IJMPR 2022, 6(5), 43-50

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

N-TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN (NTX), AS A BIOMARKER FOR OSTEOARTHRITIS PATIENTS

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Received on: 21/04/2022
Revised on: 11/05/2022
Accepted on: 31/05/2022

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ABSTRACT

Background: The early diagnosis of osteoarthritis is required to do possible intervention in early stage of disease because it is one of the leading causes of disability in advance stage. **Aim:** For this reason, this study aimed to investigate the relationship between the various biomarker anti-cyclic citrullinated peptide (anti-CCP) levels, Serum concentrations of N-terminal telopeptide of type I collagen (NTX), as bone resorption marker, alkaline phosphatase (ALP) and osteocalcin (OC) as bone formation markers, vitamin D, Calcium and phosphrous.in patients with osteoarthritis (OA). **Methods:** ninety patients with AO were included in the study. Enzyme-linked immunosorbent assay (ELISA) was used to detect Anti-CCP antibodies, NTX, OC and vitamin D. C reactive protein (CRP), phosphorus total and ionized calcium. **3.1. Age and Gender Distribution:** There was statistically significant difference between OA group and that of healthy group in addition, OA group included 70 cases of females but control group included 20 cases of males and females, there was statistically significant difference between two groups. **Results:** Among cases with negative anti-CCP group, NTX showed a significant positive correlation with OC, Anti-CCP, ALP, Phosphorus and a negative correlation with Vitamin D, Total calcium and CRP. Moreover, OC showed a significant positive correlation with Anti-CCP, ALP, Phosphorus, While the negative correlation appeared significantly with Vit D, total and ionized calcium. Among cases with positive anti-CCP group, NTX showed a significant negative correlation with osteocalcin. Osteocalcin correlates negatively with vitamin D and ionized calcium. Patients in the highest of serum NTX concentrations showed the fastest rate of bone loss. **Conclusion:** Serum NTX may provide a clinically relevant serum assay to estimate bone turnover in OA patients.

KEYWORDS: Osteoarthritis - Bone turnover- Bone formation - anti-CCP- NTX – Biomarkers OC (osteocalcin) – Alp (alkaline phosphatase) – Crp (c- reactive proteins)

1. INTRODUCTION

Osteoarthritis is the most frequent musculoskeletal disease and leads to functional decline and loss in quality of life. Osteoarthritis manifests as alteration of the whole joint structure, including progressive degradation of cartilage and ligaments, synovial inflammation and changes to the subchondral bone **(Mobasheri and Batt 2016)**. Clinically, the condition is characterized by joint pain, tenderness, crepitus, stiffness and limitation of movement with occasional effusion and variable degrees of local inflammation **(Villafañe 2018).**

The National Institutes of Health (NIH) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" **(García-Estañ and Vargas 2020)**. The measurement of biochemical markers in blood, urine or synovial fluid samples is an attractive alternative, which could reflect dynamic and quantitative changes in joint remodeling and therefore disease progression **(Roberts, Law, and Thom 2019)**. In the setting of osteoarthritis, a biochemical marker could be either an effector molecule (ie, an operator of joint damage), the result of joint damage, or both **(Lotz et al. 2013).** Such biomarkers may be useful in early phase evaluation of the efficacy and safety of drugs and may also find applications in the diagnosis of disease, the assessment of severity and the risk of progression and the monitoring of health status in the general population**(Nguyen et al. 2017).**

