Prognostic significance of IL-17, and IL-13 along with IL-23R gene polymorphisms in patients with rheumatoid arthritis in Iraqi patients

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Abstract:
Rheumatoid arthritis is a systemic disease with very complex pathogenesis and feature of chronic synovitis. The biological effect of polymorphism on expression and functionality of IL-23R such as SNP can have functional and phenotypic consequences that make IL-23R as a risk factor for RA disease. Moreover recently there is a new trends to find out a new noninvasive prognostic biomarker for RA disease which may help in fallowing up disease. Thus the aim of present work is to find out if there prognostic value for IL-13 and IL-17 in Rheumatoid arthritis through linking its expression level with disease activity score (DAS). Also To study if there is a role for IL-23R 11209026 gene polymorphism in disease susceptibility in Iraqi community by using healthy volunteer as a control group. To achieve this goal a Case control study has been conducted on 40 patient and 40 matched apparently health control. serum IL-17 and IL-13 concentration were measure by enzyme linked immunosorbent assay According to manufactural instruction, measurement of disease activity was determine according to DAS 28 Score. RF/LP PCR was used to study SNP of IL-23R gene polymorphism for patient and control group. Data were summarized, presented and analyzed using statistical package for social science (SPSS version 23). Result of present study found there was significant association between serum IL-17 and IL-13 level and RA disease (P<0.001; and P<0.001 respectively). Moreover, there is significant positive correlation between expression level of both IL-17 and IL-13 with DAS28 (0.044, and 0.034 respectively). According to Receptor operating Curve both of IL-17 and IL-13 found to have high specificity and sensitivity 100%. Regarding to IL-23 R gene polymorphism, there was no significant correlation between rs11209026 gene polymorphism and susceptibility to rheumatoid arthritis patients in Iraqi community. Thus, present study showed that the concentrations of IL-13 and IL-17 significantly correlated with disease severity and DAS 28 which reflect their prognostic value in RA. Moreover, present study demonstrated that there was no significant association between IL-23R gene rs11209026 polymorphism and susceptibility to RA in Iraqi population.

Keywords: Rheumatoid arthritis, DAS 28, IL-17, IL-13, IL-23R.

1. INTRODUCTION:
Rheumatoid arthritis (RA) is a systemic disease with very complex pathogenesis and feature of chronic synovitis. It may be affected multiple joint especially small joint of hand and feet. [1, 2] Rheumatoid arthritis manifests with asymmetric polyarthritis characterized by pain swelling loss of function and morning stiffness lasting more than one hour.[3] The prevalence estimates of RA are between 0.5% - 1.0% and may reach to more than 5% in some population[4]. RA is a multi-factorial disease of unknown aetiology genetic environmental factors and deregulated immune response contribute toward the induction and Maintenance of the disease [5]. The cytokine play important role in the pathogenesis of RA especially IL-17 that promote osteoclast differentiation that leading to bone erosion By upregulation of nuclear factor kB(RANK)-ligand expression on osteoblast or on osteoclast precursors or indirectly by stimulating cytokine release from rheumatoid synovial fibroblast or macrophage [6]. IL-13 is expressed in rheumatoid arthritis synovial fluid and synovial fluid macrophage and lookalike many function of IL-4 .The increase of biologically active IL-13 in RA backing the hypothesis that IL-13 check immune cell (including dendritic cell ) activity and mark how the varied anatomical division of cytokine may play arole in the RA disease process. The differential regulation of circulating IL-13 and M-CSF levels by TNF antagonists furthermore imples discrete roles in the rheumatoid arthritis disease process [7] Up regulated production of IL-23R that is associated with certain SNP alleles in its gene could confer risk for RA disease .IL-23/Th17 signaling pathway consisting of IL-23/IL-23R IL-17A/IL-17F encoding gene represent a candidate way for RA development with possible involvement in disease susceptibility and effect on disease progression [8]. Genetic association studies involved that IL-23 R gene visible some single nucleotide polymorphism, which play critical role in to human auto immune disease [9, 10]. Moreover, there is a new trend to a find out anew prognostic Biomarkers for RA which might help in fallowing Disease toward improvement or start second course of treatment. A study done by Lindstrom in 2010, found that Cytokine biomarker in RA is more accurate in determined outcome of disease activity than other biomarker such as auto antibody and acute phase reactant that are may be lead to error in prognostic disease outcome. [11] RA is one of the unstable disease where the condition ranging from mild to moderate to severe to remission and repeated so that we need to use biomarker that have signature predictive value of joint inflammation. Another study demonstrated that the concentration of cytokine IL-17 and IL-13 increasing proportionally with the disease activity of ERA. [12] Thus, the aims of present study are to find out if there prognostic significance for IL-13and IL-17 in Rheumatoid arthritis through linking its expression level with DAS and Disease severity. To study if there is a Role for IL-23R 11209026 gene polymorphism in disease susceptibility by using healthy volunteers as a control group.

