ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Impact of obesity on status of glutathione reductase activity and inflammatory response in patients with knee osteoarthritis

***Reham M. Shaat, *Mohamed Kamal Senna , **Naglaa Mokhtar, ** Rehan Monir, ***Adel I. Abd-El Salam,*Shereen Aly Machaly**

***Rheumatology and Rehabilitation Department Faculty of Medicine Mansoura University**

****Medical Biochemistry Department Faculty of Medicine Mansoura University**

*****Internal Medicine Department Faculty of Medicine Mansoura University**

Manuscript Info

Manuscript History:

Received: 15 July 2015
Final Accepted: 22 August 2015
Published Online: September 2015

Key words:

osteoarthritis, obesity, glutathione reductase, interleukin-6

Corresponding Author

Reham M. Shaat,Shereen Aly Machaly

Abstract

Obesity is a significant risk factor for developing osteoarthritis (OA). The exact pathogenesis of obesity-associated OA is not completely understood. However, recent studies indicate that pro-oxidant and antioxidant status together with pro-inflammatory factors may contribute to an increase in osteoarthritis risk**. Aim of work:** : was to investigate glutathione reductase (GR) activity as an antioxidant enzyme and inflammatory markers in obese and non-obese knee osteoarthritic patients

Patients and Methods: 36 patients with knee OA (21 obese and 15 non-obese), and 30 age/sex-matched controls were enrolled in this study. Western Ontario and Mc-Master University(WOMAC) score and pain were assessed. Radiological grading of knee joints by Kellgren and Lawrence (KL) score was performed. Blood samples were collected and analyzed for GR, lipid profile,C reactive protein(CRP), and interleukin-6 (IL-6). **Results:** Plasma activity of GR was significantly higher in controls than OA patients (obese and non-obese) (P=0.001, <0.05 respectively) with more lower levels in obese than non-obese OA (p=0.04). On the other hand, Inflammatory markers (IL-6 and CRP) were significantly higher in obese OA patients than controls (P<0.001) and in obese than non obese OA(P<0.001). In obese OA group significant correlation was found between IL-6 and BMI. In addition, age correlated with KL scoring of knee, and CRP with serum cholesterol level. In non-obese OA group only WOMAC score correlated significantly with BMI (P=0.041).

Conclusion: The results of this study suggest higher oxidative stress status in OA, evidenced by decreased GR activity. Association of obesity with OA adds a marked oxidative burden and inflammatory status on the joint much more than OA per se.

INTRODUCTION

OA is considered the most common arthritic condition causing chronic disability in old age**(1).** The etiology of knee OA includes several factors such as increased dynamic loading of the joint , high body mass index (BMI), trauma, female gender, aging and other factors such as genetic and neuroendocrine factors**(2).**

Since OA is a heterogeneous disorder and several factors are involved in articular cartilage degradation , several mechanisms may be involved in its development. Increase in inflammatory cytokines, which happens secondary to aging of the immune system or increased body weight may be a possible cause**(3).**
Several studies revealed associations between OA progression and inflammation using plasma CRP and synovial IL6**(4) .** However, some other studies found no significant association between any inflammatory markers and radiographic changes of OA**(5).**
The imbalance between pro-oxidants and antioxidants plays a serious role in the cellular oxidative stress , which has an important role in the progression of OA. Increase in superoxide anions and defect in antioxidant mechanisms is involved in production of ROS involved in oxidative damag.**(6).**
ROS oxidize and subsequently impair numerous components of the joint**(7).** ROS can cause damage to collagen by a direct or indirect action through the activation of latent collagenase and neutralization of protease inhibitors**(8).**
Oxygen radicals are inactivated by the glutathione system**(9)** with an involvement of GR which regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG) at the expense of NADPH**(10).**
A decreased antioxidant capacity of the glutathione system has harmful effects on articular cartilage . An increased level of endogenous ROS resulting from decreased levels of GSH can decrease the synsthesis of proteoglycan and hyaluronic acid which are components of the articular cartilage extracellular matrix (ECM) **(11).**
Therefore, the aim of the present study is to detect oxidative stress and systemic inflammation in Knee OA patients and detect the effect of obesity on oxidative stress and systemic inflammation.

