



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 6, Issue, 6, pp.4457-4460, June, 2015

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

SERUM ENZYME OF MATRIX METALLOPROTEINASE-3 IN PATIENTS WITH KNEE OSTEOARTHRITIS

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ARTICLE INFO

Article History:

Received 2nd, May, 2015
Received in revised form 10th,
May, 2015
Accepted 4th, June, 2015
Published online 28th,
June, 2015

Key words:

Serum enzyme, matrix metalloproteinase, knee osteoarthritis.

ABSTRACT

Objective: To measure serum enzyme level of matrix metalloproteinase (MMP) in patients with Knee Osteoarthritis and to compare with ESR level, age, weight and height.

Methods: An MMP3 serum level was detected and measured by using Abcam ab100607 MMP3 Human ELISA Kit (USA). The serum samples aliquots were thawed on ice and were assayed at 1:2 dilutions. The study included 23 males and 49 females were recruited who are already effected with Knee Osteoarthritis.

Results: MMP 3 were higher in Knee Osteoarthritis patients with different age group and sex. The difference between serum MMP 3 $\chi^2=30.529$, $p=0.082$ and ESR level $\chi^2=22.944$, $p=0.347$ were analyzed. The difference mean rank scores of serum MMP 3 69.38 and ESR 75.62 respectively. Since p -value $=0.082$ $0.05=$ we accept the null hypothesis both MMP 3 and ESR levels in Knee Osteoarthritis patients were elevated.

Discussion: At the 0.05 level of significance, there exists enough evidence to conclude that there is a no difference in the median test scores (and, hence, the mean test scores) among the MMP 3 and ESR levels in Knee Osteoarthritis patients. The study is concludes that, there is considerable evidence that MMP3 is capable of mediating degradation leads to knee joint destruction.

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INTRODUCTION

Osteoarthritis (OA), also often called 'osteoarthrosis or 'degenerative joint disease,' is the most common form of arthritis (Kelsey et al 1988). The name "osteoarthritis" arose from observation of the striking overgrowth of marginal and subchondral bone by the pathologists and radiologists. Global statistics reveals over 100 million people worldwide suffer from OA, which is one of the most common causes of disability (Hinman et al 2010; Heiden et al 2009). Enzymes are biological catalysts or assistants. Enzymes consist of various types of proteins that work to drive the chemical reaction required for a specific action or nutrient. Among various biological markers associated with OA, matrix metalloproteinases (MMPs) play a primary role in cartilage degradation in human joint disease and function downstream of OA signalling pathways (Benedetti et al 2010 and Ahmad et al 2009). The study shown that matrix metalloproteinases (MMPs) play an important role in the degradation of the matrix in OA and rheumatoid arthritis (RA) (Vincenti, et al. 1994).

Matrix Metalloproteinases (MMPS)

Matrix metalloproteinases are a family of structurally-related, zinc-containing enzymes that have the ability to breakdown

connective tissue. MMPs are a large family of calcium-dependent zinc-containing endopeptidases, which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan.

Stromelysin-1 (MMP-3)

MMP-3, a proteoglycan degrading enzyme, can be localized to the zones of cartilage with active proteoglycan depletion (Okada, Shinmei, Tanaka et al 1999). Stromelysin-1 can degrade aggrecan, denatured collagens and interhelical collagen domains, as well as aggrecan and link protein. Importantly, stromelysin-1 can cleave the aggrecan molecule at the MMP site, at the Asn341-Phe342 bond, to liberate the G1 domain from the remainder of the molecule (Flannery et al 1992). It has been shown that stromelysin-1 can activate the pro forms of collagenases and that this activation is a key step in cartilage degradation (Suzuki et al 1990). In osteoarthritic cartilage, stromelysin-1 is localized in chondrocytes of the superficial and transition zone (Okada et al 1992) and its strongest RNA expression is found in early degenerative articular cartilage (Bau et al 2002). Mehraban et al (1998) the study indicating that both cell types can produce stromelysin-1. It has been shown that in humans, the plasma level of stromelysin-1 was a significant predictor of joint space narrowing in knee

