

# SERUM ESTROGEN AND INTERLEUKIN-6 LEVELS IN POSTMENOPAUSAL FEMALE OSTEOARTHRITIS PATIENTS

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## ABSTRACT

The objective of the present study is to determine whether the levels of endogenous estrogen and interleukin-6 (IL-6) are associated with osteoarthritis (OA) in postmenopausal women. Forty-three patients who were suffering from osteoarthritis were studied and twenty control normal subjects having no symptoms of osteoarthritis disease, were also included in the study. The estrogen hormone and interleukin-6 were measured in the serum by Enzyme linked immunosorbent assay technique. Interleukin-6 and estrogen both were detected in serum. In osteoarthritis patients IL-6 levels were high as compared to control subjects ( $p < 0.001$ ) and estrogen levels in OA postmenopausal females was decreased significantly ( $p < 0.01$ ) when compared with control subjects. There was a minimal inverse correlation between IL-6 and estrogen ( $p < 0.05$ ) in the sera of postmenopausal osteoarthritic women. These results indicate that estrogen deficiency after menopause may cause alterations of IL-6 in postmenopausal osteoarthritic patients.

**Keywords:** Estrogen, Osteoarthritis (OA), Interleukin-6 (IL-6).

## INTRODUCTION

Osteoarthritis (OA) is a clinical classification for a combination of pathological conditions involving the progressive degeneration of articular cartilage, remodelling of sub-chondral bone, and synovitis that is usually limited to the affected joint. OA is considered the cumulative result of mechanical and biological events that induce an imbalance between the degradation and synthesis within articular joint tissues. However, in patients with rheumatoid arthritis, more severe destruction throughout the cartilage and bone tissue in the affected joint tends to occur. OA has been demonstrated to be a complex process that includes multiple changes in joint components such as cells, matrix and molecular production (Blagojevic *et al.*, 2010). It is believed that cytokines and growth factors play an important role in pathophysiology of OA. They are closely associated with functional alterations in synovium, cartilage and subchondral bone. Cytokines activate the chondrocytes, which in turn could produce catabolic factors such as proteases and proinflammatory cytokines (Chevalier *et al.*, 2009). The major proinflammatory cytokine believed to be involved in the pathology of OA is IL-6 (Hough, 1997). IL-6 stimulates proteases and prostaglandin production (Plows *et al.*, 1995). The high levels of IL-6 were also found in OA (Uson *et al.*, 1997). Both IL-6 and soluble IL-6 receptors in synovial fluids play a role in joint destruction in arthritis by enhancing osteoclastogenesis (Kalle *et al.*, 2010).

Recently, Estrogen deficiency was reported to upregulate the expression of the IL-6 receptors and to enhance osteoclastic activity (Kishimoto *et al.*, 2001, Sanchez *et*

*al.*, 2009). Several studies noted that spontaneous increase in the expression and secretion of cytokines IL-1, IL-6 and TNF alpha were associated with estrogen deficiency in ex vivo cultures of circulating monocytes (Pradeep Sherma *et al.*, 2006), (Kitawaza *et al.*, 1994), bone marrow macrophages (Bismar *et al.*, 1995), and osteoblasts.

Estrogen deficiency has also been shown to enhance the cofactors of cytokine action, thus amplifying the effects of the cytokine. In humans elevated soluble interleukin-6 receptors concentrations in circulation have been observed after surgical and natural menopause (Keller *et al.*, 2001; Yamamoto *et al.*, 2000).

The aim of study was therefore to describe the relation between estrogen and circulating levels of IL-6 among postmenopausal female osteoarthritic patients.

## MATERIALS AND METHODS

The present study was conducted in Department of Orthopaedic, DOW University of Health and Sciences, Karachi, Pakistan, from January to August 2003. A total of sixty three postmenopausal female subjects (healthy and OA subjects) were enrolled in this study. A 43 of the subjects (mean age 56.5 years) have OA. The diagnosis of OA was done by clinical and radiological evaluation. All the subjects answered a questionnaire concerning medical history, present medications, menopausal state and age at menopause. Menopause was defined as no menstruation for more than 12 months or surgical menopause with documented history of both oophorectomy and age over 45 years. Patients taking any hormone replacement therapy, having any metabolic disease, rheumatoid arthritis, gout, systemic lupus erythromatosis (SLE) and uncertain menstruation history were excluded from the

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study. Twenty healthy postmenopausal females (mean age?) were regarded as control group. A 5ml of blood was collected from all the subjects and serum was separated and stored at -70 c until used. The serum IL-6 and estrogen were estimated by enzyme linked immunosorbent assay technique (Kat #EIA-3293 and Kat # DSL-10-4300 supplied by Germany and USA respectively). Body Mass Index (BMI) was also measured and expressed as Kg/m<sup>2</sup>.

