Expression of Serum Cytokines (IL-6 & IL-8) and Oxidative Stress Marker (MDA) in Patients with Knee Osteoarthritis

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ABSTRACT:

BACKGROUND:

Osteoarthritis is a degenerative joint disease, occurring primarily in older persons. Although many factors initiate this disease, progress to overt OA needs many biological substances like IL-6 and IL-8 that reduce cartilage repair ability or like reactive species that induce cartilage destruction through direct degradation of matrix components.

OBJECTIVE:

To shed the light on the expression of serum IL-6 and IL-8 levels, and production of serum malondialdehyde level (marker of oxidative stress), then estimation of the correlation among serum IL-6, IL-8 and MDA levels in patients with knee osteoarthritis.

PATIENTS AND METHODS:

This study was conducted in AL-Kadhemiya Teaching Hospital during the period from April 2011 to December 2011. The study included two groups: knee osteoarthritis(KOA) group of patients and the control group. Each group was further sub-divided into: obese & non-obese according to their BMIs. Sixty osteoarthritic patients aged 40-70 years were chosen depending on medical history, clinical examination, and radiographic observations. 10 ml of blood needed for assessment of the above makers. Thirty healthy control (age and sex-matched) were enrolled in this study.

RESULTS:

Mean serum IL-6 and IL-8 levels were significantly higher in KOA patients compared to control group for both ($P<0.001$), with the highest levels seen in the obese KOA group. Mean serum MDA level was significantly higher in patients with KOA compared with that in control ($P<0.001$). Significant negative correlation was observed between serum IL-6 and IL-8 levels.

CONCLUSION:

IL-6, IL-8 & MDA have important role in the pathogenesis of KOA. Receptors of IL-6 and IL-8 on chondrocytes could be considered as target for future therapy of any arthritic inflammation and also to consider MDA reduction in patients with knee osteoarthritis, as part of treatment of osteoarthritis.

KEYWORDS: IL-6, IL-8, MDA

INTRODUCTION:

Osteoarthritis (OA) is a degenerative joint disease, occurring primarily in older persons, characterized by erosion of the articular cartilage, hypertrophy of bone at the margins (i.e., osteophytes), subchondral sclerosis, and a range of biochemical and morphological alterations of the synovial membrane and joint capsule [(1)]. Major factors affecting degree of risk for developing OA include: Age, joint location, obesity, genetic predisposition, gender, miscellaneous factor [(2-6,8)].

Angiogenesis and inflammation are closely integrated processes in osteoarthritis (OA) and may affect disease progression and pain. Mechanisms by which synovitis exacerbates structural damage in OA are likely to be complex [(9)]. Many substances involved in regulation of angiogenesis, some of them are stimulators like; bradykinin, prostaglandin E2, nitric oxide, angiotensin, histamine, vascular endothelial growth factor, Angiopoietin-1, Interleukin- 1, Interleukin-18, Interleukin- 8 (IL-8) , Interleukin- 6 (IL-6), …etc. Others have inhibitory action on angiogenesis like;

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Thrombospondin, Interferon-γ, Leukaemia inhibitory factor, Tissue inhibitors of matrix metalloproteinase -1 and -2, transforming growth factor-β, Hyaluronic acid (high molecular weight), Platelet factor-4 , Somatostatin, interleukin- 4, …etc[10]. Many substances and cytokines, are involved in the pathogenesis of OA. IL-6 is an IL-1 inducible protein produced by T- cells and monocytes and is spontaneously expressed by cultured fibroblast-like synoviocytes . Its receptor is of high affinity, IL-6R α-chain (p80) + gp130 ; gp 130 is non-binding and forms homodimer when complexed with IL-6R α-chain, acts to transduce signal . IL-6 plays a dual role by increasing IL-1Ra, soluble TNF receptor, and TIMP, while also enhancing immune cell function and inflammation.It also stimulate collagenase activity and MMP production from chondrocytes . IL-8 is another chemokine that is expressed at high level by chondrocyte when activated by IL-1 & TNF-α in arthritic joint.It binds to its high affinity CXCR1 & CXCR2 and modulate chondrocyte function associated with cartilage degradation. It also function as neutrophil & lymphocyte chemoattractant & activation factor [11].