[2]-Patients and methods:

2.1-Patients:

The present study was carried out on 36 patients and 30 apparently healthy subjects.
Patients were selected from the outpatient clinic of Rheumatology and Rehabilitation Department Mansoura University Hospital. All of these patients were fulfilling the classical and radiological criteria of the American College of Rheumatology (ACR) for diagnosis of primary knee OA**(12)** Patients were subdivided according to BMI into 2 groups:
Group I: included 21 knee OA patients with BMI ≥ 30 and an age range of 40-70 years (mean 52.8±6.9) 13 females and 8 males.
Group II: included 15 non obese OA patients with BMI < 30 and an age range of 38-70 years (mean 49.7±10.3) females were 8 and males were 7.
30 healthy persons were studied as control group with an age range of 38-68 years (mean 50.7 ±10.5) (12 males and 18 females).
Exclusion criteria:
Patients with erosive OA, chondrocalcinosis, trauma, surgery in knee joint, inflammation, renal insufficiency, hepatic disease, infection, gout, metabolic bone disease, neoplasms or any other systemic disease were excluded.
All patients were subjected to history taking, general examination and local examination of knee joint.

2.2-Assessment of pain, stiffness and functional status:

Using WOMAC OA index**(13).**It is a self administered questionnaire consisting of 24 items divided into 3 subscales . The scores for each subscale are summed up with a possible score range of 0-20 for pain, 0-8 for stiffness, and 0-68 for physical function. Usually a sum of the scores for all three subscales gives a total WOMAC score, higher scores on the WOMAC indicate pain , stiffness and functional limitations.

2.3-Radiological assessment:

Radiography before inclusion in the study included a weight- bearing anteroposterior tibiofemoral view in full extension and a skyline patella view .
Radiographic scoring of tibiofemoral OA and patellofemoral OA was made using KL grading scale**(14).**

2.4-Laboratory assessment:

Fasting blood samples were collected from patients then serum cholesterol, triglycerides, high density lipoprotein and low density lipoprotein were measured.
Detection of serum IL6 by ELIZA (Boster Immunoleader by Boster Biological Technology Co. Inc.) according to Ota etal (15) Detection of serum CRP by R&D system **(16)**
Colorimetric determination of GR activity in the serum according to Goldberg and Spooner **(17).**GR catalyses the reduction of glutathione (GSSG)in the presence of NADPH, which is oxidized to $NADPH^+$. The decrease in absorbance at 340 nm is measured.
$NADPH+H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH.$

This study was approved by the Ethical Committee of Faculty of Medicine Mansoura University. Written informed consent was obtained from all participants.

2.5-Statistical analysis:

All statistical analyses were performed using SPSS for windows version 19.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ±standard deviation (SD). For each parameter, continuous variables were analyzed by student's t-test and categorical variable (gender) were analyzed by chi-square test. The correlation between the inflammatory markers and GR and the parameters of lipid profile were assessed by Pearson correlation co-efficient test. *p*-values <0.05 were considered to be of statistical significance.

[3]-Results:

Demographic characteristics of study participants compared to controls are shown in table 1. Significant differences were detected between OA patients and controls as regards oxidative stress, inflammatory markers and parameters of lipid profile (P<0.001) **(table 1)**

Table 1. the demographic, clinical and laboratory data of OA patients (*Student's t test)*

	OA patients no (36)	Control no (30)	p
Age (years)	51.5 ± 8.5	50.7 ±10.5	0.776
Female (n, %)	21, 58.3%	9, 60%	0.913 (*Chi square test)*
BMI (Kg/m^2)	36.01 ± 12.2	24.3±1.3	<0.001
Total WOMAC score	58.4 ± 24.2	-	
X ray grade	2.03 ± 0.85	-	
IL-6 (pg/ml)	54.9 ± 7.5	46.8 ±1.6	0.001
CRP (mg/L)	4.5 ± 0.92	3.3 ±0.3	<0.001
GR (IU/L)	52.2 ± 8.4	63.6 ±12.3	0.001
Cholesterol (mg/dL)	193.5 ± 34.5	161.9 ±19.7	<0.001
Triglycerides (mg/dL)	103.8±34.97	87.8 ±24	0.001
HDL(mg/dL)	39.1±9.71	43.9 ±6.2	0.003
LDL (mg/dL)	135.6±34.8	100.4 ±19.6	<0.001

BMI; body mass index, IL-6; interleukin -6, GR; glutathione reductase, HDL; high density lipoprotein, LDL; low density lipoprotein

Among the OA patients GR correlated significantly with age, BMI, total WOMAC score and KL radiological score **(table 2**). Furthermore, inverse significant correlations were found between GR and inflammatory markers; IL-6 (p<0.001) and CRP (p=0.011).