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osteoarthritis. (Lohmander et al., 2005). Bassiouni and et al (2011) reveals that, the aim of the study was to examine the relationship among three different parameters used to assess cartilage in Osteoarthritis (OA) of the knee. These parameters are Phonoarthrography (Phono-A), Musculoskeletal ultrasonography (MSUS) from the 4 condyles and biochemical marker: notably (MMP-3) and tissue inhibitor of proteinase (TIMP-1). The study results shows that Phono-A values were inversely correlated with cartilage thickness and mean levels of MMP-3 is elevated and continued to rise with increasing radiological grades until grade 4. All India Institute of Medical Science (2012) the study focusing to hip resurfacing in younger patients with primary osteoarthritis. The study reveals that ruling out proximal femoral osteopenia using dual energy X-ray absorptiometry. The study conducted by Advance Knowledge of Osteoarthritis the study shows that a significant influence of the haplogroups on the serum level of MMP-3 and MMP-13 was detected ($p=0.027$ and $p=0.035$ respectively) with OA with haplogroup H showed higher serum level of MMP-3 than healthy controls. The serum levels of MMP-13 were significantly higher in patients with OA. Heinaz Farouk Abdul et al (2003) study found that the serum concentrations of MMP-3 and MMP-9 were significantly higher in RA patients than in OA patients. The ELISA sandwich method technique was used to measure the serum concentrations of MMPs. The sensitivity (*limit of detection*) of the assay system was 2.35 ng/ml for MMP-3, 0.6 for MMP-9 and 1.25 ng/ml for TIMP-1. The samples were drawn from in the different age group; the ESR level is highly significant in all the 30 RA patients. Ari Kobayashi et al (2007) study found that, the levels of MMP-3 in serum samples were collected by venous puncture from 20 osteoarthritis patients were measured by the 1-step sandwich enzyme immunoassay system. The serum samples were significantly higher in rheumatoid arthritis than in osteoarthritis, and the levels correlated directly with each other. Keenan et al (2008) the study found that ESR and CRP was more elevated in RA patients than osteoarthritis patients, the cut off values used for elevated levels for both ESR and CRP. Some consider $ESR > 30$ mm/hr as a better number for inclusion, therefore excluding some outliers (Wolfe, 1997). Normal ESR also increases with age. The upper limit of normal for males, less than 50 years of age, is 15 mm/hr, and for females, less than 50 years of age, is 20 mm/hr (Caswell, 1993). Selected biomarkers currently being investigated for the evaluation of osteoarthritis biomarkers related to other non-collagenous proteins MMP-1, MMP-3, MMP-9, MMP-13 and TIMP (Kraus V.B. et al., 2011). A study in 161 patients with knee osteoarthritis showed that levels of the Matrix metalloproteinase's MMP-1 and MMP-3 were predictive of cartilage volume loss as evaluated by quantitative MRI over 2 years (both $P < 0.05$) (Pelletier et al. 2010).

Several methods have been used for detection of MMP activity in OA. These methods include enzyme-linked immunosorbent assay (ELISA), gelatin zymography, and Western blotting. ELISA has been frequently used for measurement of total amounts of pro-form MMPs plus MMP-TIMP complexes, where- as measurement of MMP in the active form is more appropriate in assessment of the potential of matrix degradation from MMPs (Beekman et al 1996).

MATERIALS AND METHODS

The present study was conducted in Sri. Krishna Rajendra Hospital, Mysore from June 2013 to May 2014. The patients were randomly selected who are already affected with Knee osteoarthritis from the outpatient department in the section of Orthopedic, K.R. Hospital. The study included 23 males and 49 females were recruited and informed consent was obtained from each patient, including permission for the use of serum to be collected throughout the study for the assay of MMP3. The study protocol was approved by Institutional Human Ethical Committee, University of Mysore, Mysore (IHEC-UOM No. 82 Ph.D. /2013-14).

Inclusion criteria

- Age between 40-65 both male and female.
- Patients diagnosed with primary osteoarthritis
- Orthopedic doctors diagnosed knee osteoarthritis patients (radiologically confirmed)

Exclusion criteria

- Patients with any systemic illness, secondary diseases such as Diabetic, Blood Pressure and obesity etc.
- Patients who have undergone total knee replacement in both the knees, patients with OA secondary diseases like rheumatoid arthritis and gout
- Patients with restricted mobility.

Blood collection: From KOA patients 5ml of venous blood samples were collected into blood sample collection tubes. In that 2ml of blood has taken to another sterilized tube for measuring ESR levels of the KOA patients. ESR levels were measured by the method of Westergren and it is expressed in mm/hr.

Serum collection: Remaining 3ml of blood samples were allowed to coagulate for 1 hour at room temperature and then centrifuged at 2000rpm for 15 minutes. The serum samples were transferred to another sterilized appendoff's tubes and kept frozen at -20°C until analyzed. Sample analysis was performed by Abcamab100607 MMP3 Human ELISA Kit (USA), according to the manufacture's instruction. The serum samples aliquots were thawed on ice and were assayed at 1:2 dilutions.

Statistical analysis: The basic demographic characteristics of the Knee Osteoarthritis patients groups and Mean and SD were arrived. The Kruskal-Wallis H test was used to assess differences between study groups, which were further analyzed using the Mann-Whitney U test. A value of $p (0.05)$ was considered significant. Scatterplot matrix was plotted for knowing each variables association with SPSS windows 16.0.

