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Research Article

Evaluation and status of nitrite in osteoarthritis patients

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ABSTRACT

The study is based on the determination of serum nitrite levels in patients of osteoarthritis (OA) and to correlate with disease severity.Total one hundred and fifty patients with OA and fifty healthy volunteers were entered in this study. Patients were divided into two groups on the basis of disease severity. Concentration of nitrite in serum was determined by indirect griess reaction method**.** Serum nitrite levels were higher in patients having grade four osteoarthritis as compared to grade two and control subjects. In addition serum nitrite levels of both groups were found to be higher as compared to control subjects ($P<0.05$). The findings suggest that nitrite production is enhanced in patients with osteoarthritis compared with controls. In addition serum nitrite levels were increased with disease severity, which may suggest local nitrite synthesis in synovium and then in serum of the patients.

KEY WORDS: Nitrite, Osteoarthritis, chondrocytes, severity.

INTRODUCTION

Osteoarthritis (OA) is a degenerative disease involving chondrocytes, cartilage and other joint tissues, and has number of underlying factors[1,2]. Age and osteoarthritis are strongly associated with each other. Other factors which are linked to the disease are obesity and deficiency of sex hormones. Pro inflammatory factors including nitric oxide (NO) are associated with OA[3,4,5] .Nitric oxide is an inorganic gaseous free radical produce by the enzyme nitric oxide synthase (NOS). Three distinct isoforms of NOS have been identified: Inducible (iNOS), endothelial (eNOS), and neuronal (nNOS). NOS is a remarkably complex enzyme which acts on molecular oxygen and arginin in neurons, endothelial cells, platelets, neutrophils and other cells to produce NO [6,7]. Nitric oxide is a gaseous free radical with a short half-life in vivo of a few seconds or less. Therefore, the levels of the more stable NO metabolites, nitrite (NO2-) and nitrate (NO3-), have been used in the indirect measurement of NO in biological fluids [8].Nitric oxide is a unique second messenger molecule that readily diffused through cell membranes to exert a variety of biological actions in mammalian cells. Previous studies have suggested that it has several physiological roles in immune regulation, inflammation, autoimmunity and arthritis [9,10] .Increased levels of NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (RA) and osteoarthritis (OA)[11] , in animals with induced arthritis [12] and in auto immune arthritis [13].

It has been demonstrated that high local concentration of NO may exert determental effects of chordrocytes function, including inhibition of collagen and proteoglycan synthesis[14,15], activation of metalloproteinases[16] , decreased expression of IL-1 receptor antagonist, inhibition of chordrocyte proliferation and induction of apoptotic death[17,18]. On the other hand it has been suggested that nitric oxide produced inside the joints may also contribute significantly to the pathoganesis of arthritis.

In the present study we wanted to evaluate serum nitrite status responsible for the cartilage damage occurring in primarily degenerative or inflammatory joint diseases, and to compare these levels with different grades of osteoarthritis or disease severity.

MATERIALS AND METHODS

The study was conducted on 150 female patients suffering from OA with age group 45 – 65 years (mean age 55.5 years).Duration of study is about one year that is from February 2009 to December 2009. The patients were selected on the basis of signs, symptoms, history and severity of disease at joint site and X-Ray of the joints. Patients taking any hormone replacement therapy (HRT), non steroidal anti inflammatory drugs (NSAID), having metabolic disease, rheumatoid arthritis (RA), joint, systemic lupus erythromatosis (SLE) were excluded from the study. Every one filled out the questionnaire giving information about their gender, age, past health problems, present medication, menstrual state and age of menopause (Table-1). Those with uncertain menstruation history were excluded from the study.

The specimens were collected from Civil Hospital and Dow University of Health and Sciences, Karachi. The study was performed in accordance with ethics standards, permission is given by the Civil Hospital and Dow University of Health and Sciences, Karachi The patients were divided into two groups on the basis of radio graphs of their effected joints. The first group consisted of those patients who have grade 2OA, while the Second group consisted of those patients who have grade 4OA [19,20,21] (Table-2).

Control group consisted of fifty healthy female subjects (age between 40 to 60 years). Blood sample were draw and serum was immediately frozen at – 70^0C until the analysis was carried out. Nitrite levels were measured by directed griess reaction, which is the simplest and most commonly used assay method[22]. Kit is provided by assay designs, 800 Technology Drine, Amn Arbor, Michigan USA). This kit allows for the total determination of both nitric oxide products in the sample by conversion of the entire sample nitrate into nitrite, followed by the determination of the total concentration of nitrite in the sample.