Biochemical markers of bone formation and resorption constitute an evolving noninvasive and convenient tool for the assessment of bone turnover and status in various metabolic bone diseases **(Bhattoa 2018)**. Bone formation

markers include bone-specific alkaline phosphatase (ALP), which leaks from the osteoblast plasma membrane; osteocalcin, a bone matrix protein produced by osteoblasts; and procollagen Type I telopeptides (which are cleaved during collagen I formation) **(Shetty et al., 2016).** The bone resorption marker (bone turnover) assays detect the deoxypyridinoline crosslinks or the N- or C-terminal telopeptide fragments of collagen Type I, released during osteoclastic bone resorption **(Macías et al. 2020)**. Previous cross-sectional studies have used these markers in studying bone disease. Some of them reported an increase in markers of bone resorption without a compensatory increase in formation markers **(Fisher et al. 2018)**. Biomarkers provide useful diagnostic information by detecting cartilage degradation in osteoarthritis, reflecting disease-relevant biological activity and predicting the course of disease progression **(Bernotiene et al. 2020)**. Although several studies are underway, currently there are no reliable, quantifiable and easily measured biomarkers that provide an earlier diagnosis of osteoarthritis, information on the prognostic of disease and which can monitor responses to therapeutic modalities **(Bernotiene et al. 2020; Fisher et al. 2018).**

Type I collagen is the principal collagen in the skeletal system, accounting for ∼90% of the organic chemical constituents of bone **(Mahsut Dinçel 2019).** As one of the degradation products of collagen, cross linked N-telopeptide of type I collagen (NTX) is released from bone into the blood when bone is resorbed and is subsequently drained by the kidney into the urine **(Aruna, 2016)**. NTX is a bone marker which have not been widely used in the clinic for predicting bone metastases, although they can be helpful in monitoring the course of patients with bone metastases **(Macías et al. 2020)**. Bone pain in particular may be correlated with rising levels of N-telopeptide in the serum and urine and dosages of bisphosphonates may be adjusted to reduce the bone marker levels and sometimes to relieve bone pain **(Paterson 2015)**. Bone resorption markers are assumed to be superior to bone formation markers for predicting future changes in bone mass **(Park et al. 2019)**.

This study aimed to investigate the possible association between NTX as a bone resorption marker in OA patients, compared with other established markers bone formation (e.g: alkaline phosphatase and osteocalcin (OC)), and other serum biomarkers (e.g.: Calcium, phosphorus, and vitamin D) by examining the correlation of NTX with other markers in osteoarthritis patients.

2. MATERIAL AND METHODS

Information about the purpose of the study was provided to all the participants and written consent was obtained from all those enrolled into the study. The study was performed with the approval of the El- Dawley hospital (in Mansoura city) ……. ….. University (Acceptance No……..).

2.1. Subjects

The study comprised …Postmenopausal… patients aged 50…. ± …90… years …..., admitted to the ……, Damietta …...University, Egypt., due to osteoarthritis and qualified for surgery. The osteoarthritis was diagnosed based on medical history, physical examination.

The studied groups were denoted as follows

Control: the control group without regard to gender; male control subjects; female control subjects.

Patients: Osteoarthritis patients without regard to gender; was categorized to Anti-CCP-positive patients and anti-CCP-negative patients.

2.2. Biochemical determinations

A sample of blood was drawn from each participant for biochemical determinations Assays for serum calcium, and phosphate were performed on a 950 Virto's analyzer (Ortho-Clinical Diagnostics, Johnson and Johnson, Rochester, NY, USA). The analyzer uses dry chemistry slide technology and reflection spectrophotometry **(Musik et al. 2019)**. Serum total alkaline phosphatase was measured using an automated analyzer (Virto's ALKP Slides; Johnson and Johnson) **(Hunter et al. 2003).** Vitamin D was detected by Human 1,25-hydroxyvitamin D ELISA Kit (Cat. No E1919Hu) (Bioassay technology laboratory, Shanghi-China.) Serum CRP was measured by Elisa kit which is an open-reagent system that utilizes spectrophotometry to analyze samples. Serum total osteocalcin was measured with human osteocalcin/bone Gla protein ELISA kit.

NTX was measured by Human cross-linked N-telopeptide of type lcollagen ELISA kit (Bioassay technology laboratory). Anti-CCP antibodies were measured by enzyme-linked immunosorbent assay (ELISA; AIDA autoimmune diagnostic assays RA/CP Detect, Ref 10165, Germany) and considered positive at a cut-off value w15 U/mL, as suggested by the manufacturer.

