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Relationship between Disease Activity and Circulating Level of Collagen II C-Telopeptide Fragments in Papain Induced Osteoarthritis Rat Model

Humaira Majeed Khan[1], Muhammad Ashraf[1], Abu Saeed Hashmi[2], Mansur-ud-Din Ahmad[2]* and Aftab Ahmad Anjum[2]

[1]Lahore College for Women University, Lahore; [2]University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
*Corresponding author: mansuruddin@uvas.edu.pk; mansuruddin@gmail.com

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ABSTRACT

Osteoarthritis (OA) is a progressive degeneration of articular cartilage leading to failure in functional mobility of joints. It is characterized by morphological, biochemical and molecular changes in histology of cartilage. Different biological markers are used as indicators to precisely predict the stage of cartilage destruction of joints in OA patients and to evaluate the therapeutic efficacy of drugs used for OA. The present research was chalked out to establish relationship between disease activity and serum level of C-terminal telopeptide of type II collagen (CTX-II) in experimentally induced OA rat model. Out of 30 male Wistar rats, 25 were used to induce OA by injecting papain (10mg/0.5mL of 0.05M sodium acetate) in right knee joints whereas five (control) were injected with sterile normal saline solution on day 0. Blood samples (5mL each) were collected on weekly basis up to 28th days of post papain injection. Sera were separated and subjected to perform ELISA for estimating CTX-II fragments as cartilage biomarker (CartiLaps ® ELISA kit) in experimental groups. Maximum level of CTX–II (pg/mL) (40.44±3.07) was observed in sera samples of day 14 post papain injection followed by days 21 (40.22±2.01), 28 (36.82±3.81), 7 (34.48±4.17), 1 (15.08±4.22) and day 0 (2.55±0.10). The early changes in serum CTX-II from day 0 to 14 showed significant association with cartilage damage. Later on, no significant difference was observed in CTX-II level on day 14, 21 and 28 post papain injection. It is concluded that elevation in serum CTX-II level was concomitant with the onset of disease and degradation of cartilage. Moreover, CTX-II is a sensitive diagnostic biomarker to monitor joint disorder severity in papain induced OA rat experimental model on different days. These findings may be used as base line for early diagnosis of disease and initiation of therapy for successful outcome.

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INTRODUCTION

Osteoarthritis is a chronic disorder of joints characterized by degenerative changes of articular cartilage consequently disturbing biological metabolism of cartilage and underlying bone (Goldring and Goldring, 2010; Pinna *et al*., 2013). Major moiety of cartilage is aggrecan composed of glycosaminoglucan, chondroitin sulphate and keratin sulphate (Sharma *et al.,* 2013; Khan *et al.,* 2013). Due to wear and tear of cartilage in OA, the metabolic fragments first come in blood and then in urine as degradation products thus may serve as biomarkers under pathological conditions (Bay-Jensen *et al.,* 2011; Takahashi *et al.,* 2012). Cartilage degradation biomarkers including types I, II and III collagen and aggrecan synthesis (CPII and CS 846) can be used to check the extent of destruction (Svoboda *et al.,* 2013). Biomarkers of inflammation including interleukins, tumor necrosis factor, growth transforming factor and eosinophilic proteins are used to check the level of OA (Otterness *et al.,* 2000).

Early diagnosis of cartilage destruction play key role in efficient therapy and favorable prognosis. The surrogate biological markers may also be used to evaluate the ability of agents involved in structure modification (Felson and Lohmander, 2009). Costly techniques like arthroscopy, magnetic resonance imaging (MRI) and computational topography (CT) require anesthesia prior to

examination of affected joints. At early stages of OA, biomarker CTX-II is considered most reliable for accurate joint destruction (Garnero *et al*., 2003).

Blood and urine levels of biomarkers are directly related with the severity of cartilage destruction (Goode *et al.,* 2012). In OA cartilage destruction bone turnover markers decrease and serum COMP and urine CTX-II increase significantly. Increase in bio-molecules of synovial destruction is directly related with the extent of cartilaginous tissue damage. The concentration of bio-molecules can efficiently be determined using ELISA both in serum (Bay-Jensen *et al.,* 2011) and urine of different animals (Takahashi *et al.,* 2012). So, bio-molecules of joint tissues destruction may be used to determine severity of OA (Naito *et al*., 2010). In focus research was carried out to observe the relationship of CTX-II levels with extent of OA induced by injecting papain in experimental Wistar rats. CTX-II level can be estimated to determine the disease progression and efficacy of anti-osteoarthritic therapy.