RESULTS AND DISCUSSION

Statistical significance among three groups was done by ANOVA test using SPSS-16 version of statistical software. Table 1 present ANOVA used baseline characteristics of the controls and patients. Age of control subjects is significant when compared with group 1 and group 2 (p=0.029).Body mass index of patients and control subjects were calculated by measuring their weight and waist to hip ratio. BMI of group 1 and group 2 is significant when compared with the control (p=0.035).The menopause history was also collected from each subject, as it very important to correlate with the other parameters also.duration of menopause of both groups is significant when compared with controls (p=0.019).Table 3 shows the multiple comparisions of this parameter in patients and control subjects .Table 2 shows the nitrite levels in control and OA subjects. Serum nitrite levels of grade 4OA were high as compared to grade 2 OA and control. While grade 2 OA patients have higher nitrite levels as compared to control subjects (p<0.001). Figure 1 shows the percentage of serum nitrite in patients.Higher percentage was found in group 2 patients.

All the subjects included in this study are postmenopausal females and there age is specially linked with the disease correlation [23]. Because this disease is very strongly associated with the age [24], that's why selection of the patients with specific age limit is very necessary. In this study we found no correlation with BMI and osteoarthritis .In this study the most important findings was that the serum nitrite levels were increased in both OA groups compared with healthy volunteers. Also the group one has increased nitrite level when compared with group two and control group. This accords with the nitrite levels in serum and synovial fluid in previous study[25,26,27].

Karpuzoglu etal 2006 also reported high nitrite / nitrate levels in synovial tissue and serum of arthritis patients [28]. The origin of this increased of NO is not clear, widespread synovial inflammation might increase serum levels of nitric oxide when synovial fluid cleared by the lymphatic system enters the systemic circulation and equilibrates with the vascular component within the synovium [29,30,31 .]

Our findings suggest that nitrite levels provides a measure of endogenous NO synthesis and show that this level may be measured in human without complex preparatory steps.

Also these levels may be increased day by day in OA patients and disease severity. In this regard, likely beneficial of some therapeutic interventions, including antioxidants, that potential the antioxidant defense mechanism and reduce per oxidation in the management of OA is underscored.

Table 1.
Base line characteristics of the controls and osteoarthritis patients.

Variable	Controls	Group 1 Grade 2OA	Group 2 Grade 4OA	P-value
Age (years)	52.5 ± 6.2	55.4 ± 3.2	56.4 ± 4.0	0.029
BMI (Kg/m^2)	21.5 ± 5.9	23.6 ± 4.1	22.5 ± 5.0	0.035
Duration of Menopause (years)	8.5 ± 1.2	9.6 ± 1.9	10.8 ± 1.9	0.019

Values are mean ± S.D

Table 2.
Serum nitrite levels in controls and osteoarthritis patients

Variable	Controls	Group 1 Grade 2OA	Group 2 Grade 4OA
Serum nitrite levels (µmol/L)	48.6 ± 1.1 (50)	75.8* ± 1.2 (70)	115.9* ±0.7 (80)

P< 0.001
Values are mean ± S.D

Table:3 Multiple Comparisons of patients groups and control subjects

(a) serum Nitrite	(b) serum Nitrite	Mean Difference (a-b)	Std. Error	p- value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	Grp 1	-38.10100*	2.75471	.001	-44.6233	-31.5787
	Grp2	-71.45300*	2.75478	.002	-77.9753	-64.9307
Grp 1	control	38.10100*	2.75471	.001	31.5787	44.6233
	Grp2	-33.35200*	2.75471	.001	-39.8743	-26.8297
Grp2	control	71.45300*	2.75470	.000	64.9307	77.9753
	Grp 1	33.35200*	2.75470	.000	26.8297	39.8743

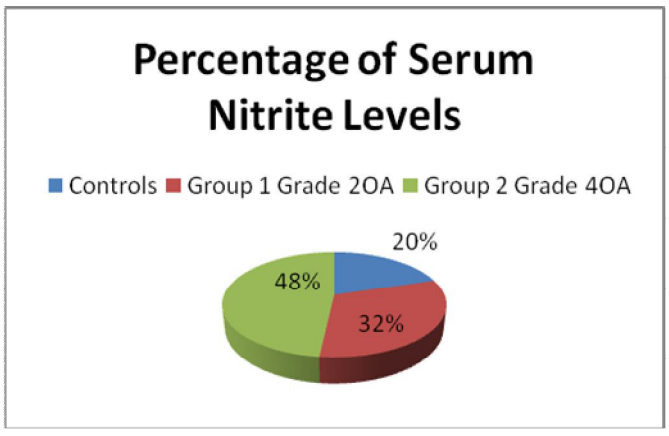

Figure 1. Percentage of serum nitrite levels

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