3. RESULTS

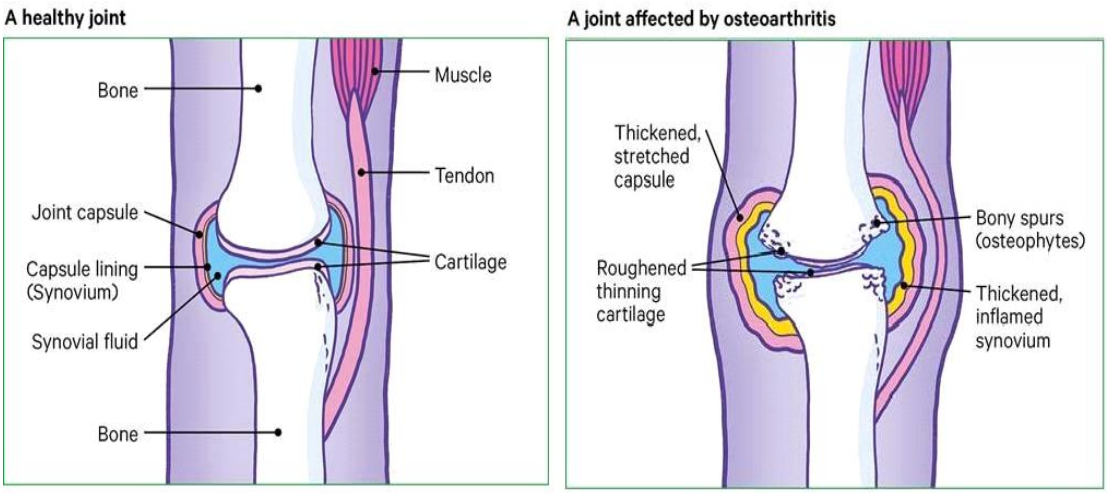
3.2. Anti-CCP

Anti-CCP antibody was positive in 26 out of 70 cases in the study group, and was negative in 42 out of 70 cases in the study group. In case of control group, the picture was different as anti-CCP antibody was negative in 20 (100%) out of 20 cases in this group. When the serological levels of anti-CCP antibody in the study group and control group was compared it was found to be significant (P = 0001).

3.3. CRP

CRP was positive in 23 out of 70 cases in the study group, and was negative in 47 out of 70 cases in the study group. In case of control group, the picture was different as CRP was positive in 7 out of 20cases in this group. CRP was negative in 14 out of 20cases in the

control group. When the serological profile of CRP in the Anti-CCP positive group and control group was compared it was found to be significant (*P* = 002) **(Figure 1)**.



Moreover, when the Anti-CCP negative group and Anti-CCP negative group was compared it was found to be significant. (P = 001), while non-significant results were detected between control group and anti-CCP negative group (*P*=0.339).

3.4. Vitamin D determination

Negative anti-CCP patients showed a significant decrease of vitamin D (*P*=0.003), compared to control subjects. Moreover, positive anti-CCP patients showed a statically significant reduction in vitamin D levels compared to control subjects (*P*= 0.001). Vitamin D levels were significantly higher in negative anti-CCP compared to positive anti-CCP patients (*P*= 0.001).

3.5. Total and ionized calcium

Negative anti-CCP patients showed a significant increase of total calcium levels and non-significant reduction in ionized calcium (P <0.869) compared to control subjects. While, positive anti-CCP patients showed a statically significant reduction in total calcium and ionized calcium levels (*P* <0.001, *P*=0.071; respectively), compared to control subjects. There was non-significant variation of total and ionized calcium levels in the negative anti-CCP patients (*P*=0.482, 0.08; respectively), compared to positive patients.

3.6. Phosphorus

Negative anti-CCP patients showed a significant increase of Phosphorus levels (*P*<0.001), compared to control subjects. Moreover, positive anti-CCP patients showed a statically significant elevation in Phosphorus levels compared to Control subjects (*P*<0.001). The results of Phosphorus were significantly (P<0.001) higher in positive anti-CCP patients compared to negative patients.