MATERIALS AND METHODS

Male Wistar rats (n=30) weighing 150-200g were selected. Feed and water were provided *ad-libitum.* Guidelines provided by International Association for the Study of Pain (IASP) was followed during experimental trials. To develop OA, 10mg papain in buffered solution (0.05M sodium acetate, pH 4.5) was injected intra-articularly (Murat *et al.,* 2007). Five rats (n=5) were injected with 0.5 mL of normal saline (0.9%) in right knee joint which served as control group.

A volume of 5 mL blood was collected without anti-coagulant on each sampling day, i.e., 0, 1, 7, 14, 21 and 28. Sera was collected and stored at -18°C till further use. CTX-II was estimated using serum preclinical CartiLaps ® ELISA kit (Immunodiagnostic system, UK. cat # AC-081) as described by Garvican *et al*. (2010) using 1.2 pg/mL detection limit. To evaluate the accuracy of ELISA kit, concentration of standards was determined from their respective absorbance values (0.122, 0.296, 0.329, 0.403, 0.542 and 0.847) at wavelength of A450/650.

Quantitative data for CTX-II were analyzed statistically and expressed as mean±SD. Differences between groups were tabulated by using one way ANOVA applying DMR using SPSS software, version 13.0 at probability level of 0.05.

RESULTS AND DISCUSSION

Level of C-telopeptides terminal of type II collagen (CTX-II) in sera samples of normal (day zero) and OA (days 1, 7, 14, 21 and 28) groups was measured from pre calibrated kit. Highest concentration of CTX-II was recorded on 14th day post papain injection followed by 21st, 28th, 7th and 1st. The lowest value was observed in control group. Differences in CTX-II concentrations on days 14, 21 and 28 were not significant statistically. Concentration of CTX-II in sera samples of 7th day was close to the 28th day. Concentrations in sera samples on 1st day and of normal group were lowest as compared with other days. All OA groups differ significantly from control group (Table 1).

Table 1: CTX II (C-terminal teleopeptide) concentrations determined by Sandwich ELISA in Sera samples of normal (control) and OA groups on different days

Control/ Sampling Days	Replicates	Mean A450-650 (nm)	Interpolated CartiLaps conc. (pg/ml)	Mean±SD
Control positive	01	0.408	40.0	
Normal Group	01	0.223	4.0	2.55±0.10^{a}
(Day zero)	02	0.176	2.5	
	03	0.166	2.0	
	04	0.133	1.4	
	05	0.191	2.9	
Osteoarthritis	01	0.324	14.0	15.08±4.22^{b}
Groups	02	0.323	14.0	
Day 01	03	0.352	22.5	
	04	0.319	12.5	
	05	0.318	12.4	
Day 07	01	0.396	38.2	34.48±4.17^{c}
	02	0.387	34.0	
	03	0.367	27.7	
	04	0.391	35.0	
	05	0.399	37.5	
Day 14	01	0.405	39.8	40.44±3.07^{d}
	02	0.392	35.5	
	03	0.410	41.0	
	04	0.415	43.0	
	05	0.414	42.9	
Day 21	01	0.399	37.5	40.22±2.01^{d}
	02	0.410	41.0	
	03	0.408	40.0	
	04	0.403	39.6	
	05	0.415	43.0	
Day 28	01	0.414	42.9	36.82±3.81^{cd}
	02	0.391	35.0	
	03	0.396	38.2	
	04	0.387	34.0	
	05	0.386	34.0	

Values (Mean±SD) carrying same superscripts in a column differ non-significantly whereas numeric values with different superscripts vary significantly.

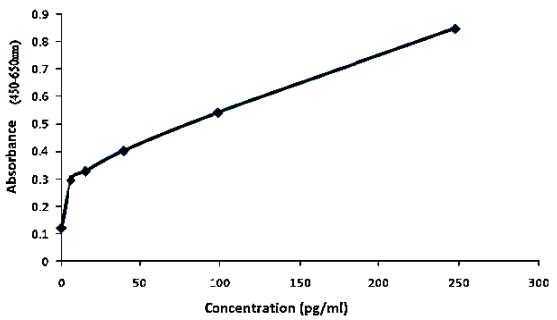

Fig. 1: Standard curve of CTX II by ELISA (Absorbance Vs Concentration)

Rise in concentration of CTX-II with progression of OA indicated that this biomarker may be related to the extent of OA.