Formation of reactive oxygen species (ROS) and reactive nitrogen species(RNS) has been suggested to play significant roles in various diseases, including OA. Oxidative stress is involved in cartilage destruction in OA and also has indirect action by activating collagenases and up-regulating genes encoding enzymes involved in matrix degradation and cytokines production . Lipid peroxidation is initiated by many reactive species among which superoxide and hydroxyl radical. Lipid peroxides are damaging substances that cause properties alteration of all cellular membranes and gradients[12]. Malondialdehyde is a marker of lipid peroxidation [13].

The aim of this study is to evaluate serum malondialdehyde (MDA) level and the cytokines IL-6 & IL-8 in obese and non obese KOA subjects, to asses degree of systemic oxidative stress and to correlate these proinflammatory markers.

SUBJECTS MATERIALS AND METHODS:

This study was conducted in AL-Kadhemiya Teaching Hospital during the period from April 2011 to December 2011, (Rheumatology and Rehabilitation Consultation Department). The study included two groups: knee osteoarthritis group of patients and the control group. Each group was further sub-divided into: obese & non-obese according to their BMIs. Sixty osteoarthritic patients aged 40-70 years were chosen depending on medical history, clinical examination, and radiographic observations.

Regarding the control group, thirty age-matched, apparently healthy subjects were selected from patients' relatives attending the Rheumatology and Rehabilitation Consultation Department.

A pre-tested questionnaire was designed to obtain information about age, gender, height, weight, and past-medical history.

Body Mass Index (BMI) was the only anthropometric parameter specified. It was calculated by weight (in kilogram) divided by the square of height (in meter), weight and height were measured by the same scale for all subjects.

BMI = weight (kg)/ square height (m^2)

Blood sampling:

About 10 milliliters of venous blood was aspirated using disposable syringes and needles. The blood was allowed to clot in plain tubes for 30-45 minutes at room temperature and serum was recovered by centrifugation at 3000 rpm for 10 minutes and transferred into plain plastic tubes and kept frozen at - 20 °C until the time of assay.

Estimation of Serum Cytokines:

The quantitative determination of serum IL-6 and IL-8 level was conducted by an Enzyme-Linked Immunosorbant Assay (ELISA) technique, using BioSource IL-6 and IL-8 EASIA kits.

Serum MDA measurement:

Malondialdehyde (MDA) was measured using the Thiobarbituric acid (TBA) method of Buege and Aust . It reacts with TBA to give a pink color that is read at (535 nm). For blank, water is used instead of serum[14] .

Statistical analysis

All data were coded and entered using the program statistical package for social sciences (SPSS) version 12 under windows XP. Descriptive data was summarized using mean, standard error (SE).Student́ s t-test was used to estimate differences between groups. Pearsoń s correlation between variables. P values < 0.05 were considered statistically significant.

RESULTS:

Clinical and Histogram distribution of subjects:

Sixty (60) patients with KOA were included in this study. Patients were classified clinically into Obese KOA (n= 36) and non-obese (n= 24).

Age distribution:

Mean age of obese KOA group was significantly higher than that of non-obese KOA (P= 0.001), and the mean age of obese-control was significantly higher than that of non-obese-control (P= 0.027).

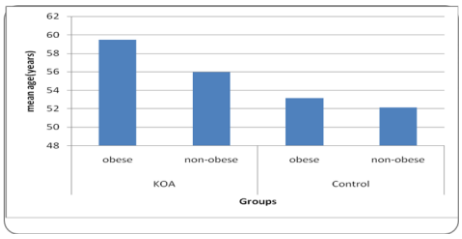


Figure 1 : The age distribution of KOA patients (obese and non-obese) and healthy control (obese and non-obese)

Gender distribution:

There was no significant statistical difference between the frequency of the male and that of female patient groups as revealed by Students t-test. (P=0.76).

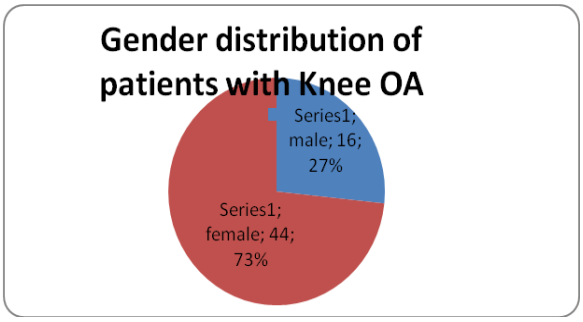


Figure 2 : Gender distribution of 60 patients with KOA.

