Study the Serum IL-6 Level between Rheumatoid Arthritis Patients and Control

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Abstract
This study involved (50) patient's that complaining rheumatoid arthritis from both genders (7 females and 43 males). Their ages range from (26-68) years there are taken from hospitals and clinics, in addition to the control which involve (30) person that look normal and their ages ranges from (23-54).
Enzyme linked immuno-sorbent assay (ELISA) has been used to measure the level of IL-6 in serum, in which the medium of IL-6 in patients was significantly higher ($p \leq 0.01$) than control group.
The study show's significant difference ($p \leq 0.01$) in level of IL-6 between males and females and between age groups.

Keywords: Interleukin 6 (IL-6), Rheumatoid arthritis (RA).

Introduction
Interleukin 6 (IL-6) is the most abundantly expressed cytokine in rheumatoid synovium [(1)]. In vitro activities of this pleiotropic cytokine have been catalogued and several activities previously ascribed to interleukin 1(IL-1) are now believed to be mediated also by IL-6. In particular IL-6 mediates acute phase protein synthesis and terminal B cell differentiation [(2)].
Previous studies have shown that IL-6 levels are higher within synovial fluid than in serum [(3, 4)]. In most patients synovial fluid macrophages do not spontaneously produce
IL-6, suggesting that IL-6 is derived from cells within the synovium[(5)]. Using cytokine probes Firestein *et al.* have shown that synoviocyte IL-6 is derived from non-T lymphocytes, type B synovial lining cells and fibroblasts [(1)]. Synoviocyte derived IL-6 in patients with rheumatoid arthritis (RA) is enhanced by IL-1 and tumour necrosis factor [(5)] and can stimulate hepatocyte synthesis of acute phase proteins in vitro.
Rheumatoid arthritis (RA) is a chronic, systemic disease characterized by inflammation and cellular proliferation in the synovial lining of joints that can ultimately result in cartilage and bone destruction. Although RA has been the subject of innumerable investigations, the etiology and pathogenesis of the disease remain incompletely understood [(5)].
It is clear, however, that cytokines play a key role in driving synovial cell activation leading to joint destruction. Among the most important of these cytokines in RA are TNF-α and IL-1β, whose capacity to induce inflammation has previously led to their historical designation, along with IL-6, as endogenous [(6)].
Pyrogens it is not surprising, therefore, that these cytokines have been targeted in the development of RA therapies. While blockade of TNF-α or IL-1β effects is efficacious for many patients with RA, not all patients respond adequately or maintain a response to these strategies [(6-8)]. Moreover, increased risk of infection (such as tuberculosis), as well as concern about malignancy and other adverse outcomes, [(9, 10)] have raised concerns about the use of currently available biologics, particularly anti-TNF-α agents. Accordingly, the search for new targets for safe and effective therapy of RA continues.
In addition to IL-1β and TNF-α, synovial fluid and synovium from RA patients contains IL-6 activity that is significantly elevated compared to control patients with osteoarthritis [(11)]. Moreover, increased IL-6 activity correlates with elevations of acute phase reactants, as well as other signs of inflammation, including fever and anemia [(12)].
Objective: In this article, we examine the role of IL-6 in RA pathogenesis. We review the basic biology of IL-6, and discuss the clinical data.

Materials and Methods
Subjects Selection
Patients were excluded from the study they had comorbid diseases, overlapped with other connective tissue diseases or inflammatory arthritis, and vasculitis. They were recruited from private clinic in different region of Salahaddin governorate during the period from (September 2013-December 2013). The study involved (50) patient's that complaining rheumatoid arthritis from both genders (7 females and 43 males) and 30 healthy individuals (20 females and 10 males; age: matching with patients) Their ages range from (26-68) years, who were staff of Tikrit University and hospital workers were considered as a control group.
We used paper clinical research form through interview and questionnaires. We asked the patients about age, sex, disease duration, and disease activity. Full history was taken and complete clinical exam of participants was done.
Sample Collection
Under complete aseptic conditions, (3) ml of venous blood were collected from all the participants at the time of clinical examination, then were left to clot serum was separated, then kept at -20°C for determination of IL-6 levels.
IL-6 value
Serum IL-6 levels were value in both RA and healthy controls using Enzyme linked immuno-sorbent assay (ELISA) according to the manufacturer's instructions (Abcam-UK).

Results and discussion

Frequency of male was more than female in patient and control (86.6 % and 86 % respectively). Ages of patients range between (26-68) years with median (45.16) years while in control, ages range between (23-54) years with a median (42.9) years (Fig 1). And there was correlation between IL-6 and age p > 0.01 (Fig2)

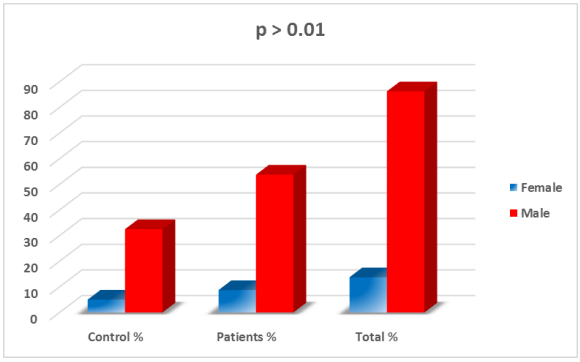

Fig: (1) The proportion of the study samples.