Table 2. correlations between oxidative stress and inflammatory markers and clinical or lipid profile parameters in OA patients (*Pearson correlation co-efficient test*)

	GR		IL-6		CRP	
	r	p	r	p	r	p
GR(IU/L)	-	-	-.577	<0.001	-.417	.011
IL-6(pg/ml)	-.577	<0.001	-	-	.625	<0.001
CRP(mg/L)	-.417	.011	.625	<0.001	-	-
Cholesterol(mg/dL)	-.111	.518	.415	0.012	.529	.001
Triglycerides(mg/dL)	-.236	.166	.465	.004	.615	<0.001
HDL(mg/dL)	.178	.298	-.232	.173	-.412	0.013

LDL(mg/dL)	-.107	.534	.367	0.028	.466	.004
Age (years)	-.385	.020	.285	.092	.222	.193
BMI (Kg/m^2)	-.372	.026	.662	<0.001	.627	<0.001
Total WOMAC score	-.398	.016	.486	0.003	.678	<0.001
X ray grade	-.344	0.040	.250	.141	.565	<0.001

When OA patients were further subdivided into 2 groups according BMI to detect the effect of obesity on the clinical, oxidative stress and inflammatory status of OA patients, significant differences in WOMAC score and x ray grade had exhibited between obese and non-obese OA **(table 3).** These differences existed also as regards inflammatory markers (IL-6 and CRP), GR, and variables of lipogram **(tables 3&4).**

Table 3. Comparison of demographic and clinical data between subgroups of OA

	Obese OA no (21)	Non-obese OA n (15)	Control n (30)	p
Age (years)	52.8 ±6.9	49.7 ±10.3	50.7 ±10.5	0.596*
Female (n, %)	13, 61.9%	8, 53.3%	9, 60%	0.870**
BMI (Kg/m^2)	42.7 ±12.1	26.7 ±2.2	24.3 ±1.3	<0.001***
Total WOMAC score	74.3 ±13.5	36 ±16.8		<0.001***
X ray grade	2.5 ±0.7	1.4 ±0.6		<0.001***

-* *ANOVA test, ** Chi square test, *** Student's t test*
- *(data expressed as mean ± SD)*

Table 4. the laboratory data among the groups *(ANOVA test)*

	Obese OA no (21)	Non-obese OA no (15)	Control no (30)	p
IL-6(pg/ml)	58.5 ±6.5	50 ±6	46.8 ±1.6	<0.001
CRP(mg/L)	5.2 ±0.2	3.5 ±0.5	3.3 ±0.3	<0.001
GR(IU/L)	49.8 ±6.4	55.5 ±9.8	63.6 ±12.3	<0.001
Cholesterol(mg/dL)	210 ±22.2	170.5 ±35.9	161.9 ±19.7	<0.001
Triglycerides(mg/dL)	123.9 ±17.4	75.7 ±34.3	87.8 ±24	<0.001
HDL(mg/dL)	35.7 ±6.3	43.7 ±11.8	43.9 ±6.2	0.005
LDL(mg/dL)	149.7 ±23.3	115.8 ±39	100.4 ±19.6	<0.001

* *Data is expressed as mean ± SD*

Interestingly, the levels of GR in non-obese OA patients were significantly lower than controls and levels of IL-6 were significantly higher than controls (P<0.05) **(table 5)**

Table 5. the comparison of the laboratory findings between subgroups of OA *(paired sample student's t test)*