Participation in the study was voluntary and based on the participants' informed consent. The Hospital approved the study protocol.

Statistical analysis: The data are expressed as mean ± SEM unless otherwise stated. Student-t test and pearson coefficient correlation test were used. Values of P < 0.05 were regarded as significant.

## RESULTS

A total of 43 female postmenopausal osteoarthritis patients and 20 control subjects participated in the study. Table 1 shows the age and BMI of controls and osteoarthritic subjects, but no significant differences were found between BMI of patients and control subjects.

Table 2 shows the status of IL-6 and estrogen hormone in osteoarthritic female patients and control group. IL-6 was statistically increased (p<0.001), and estrogen hormone was significantly decreased (p<0.01), in female patients as compared with control group.

Fig. 1 shows the inverse mild relationship of IL-6 and estrogen hormone in OA female group (p<0.05).

**Table 1:** Age and BMI of control and osteoarthritis postmenopausal female patients.

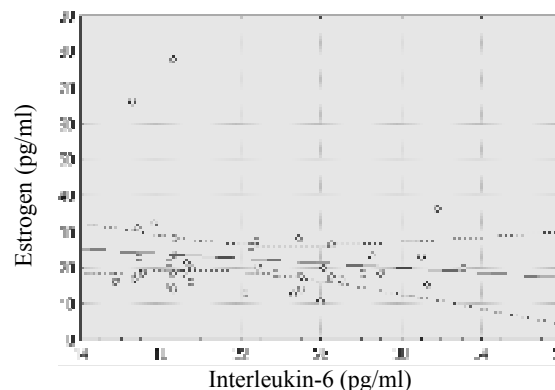
Characteristic	Control (n=20)	Patients (n=43)
Age (years)	56 ± 2.02 (49-63)	57 ± 1.9 (48-65)
BMI (Kg/m <sup>2</sup> )	20.9 ± 0.73	21.6 ± 0.92

Values are the mean ± S.E.M.

**Table 2:** Status of Interleukin-6 and estrogen hormone in osteoarthritis postmenopausal female patients.

Characteristic	Control (n=20)	Patients (n=43)
Interleukin-6 (pg/ml)	4.57 ± 0.15	22.21 ± 0.77*
Estrogen (pg/ml)	31.13 ± 2.3	22.63 ± 1.89**

Values are the mean ± S.E.M., \*p<0.001, \*\* p<0.01



Correlation: r = -0.1387

**Fig. 1:** Correlation of interleukin-6 vs. Estrogen in female osteoarthritis patients.

## DISCUSSION

Osteoarthritis is a complex disease whose pathogenesis includes the contribution of biochemical and metabolic factors altering homeostasis of articular cartilage and subchondral bone (Blagojevic *et al.*, 2010). During OA degradation of cartilage of knee joints causes inflammation may be due to the release of inflammatory cytokines, which are responsible for proteolytic digestion of cartilage in joints. In our study female subjects suffering from OA showed a decreased levels of estrogen hormone (p< 0.001) and IL-6 increased significantly p<0.01 as compared to healthy control subjects (table 2). This is consistent with the studies of Masahiko *et al.* (2005), Iannone and Lapadula (2003) and Fernandes *et al.* (2002).

Increased secretion of IL-6 is directly related to activation of osteophytes in synovial fluid of OA patients. Cytokines also stimulate chondrocyte, which are responsible for enhanced activity of proteolytic enzymes i.e. matrix metalloprotease enzyme (MMPs) (Fernandas *et al.* 2002). The cartilage are prime site of OA disease and is very sensitive to change in sex hormones level. The menopause coincides with the appearance of many symptoms, which are associated with OA. In humans the relationship between estrogen and IL-6 in OA remain to be clarified. The biological significance of serum levels of IL-6 and the mechanisms by which its production is regulated in vivo have not been fully elucidated. During inflammatory joint disease, chondrocytes are likely to contribute to intra-articular IL-6. IL-1 seemed to be the most potent inducer of IL-6 in chondrocytes (Bonnetand Walsh 2005). Estrogen withdrawal might also cause the release of higher levels of soluble IL-6 receptors in the bone marrow cells. In endometrial stromal cells, the estrogen strongly inhibited IL-6 production (Deswal *et al.*, 2001, Livshits *et al.*, 2009).

In the context of postmenopausal OA, it can be concluded that the increased production of cytokines (IL-6) may be due to estrogen deficiency. Though further studies are suggested, IL-6 may be used as a suggestive marker for the assessment of OA.

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