RESULTS

The female and male Knee Osteoarthritis patients were classified into different age group. In 72 total Knee Osteoarthritis patients, 49 female and 23 male were selected.

Table 1 Demographic of Knee Osteoarthritis patients

	Female (N=49)			Male (N=23)			Total (N=72)		
	MMP 3 (ng/ml)	ESR (mm/hr)	AGE	MMP 3 (ng/ml)	ESR (mm/hr)	AGE	MMP 3 (ng/ml)	ESR (mm/hr)	AGE
Min.=Max.	11.33=87.46	15=90	40=64	23.26=79.20	10=85	42=65	11.33=87.46	10=90	40=65
Mean ±SD	30.56±15.67	46.84±18.13	50.08±6.32	50.66±15.36	26.52±19.09	55.26±7.14	36.98±18.12	40.35±20.65	51.74±6.98

Measurement of serum MMP 3 (ng/ml) levels in female, minimum is 11.33 and maximum is 87.46, whereas in male minimum is 23.26 and maximum is 79.20 (ng/ml). The Erythrocyte Sedimentation Rate (ESR) has been shown to be useful for diagnosis of inflammation in Knee osteoarthritis patients. The ESR in Knee Osteoarthritis patients, out of 72 subjects, about 51 (70.83%) subjects was having ESR level of 30 and above mm/hr and rest of it 21 (29.17%) subjects were having below 30mm/hr. Some consider ESR>30 mm/hr as a better number for inclusion, therefore excluding some outliers (Wolfe, 1997) and normal ESR also increases with age. The mean average of female is 46.84mm/hr, male 26.52mm/hr and standard deviation of female is 18.13, and male is 20.65mm/hr. It indicates that female ESR level is more while compare male among the Knee osteoarthritis patients (Table 1).

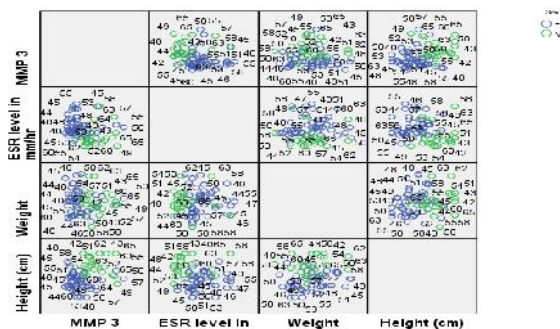
DISCUSSION

A Kruskal –Wallis H test the Mann-Whitney U test showed that there was a statistically significant correlation between Serum MMP 3 level as well as ESR levels in Knee Osteoarthritis patients. The difference between serum MMP 3 $x^2=30.529$, $p=0.082$ and ESR level $x^2=22.944$, $p=0.347$. The difference mean rank scores of serum MMP 3 69.38 and ESR 75.62 respectively. Since p -value =0.082 0.05= we accept the null hypothesis both MMP 3 and ESR levels in Knee Osteoarthritis patients were elevated. At the 0.05 level of significance, there exists enough evidence to conclude that there is a no difference in the median test scores among the MMP 3 and ESR levels in Knee Osteoarthritis patients(table 2).

Table 2 Test Statistics^{a,b}

	MMP 3 (ng/ml)	ESR level in mm/hr
Chi-Square(x^2)	30.529	22.944
Degree of freedom (df)	21	21
Asymp. Sig.	.082	.347

a. Kruskal Wallis Test
b. Grouping Variable: Age



Graph 1 Scatterplot (Matrix) =MMP3, ESR level in mm/hr, weight and height by Sex and Age

A scatterplot matrix were used to know the relationship between a pair of variables such as MMP3, ESR level, Weight and Height of Knee Osteoarthritis patients comparing by age and sex.

Scatterplots were organized into a grid (Matrix); each scatter plot shows the relationship between a pair of variables. All four pairs of variable have dissected by sex and age; there is no linear correlation among the variables (Graph 1).

CONCLUSION

The MMPs play an important role both in normal physiological and pathological conditions such as arthritis. This study provides an extensive analysis of systemic serum levels of MMP3 in Knee Osteoarthritis patients with different age group and sex. Early detection of elevated serum levels of MMP 3 markers may herald progressive course and may modulate the lines of treatment. This study concludes that, there is considerable evidence that MMP3 is capable of mediating degradation leads to knee joint destruction. Our finding suggests that serum MMP 3 is a marker of systematic inflammation of Knee Osteoarthritis patients.

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How to cite this article:

M.S. Radha et al, Serum Enzyme Of Matrix Metalloproteinase-3 In Patients With Knee Osteoarthritis. *International Journal of Recent Scientific Research* Vol. 6, Issue, 6, pp.4457-4460, June, 2015