	Obese OA	Non-obese OA	control	p
IL-6	58.5 ±6.5	50 ±6	46.8 ±1.6	P1<0.001
				P2<0.001
				P3=0.049
CRP	5.2 ±0.2	3.5 ±0.5	3.3 ±0.3	P1<0.001
				P2<0.001
				P3=0.068
GR	49.8 ±6.4	55.5 ±9.8	63.6 ±12.3	P1=0.044
				P2<0.001
				P3=0.05
Cholesterol	210 ±22.2	170.5 ±35.9	161.9 ±19.7	P1<0.001
				P2<0.001
				P3=0.427
Triglycerides	123.9 ±17.4	75.7 ±34.3	87.8 ±24	P1<0.001
				P2<0.001
				P3=0.274
HDL	35.7 ±6.3	43.7 ±11.8	43.9 ±6.2	P1=0.012
				P2<0.001
				P3=0.969
LDL	149.7 ±23.3	115.8 ±39	100.4 ±19.6	P1=0.003
				P2<0.001
				P3=0.183
Glucose	100 ±21.3	101.5 ±54	101.7 ±17.1	P1=0.906
				P2=0.804
				P3=0.993

P1= comparison between obese OA and non-obese OA patients
P2= comparison between obese OA patients and controls
P3= comparison between non-obese OA patients and controls

Noteworthy, age correlated inversely with GR in obese and non-obese OA groups (p=0.037) and (p=0.032) respectively. Also, BMI correlated with GR negatively (p=0.042) and (p=0.04) in obese and non-obese groups respectively.

Among the non-obese OA group, BMI correlated significantly with WOMAC score (p=0.041) and with IL-6 (p=0.004). Meanwhile, GR correlated with IL-6 and inversely with BMI (p<0.001). While among the obese OA group; IL-6 correlated with BMI (p=0.017), and x ray grade with age (p=0.027) .

[4]-Discussion:

OA involves progressive destruction of articular cartilage as a result of various causes including increased oxidative stress with advanced age which has not yet been controlled **(18).**

OA is characterized by increased markers of oxidative stress.Recent studies have suggested that human articular chondrocyte can actively produce ROS .ROS are released during inflammation of synovial membrane of synoviocytes.These radical oxygen species with oxidative activity play an important role in the chondrocyte catabolic program being the mediators and effectors of cartilage damage.The damaging effect of the process is initiated by a chain reaction that provides continue supply of free radicals which initiates further peroxidation**.(19).**

The results as shown in table (1) demonstrate that there is significant difference between OA patients and healthy controls in oxidative stress .Reduced glutathione levels were found to be significantly decreased in patients with OA than in healthy subjects indicating inadequate antioxidant mechanism in patients suffering from OA**.(20).**

IL6 is one of the main regulators of CRP production, may have important role in the inflammatory process. In our results there was significant elevation in IL6 and CRP in serum of OA patients in comparison with control .These results goes with Alonzi etal.**(21)**and Bhattacharya etal. **(18).**Some of KOA were obese in whom serum leptin increases and in addition of being an adipokine it can alone or in combination with IL1 enhance the expression of (iNOS), cyclooxygenase(COX2) and production of nitric oxide(NO),prostaglandin(PGE2),IL6 and IL8**.(22).**

In our study we found significant correlation between GR and IL6,CRP and BMI these results could be explained by the fact that in obese there is increase in leptin hormone which enhances (NO) production in OA cartilage in a dose dependent manner.Western blot analysis with human NOS antibody showed that leptin (10g/ml) induced also NOS expression in cultured cartilage tissue.In addition, leptin (10g/ml) increased (PG) production and (COX2)expression and IL6 and IL8 production in human OA cartilage during 48 h. incubation**.(22).**

There were significant correlation between GR , age , total womac, BMI, KL radiological score indicating its association with severity of OA .These results showed that OA and oxidative stress increase with age and obesity.The reason of KOA in old aged obese patients could be due to the fact that OA is the most common chronic disease in later life , leading to reduction of movement and ultimately gaining of weight.Moreover the subject may already be obese and this obesity has increased the force at weight –bearing joints and may change posture, gait, and physical activity level.Any or all of which may further contribute to altered joint biomechanics that eventually end with old obese individual with knee OA**.(23).**

In contrast to our study Angthong etal. **(24)** found no correlation between glutathione in synovial fluid and KL score and severity of OA .This could be explained that MRI imaging or histological evaluation of articular cartilage may be more useful to assess the severity of OA.