3.7. Alkaline phosphatase

Negative anti-CCP patients showed a significant increase of alkaline phosphatase levels (*P* <0.001), compared to control subjects. Moreover, positive anti-CCP patients showed a statically significant elevation in alkaline phosphatase levels compared to Control subjects (*P*<0.001). The results of ALP were significantly (*P*<0.001) higher in positive anti-CCP patients compared to negative patients.

3.8. Osteocalcin

They were significant elevation of osteocalcin levels in both anti-CCP positive and negative patients (*P*=0.001), relative to control group. Moreover, a significant elevation was detected in anti-CCP positive patients compared to anti-CCP negative patients (*P*=0.002).

3.9. Human cross linked N-telopeptide of type l collagen (NTX)

Negative and positive anti-CCP patients showed a significant increase of **NTX** levels (*P* <0.001), compared to control subjects. While there was non-significant variation in NTX level among negative and positive Anti-CCP cases.

4. DISCUSSION

Anti-cyclic citrullinated peptide (anti-CCP) antibody testing is particularly useful in the diagnosis of osteoarthritis, with high specificity, presence early in the disease process, and ability to identify patients who are likely to have severe disease and irreversible damage **(Taylor et al. 2011).** However, its sensitivity is low, and a negative result does not exclude disease. Immunization against citrullinated proteins is a feature almost unique for osteoarthritis, although ACPA may occur long before the onset of symptoms **(de Brito Rocha, Baldo, and Andrade 2019)**. Even if the presence of ACPA does not seem to reveal a distinct arthritis phenotype at symptom onset, it predicts an aggressive disease course with unfavorable outcome **(de Brito Rocha et al. 2019; Skogh 2005)**.

Despite the very high diagnostic specificity osteoarthritis, ACPA-positivity does not always accord

with a traditional diagnosis of osteoarthritis at clinical presentation. However, even when these patients are judged to suffer from mild undifferentiated arthritis, they call for follow-up **(Skogh 2005; Ziegelasch 2019).** The existence of the anti-CCP antibody in non-OA patients, such as those with autoimmune hepatitis **(Vannini et al. 2006),** tuberculosis **(Kakumanu et al. 2008)**, and systemic lupus erythematosus (SLE) **(Kakumanu et al., 2009)**, is not dependent on citrullination. The anti-CCP-positive sera of patients may also react with cyclic arginine peptides (CAP), in which the citrulline residues of CCP peptides are substituted with arginine residues **(Iwasaki et al. 2020)**. In the present study, in osteoarthritis patients anti-CCP antibody was positive in (38.1%) and negative in (61.9%) of cases in the study group. In case of control group, anti-CCP antibody was negative in all cases in this group. When the serological levels of anti-CCP antibody in the study group and control group was compared it was found to be significant (P = 0001). Thus, osteoarthritis (OA) patient was categorized as negative anti-CCP patients and negative anti-CCP.

Biochemical markers of bone formation and resorption constitute an evolving noninvasive and convenient tool for the assessment of bone turnover and status in various metabolic bone diseases **(Kasiske et al. 2016).** Therefore, this study aimed to investigate the possible association between NTX as a bone resorption marker in OA patients, compared with other established markers bone formation (e.g.: alkaline phosphatase and osteocalcin (OC) and other serum biomarkers (e.g.: Calcium, phosphorus, and vitamin D).