Many types of cleaved CTX-II fragments are considered to be the potential biomarkers of cartilage biosynthesis or breakdown. These included pyridinoline (PYD), triple helix fragments, CTX-II, core protein fragments, N and C propeptides (Garnero *et al*., 2002). Relationship of biological markers with the destruction of cartilaginous tissues played key role in the development of highly sensitive assays (Christgau *et al*., 2001). Bleasel *et al*. (1999) observed and reported the relationship of degradation molecules with the severity of joint disorders.

Relation between degradation of cartilage with raised levels of CTX-II in sera of OA patients has been used as

biomarker by a number of researchers. Garnero *et al.* (2001) determined relationship between bone, cartilage and synovial markers with severity of joint destruction. It was concluded that all bone turnover markers decreased in OA patients compared with healthy controls whereas markers of cartilage turnover were significantly increased. Measurement of CTX-II concentration in OA rat sera carried out in present study was in accord with the biomarker of collagen degradation used by Garnero *et al*. (2003). Svoboda *et al.* (2013) used collagen types I and II and aggrecan synthesis molecules to detect early cartilage degenerative changes in sera of young athletes.

In another study, urinary CTX-II and free deoxypyridinoline (DPD) levels in patients with rapidly destructive and slowly progressive hip OA were investigated. Increased CTX-II levels were associated with rapid destructive OA (Garnero *et al.,* 2003). Raised levels of biomarker in serum indicated subsequent structural changes in knee joint in present study. These findings are in corroboration with clinical investigations reported by Garnero *et al*. (2002). Garnero *et al*. (2001) related CTX-II levels with radiological assessment of damage to knee joint cartilage. Urinary CTX-II has been used as biomarker in diagnosis and quantitative degradation of cartilage by Mazières *et al*. (2006) and Meulenbelt *et al*. (2007). This biomarker has been included to evaluate histological grading of localized affected joints and hypertrophic OA (Lorenz *et al*., 2005). Conrozier *et al.* (2012) used CTX-II as indicator biomarker in serum and urine to evaluate the effects of hyaluronic acid in knee OA patients.

In agreement with present study, circulating levels of CTX-II determined were consistent with previous investigations of urinary CTX-II levels in rats (Ishikawa *et al*., 2004). Raised CTX-II concentration in sera of rats was significant at early stages of joint inflammation (Oestergaard *et al*., 2006). The diagnostic value of serum CTX-II at early stage of cartilage degradation due to joint inflammation was evaluated by Oestergaard *et al*. (2006). The predictive importance of CTX-II molecular marker for cartilage destruction has been undertaken in other models of destructive arthritis (Hoegh-Andersen, 2004) and arthritis induced by adjuvant (De Ceuninck *et al*., 2003). CTX-II was used as an early indication of joint inflammation in collagen induced arthritis and was a reflection of progressive cartilage degradation (Oestergaard *et al*., 2006). Significantly higher levels of CTX-II were observed by Matyas *et al.* (2004) in sera of OA dogs than normal on surgical induction. Elevated levels of CTX-II persisted in sera of rats for long period in present study which is in agreement with the findings of Goranov (2007) in human OA.

In contrast results has been reported in cases under natural conditions (Hayashi *et al.,* 2009). Higher concentrations of CTX-II were observed in serum of horses at early stage of OA (Frisbie *et al*., 2008). Similar studies were undertaken in sheep following injury to cartilage by Lu *et al*. (2006) and in guinea pigs by Huebner and Kraus (2006).

ELISA is a reliable and sensitive tool to measure the type II collagen molecules of cartilage destruction for the assessment of OA in various samples of animals (Takahashi *et al.,* 2012). Bay-Jensen *et al.* (2011) used ELISA to determine the concentration of type II collagen in serum samples and related the levels with degenerative joint diseases. Similarly, type III collagen biomarkers were measured in urine samples by ELISA and related with the progression of cartilage degradation by Barascuk *et al.* (2011).

In view of present study along with previous research work, maximum CTX-II concentration was observed in sera of OA rats on 14th day post papain injection that has no significant difference with 21st and 28th CTX-II levels. So the treatments may be started from any of the three days post injection to assess the therapeutic efficacy of drugs in this rodent model, moreover if correlated with histological findings of Murat *et al*. (2007), the starting point of disease may be 28th day of post papain injection in rodent model.

In conclusion, it is demonstrated that measurement of serum CTX-II provides a useful estimate of disease progression in relation to time. Based on these findings, it seems reasonable to use CTX-II biomarker to monitor the effects of chondroprotective drugs in papain induced OA model.

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