When we further divides OA patients into 2 groups obese and non obese we found that obesity increases the severity of OA , inflammatory markers and oxidative stress and there were significant increase in lipogram between obese and non obese .This is explained by the effect of obesity in increasing the risk of OA by altering knee joint loading patterns such as occurs with varus and valgus laxity and knee joint malalignmen**(25).**

Obesity is also involved in developing OA in non weight bearing joints suggesting that systemic inflammatory mediators contribute to the increased risk of OA with obesity. Proinflammatory cytokines secreted from adipose tissue which are often referred to as adipokines , many of them are found elevated with obesity and metabolic syndrome and are involved in synovitis and cartilage degradation**(26).**

OA ,like cardiovascular disease , involves altered lipid metabolism coupled with increased systemic and cellular expression of pro-inflammatory mediators. A growing number of studies although not all support a role for metabolic factors in OA**.(27).**

As with obesity , a central cellular characteristic of OA is an increase in the production of ROS, cellular oxidation and apoptosis.It seems that with age the antioxidant enzyme system such as glutathione and catalase appear to be impaired leading to defect in oxidative homeostasis ,increase in oxidative stress and increase in cartilage degradation**.(28).**

In none obese OA patients significant increase in CRP and IL6 than healthy control group was found this goes with results of Bhattacharya etal. **(18).**

IL6 one of the main regulators of CRP production was found elevated in synovial fluid of osteoarthritic patients and is involved in inflammatory process and degeneration in OA**.(21).**

In non obese OA there was significant reduction in GR in non obese than healthy control suggesting the role of oxidative stress in OA this goes with the results of Surapaneni and Venkataramana **(29)**showing that altered antioxidant enzyme activities and lipid peroxidation are considered to be a major phenomenon by which ROS can cause cartilage collagen degradation.

Conclusion:

From our results we conclude that oxidative stress plays an important role in pathogenesis of OA and obesity increases OA not only through mechanical overload but through increase in oxidative stress and inflammatory markers also metabolic inflammation associated with obesity is believed to exacerbate the condition.

References:

[1]. Anandacoomarasamy A. and March L. Current Evidence for Osteoarthritis Treatments. Ther Adv Musculoskelet Dis. 2010; 2: 17–28.

[2]. Takeda H, Nakagawa T , Nakamura K and Engebretsen L:Prevention and management of knee osteoarthritis and knee cartilage injury in sports. Br J Sports Med.2011 ;45:304-309.

[3]. Livshits G,Zhai G,Hart DJ,Kota BS ,Wang H , Williams FM, etal.:Interleukin 6 is a significant predictor of radiographic knee osteoarthritis.Arthritis Rheum.2009 ;60:2037-2045.

[4]. Pearle AD,scanzello CR,George S,Mandl LA,Dicarlo EF, Peterson M etal.:Elevated high sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis.Osteoarthritis Cartilage.2007;15:516-523.

[5]. Vlad SC, Neogi T,Aliabadi P,Fontes JD and Felson DT:No association between markers of inflammation and osteoarthritis of the hands and knees.J Rheumatol in press.2011;38:1665-70.

[6]. Martin JA and Buckwalter JA:Aging ,articular cartilage chondrocyte senescence and osteoarthritis.Biogerontology.2002;3:257-264.

[7]. Ostalowska A,Birkner E,Wiecha M ,Kasperezyk S,Kasperezyk A,etal.:Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint.Osteoarthritis Cartilage.2006;14:139-145.

[8]. Rajagopalan S,Meng XP,Ramasamy S,Harrison DG and Galis ZS:Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro:implications for atherosclerotic plaque stability.J Clin Invest.1996 ;98:2572-2579.

[9]. Ostrowska J,Makieta M and Zwierz K:Contribution of oxidative stress in liver injury.Hepatologia.2004;4:28-32.

[10]. Bazzichi L,Ciompi ML, Betti L , Rossi A,Melchiorre D,Fiorini M ,etal.:Impaired glutathione reductase activity and levels of collagenase and elastase in synovial fluid in rheumatoid arthritis.Clin Exp Rheumatol.2002;20:761-766.