OA co-exists frequently with vitamin D deficiency in older people. Vitamin D deficiency. Vitamin D has multiple biological functions in cartilage, bone and muscle by acting on vitamin D receptors, and may have beneficial **(Sirajudeen, Shah, and Al Menhali 2019).** The primary functions of vitamin D are calcium homeostasis and regulation of bone metabolism; and it has a wide range of effects on different cell and tissue types **(Veldurthy et al. 2016).** Acting via the vitamin D receptor (VDR), vitamin D regulates circulating calcium and phosphate homeostasis through altering kidney reabsorption and intestinal absorption **(Mabey and Honsawek 2015)**. Parathyroid hormone (PTH) and fibroblast growth factor- (FGF-) 23, a bone-derived phosphaturic hormone produced in the presence of active vitamin D, are also major players involved in the maintenance of these circulating ion levels **(Veldurthy et al. 2016)**. PTH is secreted by the parathyroid glands in response to low calcium levels and acts to stimulate active vitamin D synthesis. This is achieved by inducing the release of calcium into the circulation via increased bone turnover to prevent hypocalcaemia **(Khan, Jose, and Sharma 2020; Veldurthy et al. 2016).**
Low vitamin D may lead to vascular smooth muscle cell proliferation, endothelial cell dysfunction, vascular dysfunction and increased inflammation; all of these may play roles in the aetiology of OA **(Park, 2019)**. In the present study low levels of vitamin D were detected in both negative and positive anti-CCP patients compared to control subjects (P= 0.001). While, vitamin D levels were significantly higher in negative anti-CCP compared to positive anti-CCP patients. This was confirmed by the statically negative correlation between Anti-CCP and Vit D among cases with negative anti-CCP. Moreover, Osteocalcin correlates negatively with vitamin D in both positive and negative Anti-CCP patients. A previous cohort study demonstrated that higher serum levels of 25-(OH) D predicted reduced loss of cartilage volume over 2 years, and increases in 25-(OH) D levels were associated with further protective association **(Cao et al. 2013)**. Moreover, Vitamin D deficiency results in hypocalcemia and hypophosphatemia in addition to increase in PTH secretion **(Underland, Markowitz, and Gensure 2020).**

Calcium is an essential nutrient which plays a key role in regulating a great diversity of physiology processes, including muscle contraction, neurotransmitter release, endocrine and exocrine secretion, and blood clotting **(Li et al. 2016).** In vitro studies have investigated the pathophysiological mechanism of calcium on chondrocyte. For chondrocytes, calcium is involved in matrix synthesis, cytoskeletal remodeling, cell hyperpolarization, and cell death **(Suzuki et al. 2020).** In the present study, Negative anti-CCP (OA) patients showed a significant increase of total calcium levels and non-significant reduction in ionized calcium compared to control subjects. While, positive anti-CCP patients showed a statically significant reduction in total calcium and ionized calcium levels compared to control subjects. There was non-significant variation of total and ionized calcium levels in the negative anti-CCP patients compared to positive patients. Total calcium showed a significant negative correlation with NTX, osteocalcin while ionized calcium showed a significant negative correlation with osteocalcin in negative and positive anti-CCP.

The maintenance of appropriate phosphorus homeostasis is critical for the well-being of the organism and for an optimal calcium–phosphate product for the mineralization of bone without its deposition in vascular and other soft tissues **(Heron 2019; Penido and Alon 2012)**. In biological systems, phosphorus is present as phosphate, and these two terms are commonly used interchangeably.

In the present study, Negative and positive anti-CCP patients showed a significant increase of phosphorus levels, compared to control subjects. However, phosphorus levels were significantly higher in positive anti-CCP patients compared to negative cases. Moreover, correlation between CRP and phosphorus levels among control group (P<0.01). Phosphorus levels showed a positive correlation between NTX and osteocalcin among negative Anti-CCP patients. Additionally,

appositive correlation was detected with CRP and Anti-CCP among Anti-CCP negative patients.