Body Mass Index (BMI):

36 (60%) of the patients had a BMI> 25 (i.e. obese). Students t- test revealed a significant differences between patients and control group (p= 0.025).

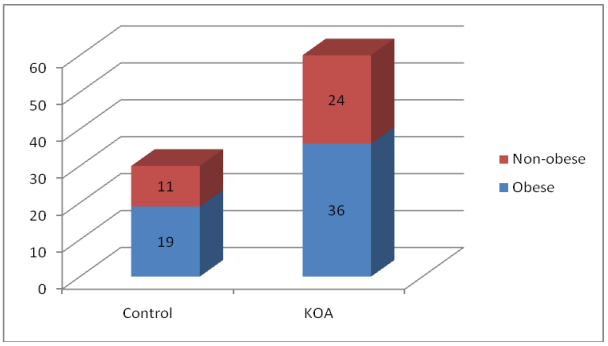


Figure 3 : Obese and non obese distribution of patients and healthy controls.

Family history (FH):
The number of subjects with positive FH in the control group was 0, while the number of patients with positive FH in the KOA group was significantly higher than that of control (P< 0.001).

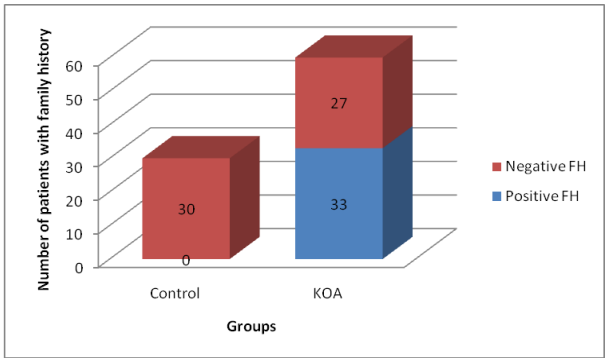


Figure 4 : Distributions of patients with family history and patients without family history in 60 patients with KOA and healthy Controls.

Cytokine profile in Knee osteoarthritic patients and healthy controls:-
Serum levels of IL- 6 & IL-8:

There was a highly significant difference between controls and disease group (P< 0.001), with the highest level seen in the obese KOA group as shown in Table-1.

Table 1 : Serum Cytokine Profile in KOA and Control Groups.

Parameter IL- 6				
Groups	obese-control	Non-obese-control	Obese-KOA	non-obese-KOA
Number	19	11	36	24
Mean	5.71	5.26	188.88	119.75
Standard Error of Mean	0.36	0.69	7.597	3.33
P value	t-test significant difference between obese – control & non obese-control (P< 0.001)			
Parameter IL-8				
Groups	obese-control	Non-obese-control	Obese-KOA	non-obese-KOA
Number	19	11	36	24
Mean	21.75	18.26	329.95	309.70
Standard Error of Mean	2.16	1.39	8.575	6.679
P Value	t-test significant difference between obese – control & non obese-control (P< 0.001)			

Serum Malondialdehyde (MDA):
The mean MDA level was significantly higher in patients with KOA than that in control (P<0.001).

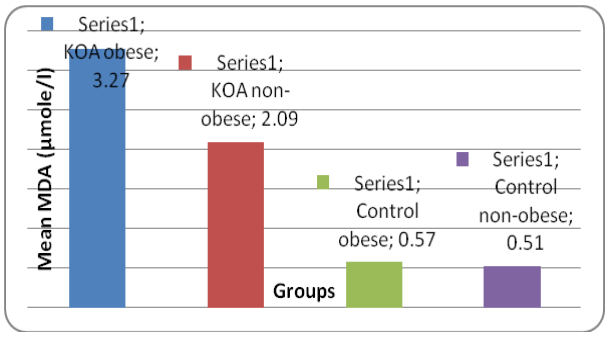
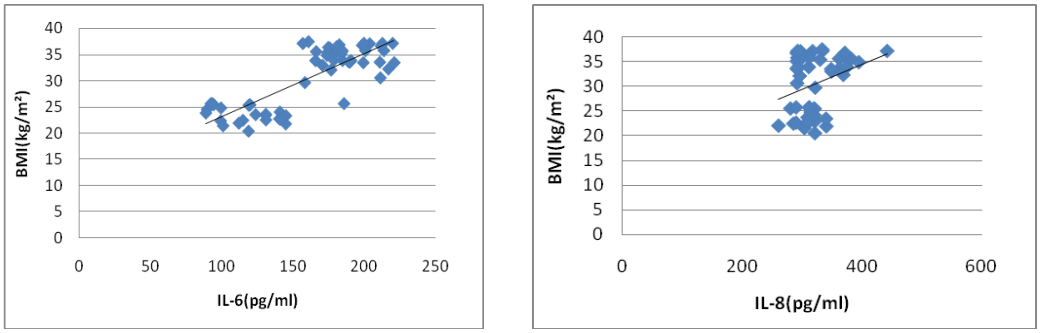


