insignificant. Besides, for TMJ OA diagnosis, there are few studies on urinary PYD, DPD, and CTX-II levels and on the association between the clinical and radiographic findings.

PYD and DPD levels in the urine showed a significant correlation with radiological OA erosion findings in this study ($R^2 = 0.85$, $p < 0.05$). This is because PYD and DPD, as the main biomarkers of the bone, reflect TMJ erosion, which in turn reflects the degradation and remodeling of bone; thus, these components can serve as effective markers of OA [17].

Meanwhile, Tanimoto et al. [13] suggested that the PYD/DPD ratio could be used as a useful indicator in the initial differential diagnosis of TMJ OA. In this study, the correlation coefficient between PYD and DPD was found to be high ($R^2 = 0.85$), but the PYD/DPD ratio was not significantly different between the two groups ($p < 0.05$). DPD is mostly present in the bones, and PYD is present in both the bone and the cartilage. In the study by Tanimoto et al. [13], the control group and the patient group showed no difference in DPD levels, but the patient group showed twice as much PYD as the control group, indicating a high PYD/DPD ratio in the patient group. This study indicated that PYD levels were much higher than those in the control group. In other previous studies, a high PYD/DPD ratio was considered to be a result of higher levels of PYD than those of DPD in the cartilage, which causes a sharp increase in bone degradation [13,18,19]. In this study, DPD and PYD were higher in the TMJ OA patient group than in the control group, but the PYD/DPD ratio was not different. This was due to the fact that this study design was aimed at patients with early TMJ OA. PYD in early TMJ-OA patients was higher than in the control group, but it did not show a sharp increase. Based on the AUC results of the ROC, PYD (AUC = 0.691) and DPD (AUC = 0.693) showed higher sensitivity and specificity as biomarkers of the patients who showed bone remodeling-like erosion among the TMJ OA patients. Therefore, PYD and DPD were considered more efficient indicators than CTX-I (AUC = 0.561) and CTX-II (AUC = 0.532). The cutoff values for PYD (23.0 mmoles/liter) and DPD (5.0 mmoles/liter) are indicators of the diagnostic value of these biomarkers in TMJ OA patients; these biomarkers could also effectively help monitor treatment outcomes in these patients. Hosnijeh et al. [17] also reported that the urine biomarker that appears in remodeling of cartilage and bone shows a lower detecting threshold than diagnosis based on a radiological image. In addition, the precise values of these biomarker concentrations enable more detailed classification than is possible in existing methods of clinical diagnosis based on signs and symptoms of the patient and radiological images [14]. However, one limitation of these biomarkers is that the urinary concentrations reflect the general condition of the body

and that TMJ has a smaller area compared to other joints in the body. Therefore, in future studies, the stages of TMJ OA must be classified and evaluated by measuring factors in different symptomatic stages and by comparing them to radiographic findings in order to determine the concentrations of the biomarkers specific to the stages of TMJ OA. Furthermore, for clinical application, a larger sample size is required, and the study must involve the measurement and comparison of changes in biomarker levels following improvement of symptoms from treatment.

Conclusion

On the basis of the current study's finding, PYD and DPD concentrations in the urine were significantly higher in TMJ OA patients. PYD and DPD levels in the urine showed a significant correlation with radiological OA erosion findings in this study ($R^2 = 0.85$, $p < 0.05$). The cutoff value of PYD was 22.0 mmole/liter and that of DPD was 5.0 mmole/liter.

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Figure 1.

(a) Concentration of pyridinoline (PYD) in the non-symptomatic and OA groups. (b) Concentration of deoxypyridinoline (DPD) in the non-symptomatic and OA groups. (c) Concentration of C-terminal telopeptides type I collagen (CTX-I) in the non-symptomatic and OA groups. (d) Concentration of C-terminal telopeptides type II collagen (CTX-II) in the non-symptomatic and OA groups.

*Differences were statistically significant at $p < 0.05$. N.S.: not significant.

Figure 2.

Optimal cutoff level by ROC analysis. (a) Pyridinoline (PYD) level in the non-symptomatic ($n = 36$) vs. OA group ($n = 31$). AUC = 0.691, cutoff value is 23 mmol/L. (b) Deoxypyridinoline (DPD) level in the non-symptomatic ($n = 36$) vs. OA group ($n = 31$). AUC = 0.693, cutoff value is 5 mmol/L. (c) C-terminal telopeptides type I collagen (CTX-I) level in the non-symptomatic ($n = 36$) vs. OA group ($n = 31$) AUC = 0.561, cutoff value is 193.53 ng/mmol. (d) C-terminal telopeptides type II collagen (CTX-II) level in the nonsymptomatic ($n = 36$) vs. OA group ($n = 31$) AUC = 0.532, cutoff value is 286.97 ng/mmol.

Figure 3.
Correlation between pyridinoline (PYD) and deoxypyridinoline (DPD) values.
Note: R^2 is 0.847. $PYD = 5.35 + 4.05 \times DPD$ ($p < 0.001$).

Table 1

Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) for osteoarthritis of the temporomandibular joint (TMJ).

Parameters	RDC/TMD: Osteoarthritis
Diagnostic signs	No signs and symptoms of joint pain (arthralgia) <ul style="list-style-type: none"> • Coarse crepitus from affected TMJ, or • Positive imaging findings
Findings or comments	There is no pain on function, loading, or palpation
Imaging	Tomograms show: <ul style="list-style-type: none"> • Cortical erosion, or • Sclerosis, or • Flattening, or • Osteophytes
Authors' comments	As discussed in the text, the authors opt not to use osteoarthritis, since patients with osteoarthritis (OA) may be labile and frequently fluctuate between symptomatic and asymptomatic states. This probably reflects levels of inflammatory mediators within the joint and not separate diagnoses. The authors thus recommend the use of OA as a diagnosis with additional qualification as to the presence of pain or dysfunction as relevant

Table 2

Concentrations of pyridinoline (PYD), deoxypyridinoline (DPD), PYD/DPD, C-terminal telopeptides type I collagen (CTX-I), and CTX-II in the urine samples of both the non-symptomatic and osteoarthritis (OA) group.

Groups	PYD (mmoles/liter)		DPD (mmoles/liter)		PYD/DPD		CTX-I (ng/mmol)		CTX-II (ng/mmol)	
	Non-symptom	OA	Non-symptom	OA	Non-symptom	OA	Non-symptom	OA	Non-symptom	OA
Subjects	36	31	36	31	36	31	36	31	36	31
Mean $\pm$ standard deviation	25.3 $\pm$ 10.0	32.9 $\pm$ 17.0	5.0 $\pm$ 2.0	6.5 $\pm$ 2.8	5.1 $\pm$ 1.4	5.1 $\pm$ 1.1	148.7 $\pm$ 65.3	171.5 $\pm$ 94.3	278.5 $\pm$ 156.2	392.7 $\pm$ 365.0
<i>p</i> -value	<0.05*		<0.05*		>0.05		>0.05		>0.05	