Crucial to the activity of osteoblasts and osteocytes in the process in of matrix mineralization is the maintenance of adequate inorganic phosphorus levels. Inorganic phosphorus is one of the two main ionic components required for hydroxyapatite formation during the mineralization of the extracellular matrix **(Gomes et al. 2019)**. The enzymatic activity of alkaline phosphatase is required to generate enough of the free mineral. This enzyme is located at the plasma membrane with its catalytic unit oriented outside **(Penido and Alon 2012)**. The enzyme cleaves phosphorus from beta-glycerol phosphate, and the free mineral enters the cell via a sodium-dependent phosphate transporter and is maintained intracellularly within a narrow range **(Tiosano and Hochberg 2009)**. In cases of hypophosphatemia, alkaline phosphatase activity rises in order to try to provide more phosphate to the bone cells. Once adequate amounts of phosphate are provided, the enzyme's activity decreases; as such, it is a good indicator of phosphate homeostasis within the bone **(Khan et al. 2020).** In the present study, Negative and positive anti-CCP patients showed a significant increase of alkaline phosphatase levels compared to control subjects. However, ALP levels were significantly higher in positive anti-CCP patients compared to negative patients. This could have referred to the association between serum ALP level to low-grade inflammation, which may cause an inflammatory response in chondrocytes **(Seo et al. 2019)**. Emerging studies have demonstrated that serum ALP level is positively and independently associated with inflammatory markers such as CRP level and leukocyte counts **(Park et al. 2020)** . In the present study ALP was found to correlate positively with NTX and osteocalcin while a negative co relation was detected with Anti-CCP and Vit D among cases with negative anti-CCP group.

Serum CRP is perhaps the most widely used clinical marker of systemic inflammation. While some studies have reported elevated levels of systemic CRP among individuals with OA compared to controls **(Ciubotaru, Potempa, and Wander 2005; Ridker 2003)**, In the present study, CRP was positive in (49.3%) cases in the study group, and was negative in (50.77%). In case of control group, the CRP was positive in (36.8.%) of cases in this group. When CRP levels in Anti-CCP positive group and control group was compared it was found to be significant. (P = 002). Moreover, when the Anti-CCP negative group and Anti-CCP negative group was compared it was found to be significant., while non-significant results were detected between control group and anti-CCP negative group (P=0.339). Our results agreed with a recent meta-analysis, however, suggested that circulating levels of CRP were indeed elevated, though modestly, in OA patients as compared to healthy controls. CRP, one of the most useful markers of systemic inflammation, has recently been identified as a marker of OA with clinical significance as CRP levels are modestly elevated in patients with OA as compared with normal controls (Perruccio et al. 2017), The increased CRP levels have been associated with disease progression as well as with clinical severity **(Pearle et al. 2007; Sproston and Ashworth 2018)**.

Moreover, Within OA, this systematic review and meta-analysis also examined CRP in relation to the extent of OA symptoms, and reported overall weak but statistically significant positive correlation between CRP levels and pain scale scores **(Zhang 2018).** The authors conclude by suggesting that low grade inflammation may play a greater role in symptoms rather than radiographic changes in OA. Additionally, it has been reported that high-sensitivity CRP (hs-CRP) levels may be a prognostic feature of rapid progressive hip and knee OA **(Jin et al. 2015)**. In the present study, a negative correlation was detected between CRP and NTX among cases of negative anti-CCP group. Moreover, a positive significant correlation was detected among CRP and phosphorus levels among cases of positive anti-CCP group.

Osteocalcin is produced by osteoblasts, and is generally regarded as a marker of bone formation, but it seems to be involved in the process of mineralization rather than matrix production. Plasma concentrations of intact osteocalcin are markers of bone formation, and circulating concentrations generally correlate well with histological measures of bone formation rate **(Moser and van der Eerden 2018)**. Serum OC level is higher in conditions characterized by increased bone turnover, both physiological (rapid growth during puberty) and pathological (primary hyperparathyroidism, hyperthyroidism, Paget disease, acromegaly, bone metastases **(Szulc and Bauer 2013)**. By contrast, serum OC is decreased in hypoparathyroidism, hypothyroidism, Cushing disease, in glucocorticoid-treated patients and in some patients with multiple myeloma and malignant hypercalcemia **(Bhattoa 2018; Szulc and Bauer 2013)**.