Figure 5 : Serum MDA concentration among the study groups.

Correlations:

Correlation between Serum Cytokine Profile and BMI in KOA:

A significant positive correlation was observed between Cytokine profile (IL-6, IL-8) and (BMI) in patients with KOA(r= 0.59,P < 0.004 ; r= 0.37, P < 0.01, respectively).



A B

Figure 6 : Correlations between Serum cytokines and BMI; (A) IL-6 Level and BMI; (B) IL-8 and BMI in KOA patients.

Correlation between Serum MDA and BMI in KOA:

A significant positive correlation was observed between Oxidative stress marker (MDA) and (BMI) in patients with KOA(r= 0.70 , P < 0.003) .

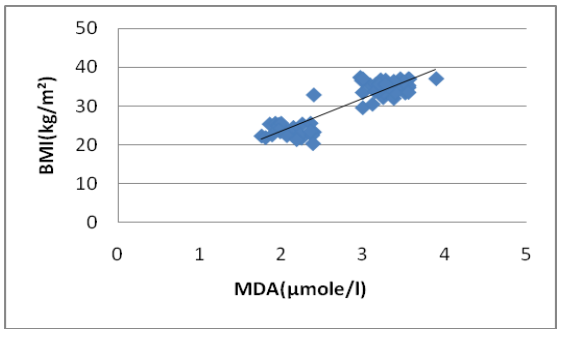


Figure 7 : Correlation between Serum MDA level and BMI in KOA patients.

Correlation between Serum Cytokines (IL-6 and IL-8) and *Serum* Oxidative stress marker (MDA) in KOA patients:

There was significant positive correlation between serum levels of IL-6 and MDA (r= 0.65, in KOA group.(0.01) 0.001 < 0.001)as well as between IL-8 and MDA (r= 0.53, P <, P

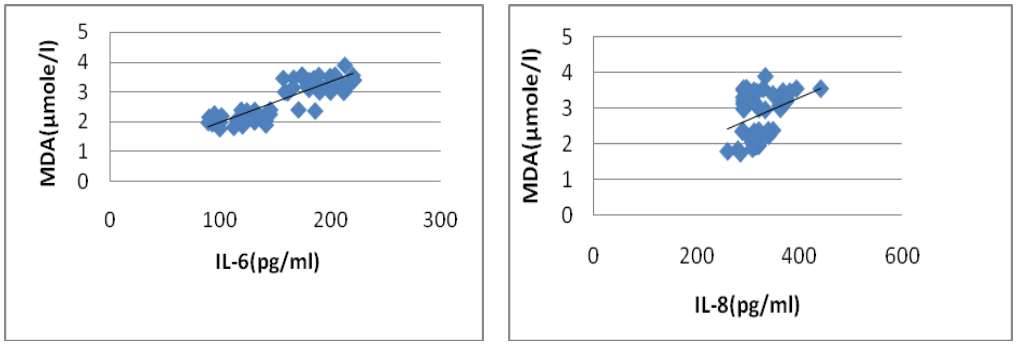


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Figure 8 : Correlations between serum cytokines and MDA; (A)Serum IL-6 Level and MDA; (B) serum IL-8 level and MDA in KOA patients.

Correlation between Serum IL-6 and IL-8:

A significant Negative correlation was observed between IL-6 and IL-8 in patients with KOA as seen in Table-2.