[11]. Carlo MD and Loeser RF:Increased oxidative stress with aging reduces chondrocyte survival :correlation with intracellular glutathione levels.Arthritis Rheum.2003;48:3419-3430.

[12]. Altman R, Asch E and Bloch D. Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum 1986; 29: 1039-49.

[13]-Bellamy N, Campbell J, Stevens J, Pilcher L, Stewart C and Mahmood Z.: Validation study of a computerized version of the Western Ontario and McMaster Universities VA3.0 Osteoarthritis Index *J Rheumatol.* 1997;24:2413.

[14]. Kellgren J and Lawrence J. Radiological assessment of osteoarthrosis. Ann Rheum Dis.1957; 16:494-501.

[15]. Ota N ,Nakajima T, Nakazawa I,Suzuki T, Hosoni T,Orimo H etal.:Nucleotide variant in the promoter region of the interleukin-6-gene associated with decreased bone mineral density.J.Hum.Genet.2001;46:267-272.

[16]. Chen HY,Chung AC,Fu P,Huang XR,Lan HY,Liu F,etal.C- reactive protein promotes diabetic kidney disease in mouse modle of type 1 diabetes.Diabetologia.2011; 54:2713-23.

[17]- Goldberg DM and Spooner RJ:In Methods of Enzymatic Analysis(Bergmeyen,H.V.Ed.)3rd edn.1983.Vol 3 ,258-265,Verlog Chemie,Deerfield beach,FL.

[18]-Bhattacharya I, Saxena R and Gupta V. efficacy of vitamin E in knee osteoarthritis management of North Indian geriatric population. Ther Adv Musculoskeletal Dis. 2012; 4:9-11.

[19]-Mane KK,Sardeshmukh AS ,Rathi DB and Suryakar AN :Lipid peroxide and antioxidants in arthritis.Medical Journal of Western India.1999;27:108-110.

[20]-Maneesh M,Jayalekshmi H,Suma T,Chatterjees S and Singh TA:Evidence for oxidative stress in osteoarthritis.Indian journal of clinical biochemistry.2005;20:129-130.

[21]- Alonzi T, Fattori E,Lazzaro D,Costa P, Probert L,Kollias G etal.:Interleukin 6 is required for the development of collagen induced arthritis.J Exp Med.1998; 187:461-468.

[22]-Munaf SD, Israa AA and Mohammed IH:Expression of serum cytokines (IL6 and IL8) and oxidative stress marker (MDA) in patients with knee osteoarthritis.The Iraqi Post Grad. Med. J.2014;13:187-194.

[23]- Messier SP,Daries AB,Moore DT,Davis SE,Pack RJ and Kazmar SC:Severe obesity effects on foot mechanics during walking.Foot Ankle Int.2004;15:29-34.

[24]- Angthong C, Morales NP, Sutipornpalangkul W, Khadsongkram A, Pinsornsak P and Pongcharoen B.:Can levels of antioxidants in synovial fluid predict the severity of primary knee osteoarthritis:a preliminary study. Springerplus. 2013;2:652.

[25]- Felson DT,Goggins J,Niu J,Zhang Y and Hunter DJ:The effect of body weight on progression of knee osteoarthritis is dependent on alignment.Arthritis Rheum.2004; 50:3904-9.

[26]- Lago F,Dieguez C,Gomez-Reino J and Gualillo O :The emerging role of adipokines as mediators of inflammation and immune responses.Cytokine Growth Factor Rev.2007; 18:313-25.

[27]-Griffin TM,Huebner JL,Kraus VB,Yan Z and Guilak F:Induction of osteoarthritis and metabolic inflammation by a very high fat diet in mice:effects of short term excecise.Arthritis Rheum.2012;64:443-53.

[28]- Rhee SG,Yang KS,Kang SW,Woo HA and Chang TS.:Controlled elimination of intracellular H(2)O(2):regulation of peroxiredoxin , catalase and glutathione peroxdase via post-translational modification.Antioxid Redox Signal.2005;7:619-26.

[29]- Surapaneni KM and Venkataramana G:Status of lipid peroxidation , glutathione, ascorbic acid,vitamin E and antioxidant enzymes in patients with osteoarthritis.In J Med Sci.2007 ;61:9-14.