In the present study, a significant elevation of osteocalcin levels in both anti-CCP positive and negative patients (P=0.001), relative to control group. Moreover, a significant elevation was detected in anti-CCP positive patients compared to anti-CCP negative patients. suggesting increased bone turnover in both positive and negative Anti-CCP patients. This agreed with another research, which demonstrated an increase in the OC mRNA in moderate and severe osteoarthritic lesions. Osteocalcin has a particular advantage in having greater sensitivity than ALP in the detection of low rates of bone formation. As with plasma ALP activity, plasma osteocalcin and growth rate are closely correlated. Plasma concentrations rise when osteoblasts are stimulated by the administration of 1,25(OH)**2D (García-Martín et al. 2011)**. There were in negative Anti-CCP patients osteocalcin showed a positive correlation with NTX, Anti-CCP, ALP and Phosphorus,

While the negative correlation appeared significantly with Vit D, total and ionized calcium on the other context, osteocalcin showed a negative correlation with NTX vitamin D and ionized calcium.

A central hallmark in OA pathogenicity is a gradual destruction of articular cartilage and local denudation leading to loss of joint function.9 The turnover of cartilage is normally maintained by a balance between catabolic and anabolic processes; however, in the case of pathological matrix destruction, the rate of cartilage. degradation exceeds the rate of formation, resulting in a net loss of cartilage matrix **(Mobasheri and Batt 2016)**. Type I collagen is degraded during the bone resorption process, liberating small fragments, such as NTX, β-CTX, PYD, and DPD, into the blood and then into the urine and making serum and urinary concentrations of these fragments available as biochemical markers of bone resorption **(Shetty et al. 2016.)**.

NTX as a bone resorption marker is a highly sensitive marker of bone resorption is cross-linked N-terminal telopeptide of collagen type I, also referred to as NTX. The assay is based on a monoclonal antibody raised against a peptide isolated from urine of a patient with Paget's disease of bone(Greenblatt et al. 2017). In vitro studies show that NTX can be quantitatively released from bone by the action of cathepsin K, a specific protease of the osteoclast. and good relationships with calcium kinetics and histomorphometry data on bone resorption have been shown for NTX in urine **(Lotz et al. 2013)**.

In the present study NTX was detected in serum of patient of OA. Negative and positive anti-CCP patients showed a significant increase of NTX levels (P <0.001), compared to control subjects. While there was non-significant variation in NTX level among negative and positive Anti-CCP cases reflecting the increase of bone turnover in osteoarthritis patients. this as confirmed by the significant positive correlation of NTX with osteocalcin, Anti-CCP, ALP, Phosphorus and the negative correlation with Vitamin D in negative Anti-CCP cases. Moreover, NTX showed a significant negative correlation with osteocalcin among in positive Anti-CCP cases.

CONCLUSION

Biochemical markers of bone formation and resorption constitute an evolving noninvasive and convenient tool for the assessment of bone turnover and status in various metabolic bone disease. Bone formation markers include bone-specific alkaline phosphatase (ALP), which leaks from the osteoblast plasma membrane; osteocalcin, a bone matrix protein produced by osteoblasts; and procollagen Type I telopeptides (which are cleaved during collagen I formation. The bone resorption marker (bone turnover) assays detect the deoxypyridinoline crosslinks or the N- or C-terminal telopeptide fragments of collagen Type I, released during osteoclastic bone resorption.

This study aimed to investigate the possible association between NTX as a bone resorption marker in OA patients, compared with other established markers bone formation (e.g.: alkaline phosphatase and osteocalcin (OC) and other serum biomarkers (e.g.: Calcium, phosphorus, and vitamin D) by examining the correlation of NTX with other markers in osteoarthritis patients.

In the present study, eighty patients with AO were included in the study. Enzyme-linked immunosorbent assay (ELISA) was used to detect Anti-CCP antibodies, NTX, OC and vitamin D. C reactive protein (CRP), phosphorus total and ionized calcium was also estimated.

Improved knowledge of the pathogenesis of OA and of its molecular and anatomic pathology and the wealth of information relating biomarkers of disease with clinical outcomes will permit a better means for the assessment of the effects of new therapeutic interventions in OA. Elevated serum NTx could be a useful predictor of bone loss in patients with OA.

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