Table 2: Correlation between Serum IL-6 and IL-8 in KOA patients.

KOA				
Correlation	Type	r	P	Significance
IL-8 and IL-6	Negative	-0.407	P= 0.001	Significant

DISCUSSION:

The results shown in Fig.-1 indicate that the KOA that appears in non-obese patients was at younger age and is most probably due to previous trauma ; this is in accordance with Frobell et al [15], or to repetitive high impact sports which strongly associated with joint injury that increase the risk for lower limb OA;this is in accordance with Chaudhari et al[16].Other less probable causes are genetic predisposition, Vitamin D and or C deficiency, metabolic diseases (gout, homocystinurea,..), joint infections and allergies as reported by Harris et al [11] . As most of the above diseases appear early in life, so joints would undergo morphologic and structural changes in articular cartilage pre-, intra-, or just post- adulthood, leading ultimately to OA.

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.Moreove, the subject may already be obese, and this obesity has increased the forces 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 [5,6].

The data presented in Fig.-2 showed no significant difference between frequency of male and that of female patient group.This could be explained by the fact that the age of the female patient group included both pre- and post-menopause, and not one of them. Clearly women have a lower prevalence of OA than men before age of 50 years, while there is marked increase in prevalence among women after 50, particularly in the knee [10].

Nuclear estrogen receptors (ERs) have been detected in articular chondrocytes of humans, and

in human growth plate chondrocytes. One direct effect of estrogen on cultured chondrocytes is up-regulation of ER. An increase in ER has been associated with increased proteoglycans synthesis in rats[8,17].Changes in the prevalence of KOA pre-& post-menopause women is due to estrogen deficiency[12].

Fig.-3 showed that there was a high significant difference between obese and non-obese patients with KOA. Obesity is an important risk factor for osteoarthritis (OA) in weight-bearing joints, but also in hand joints, pointing to an obesity-related metabolic factor that influences on the pathogenesis of OA.

Patients with positive family history of KOA were significantly higher than those with negative family history.This is shown in Fig.-4 . This result is expected due to inheritance of OA related- genes, or inheritance of mutant and or deleted genes, those that affect type or strength of articular collagen fibers, structural protein of extracellular matrix, integrity of articular cartilage, or causing generation of autoimmune antibodies directed against articular structures [11].

In Table-1, a significant difference in serum level of IL- 6 and IL-8 was observed between control subjects and patients with KOA, most obvious in obese patients.This could be explained by the fact that patients in this study were obese, a factor stimulating expression of IL-6 and IL-8 in serum, i.e. serum leptin level increases in obese people, and in addition of being an adipokine it can alone or in combination with IL-1 enhance the expression of (iNOS), (COX-2), and production of NO, PGE2, IL-6, and IL-8[12] . Concerning the non- obese patients, there was still significant difference between their serum IL-6 and IL-8 levels and that of control.The most probable explanation is that most of non-obese patients were young, bringing to mind the possibility of presence of systemic diseases (due to genetic predisposition) that stimulate the occurrence of OA earlier than patients with a single stimulating factor (obesity) [11]. There was a negative correlation between IL-6 level and IL-8 as shown in Table-2.The probable increment in one of them could act as inhibitor to the expression of the other, by negative feedback mechanism.

In Fig.5 , a significant difference was observed between serum MDA of patients with KOA compared with the control, indicating that oxidative stress in OA is much higher than the ability of protective reducing system to overcome [2] . Moreover there was a significant positive correlation observed between each of IL-6 & IL-8 with BMI and MDA in patients with KOA as shown in Figs.-6 & 8,respectively . This is mostly due to the effect of leptin, as it enhances NO production in OA cartilage in a dose-dependent manner.Western blot analysis with human iNOS antibody showed that leptin (10 g/mL) induced also iNOS expression in cultured cartilage tissue. In addition, leptin (10 g/mL) increased PG production and COX-2 expression, and IL-6 and IL-8 production in human OA cartilage during 48 hours incubation [12] . Also, Fig.-7 shows a significant positive correlation between oxidative stress marker MDA and BMI in patients with KOA. These results could be explained by the fact that most of candidate were obese, in whom leptin, (a hormone that is synthesized by white adipocytes and has a strong correlation with the amount of adipose tissue and with BMI) is higher than non-obese [12].

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