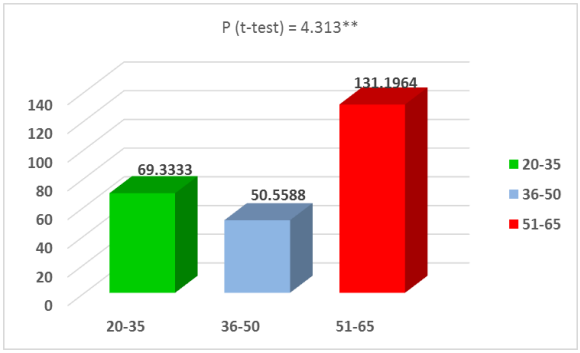

Fig: (2) Correlation between IL-6 and age.

However, while RA-related autoantibodies are likely highly predictive of future symptomatic disease, they may be present for many years prior to the onset of articular symptoms, and are therefore perhaps less useful in isolation for prediction of imminent symptomatic disease [13,14]. As such, assessment of additional biomarkers in the preclinical period of RA may aid in the development of models to predict accurately the timing of the onset of symptomatic disease. Additionally, as demonstrated by our prior findings [15] and those of Bos *et al.* [16] Showing that individuals with an older age at diagnosis of RA have a longer, age-related duration of other preclinical elevations in biomarkers may influence the development of models to predict the timing of the onset of symptomatic future RA.

Serum IL-6 level were significantly higher in patients with RA than the control (3.3 ± 0.45 pg/ml in control and 130.83 ± 197.96 pg/ml in patients) Fig 3.

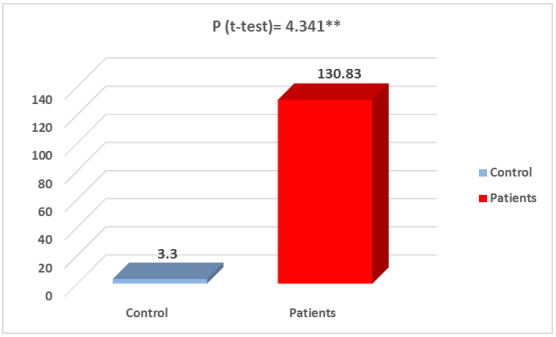

Fig: (3) Serum Il-6 level in patient and control.

RA was one of the first inflammatory diseases in which an important increase of the expression of IL-6 was described, both in plasma as in synovial tissue [17]. Its increase in plasma fluctuates rapidly in the same direction as activity, severity, and positive response to therapy, in the same way as its indirect marker, CRP [18, 19].

The main effects of the systemic increase of IL-6 in RA are the increase in APR and, therefore, secondary amyloidosis (SAA), chronic disease anemia and possibly, systemic osteoporosis and an increase in vascular risk.

In the synovial tissue, both in the mononuclear cells of the infiltrate as in synovial fibroblasts or synoviocytes, seem to contribute to the excessive synthesis of IL-6 [1]. Synoviocyte hyperplasia, the effects of cytokines such as TNF or IL-1 on them and finally a stable phenotypical alteration, contribute to overproduction of IL-6 by these cells, a property that they maintain even when they are cultured ex vivo [20]. Many of the cells implicated in synovitis (chondrocytes, synoviocytes, fibroblasts, endothelial cells) do not have an IL-6R and, however, are sensitive to the effects of IL-6 through a trans-signaling mechanism. There is abundant soluble IL-6R in the joint environment which comes from infiltrating leukocytes, guaranteeing the action of IL-6 on all of these cell elements [21].

In Fig 4 seen there were difference in IL-6 level between men (90.992 ± 21.461 pg/ml in male, and 32.909 ± 17.146 pg/ml in female).

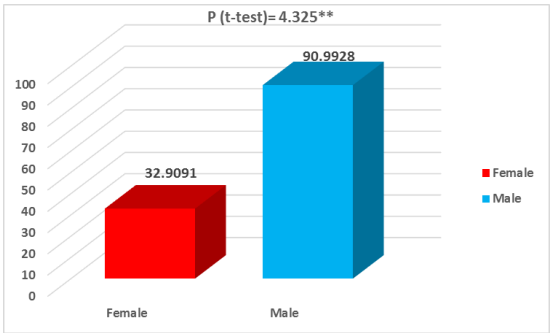

Fig: (4) Serum IL-6 level in male and female.

Estrogens are well known inhibitors of IL-6 secretion [22]. As proposed by Straub *et al.* [25], adipose tissue-derived estrogens in postmenopausal women would not be sufficient to reduce IL-6 in a similar way as endogenous estrogens in premenopausal women, *i.e.* those subjects who have been evaluated in this study.

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دراسة مستوى IL-6 في مصل مرضى المفاصل الرثوي والأصحاء

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الملخص

شملت هذه الدراسة (50) مريضاً بالالتهاب المفاصل الرثوي من كلا الجنسين (7 إناث و 43 ذكور) تراوحت أعمارهم بين (26–68) سنة من ضمن الحالات الواردة إلى المستشفيات والعيادات الخارجية، فضلاً عن مجموعة السيطرة التي شملت (30) شخصاً من الأصحاء ظاهرياً وقد تراوحت أعمارهم بين (23–54).

تم استخدام اختبار الامتزاز المناعي المرتبط بالانزيم Enzyme Immuno-sorbent assay (ELISA) لقياس تركيز IL-6 في المصل وقد كانت قيمة المتوسط الحسابي لـ IL-6 لدى المرضى أعلى وذات فرق احصائي عالي المعنوية ($P \leq 0.01$) عنها في مجموعة السيطرة.

وقد أظهرت الدراسة اختلاف عالي المعنوية ($P \leq 0.01$) في مستوى IL-6 بين الذكور والإناث وكذلك بين المجاميع العمرية.