2. MATERIAL AND METHOD:
2.1. Patient and sampling
A blood sample from 40 Iraqi patient with RA, who attended the consultant clinic for Rheumatology in AL-Diwaniyah teaching hospital in the period from 1January 2017 to 10 May 2017 under the supervision of orthopedic specialists (ESR, CRP, RF, ACPA) was performed in the Lab. Disease activity was assessed by measuring the disease activity score for 28 joint ,the DAS28 include 28 tender and swollen joint count , patient global health using the100 mm visual analog scale (0=best,100-worse) and the level of acute phase reactant (ESR/mm/hours). In addition to that about 40 healthy volunteers were included as a control group. Blood sample were collected by venipuncture from 40 patients and healthy controls. Two milliliters of blood collected directly in a sterile tube containing EDTA for DNA extraction, and. RF/LP PCR technique, to study IL-23R gene polymorphism, these samples should be frozen immediately at -20°C. in addition three milliliters were collected in EDTA free plane tube and allowed to clot then serum was separated by centrifugation at3000rpm for5 minute. The serum has been collected in Eppendorf tube then
stored at -20°C to be used for ELISA test to measure IL-17 and IL-13 concentrations.

2.2. ELISA technique:
The analysis of serum parameters was based on a quantitative sandwich ELISA according to the manufacturer’s instructions (Elabscience ELISA Kit -China). The investigations of human serum of IL-13 and IL-17 concentration was achieved using ELISA Kit Elabscience. The test principle was the micro ELISA plate has been pre-coated with an antibody specific to IL-13, IL-17. Then, antigen is bound to immobilized capture antibody, standard or samples are pipetted into the wells and any IL-13 and IL-17 present is bound by the immobilized antibody. Biotin – conjugated antibody specific for IL-13, IL-17 was added to the wells and Avidin conjugated Horse radish peroxidase (HRP) was added to each microplate well and then incubated and then washed to remove any unbound Avidin – enzyme reagent, substrate solution was added specific to the enzyme in the well. The color intensity produced is directly proportional to the amount of IL-13, IL-17 bound in the initial step. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm by Automatic ELISA reader (paramedical-Italian) . The OD value is proportional to the concentration of IL-13 and IL-17 and then calculate the concentration of IL-13 and IL-17 in the sample by comparing the OD of the samples to the standard curve.

2.3. RFLP-PCR:
Genomic DNA from blood samples were extracted by using Genomic DNA mini kit extraction kit (Genaid-USA). The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (Thermo-USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260/280 nm) the PCR master mix component placed in standard AccuPower PCR Premix Kit (Bioneer-Korea) that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and loading dye). Then placed in PCR Thermocycler (Mygene-Korea). The PCR products were analyzed by agarose gel electrophoresis, RFLP-PCR mix was prepared by using Hpy188I restriction enzyme (New England Biolabs.-UK).

2.4. Statistical analysis:
Data were summarized, presented and analyzed using statistical package for social science (SPSS version 23) and Microsoft Office Excel 2016. Numeric data were presented as mean, standard deviation, range, median and interquartile range (IQR) after performance of Kolmogorov-Smirnov normality test and making decision about normally and non-normally distributed variables. Kruskall Wallis test was used to study difference in mean rank among more than two groups while Mann Whitney U test was used to study differences in mean rank between any two groups provided that the variable was non-parametric. Chi-square test was used to study association between any two categorical variables. The level of significance was considered at P-value of 0.05 or less and highly significant level at 0.01 or less.

3. RESULT AND DISCUSSION:
Median IL-13 was 33830.00 pg/ml and 117.66 pg/ml in study group and control group respectively, and median IL-17 was 34565.00 pg/ml and 156.65 pg/ml in study group and control group respectively significantly higher in study group than in the control group (P<0.001, and P<0.001) respectively as in the Table 1. When the expression level of both IL-13, and IL-17 were compared along with disease severity, we found that, there was significant correlation between disease severity and the level of both IL-13 and IL-17, P-value equals to (0.047 and 0.049) respectively as shown in table 2. These results reflect a proportional correlation between serum concentrations of these investigated Biomarkers and clinical severity of disease. Thus, Such higher expression of these cytokines reflect their contribution in inflammatory process that responsible for RA, moreover, scientific literature mentioned that IL-17 activate macrophage to release pro-inflammatory cytokine such as (IL-1, TNF, IL-6) and chemokine. IL-17 also stimulate production of matrix metalloproteinase (MTM) material that responsible for cartilage destruction [13, 14]. Moreover, IL-13 also demonstrated significant high expression level in RA patients and such high expression might either have a pro-inflammatory through activation of B cells to produce auto-antibodies and stimulation to release IL-6 cytokine that contribute to osteoclast formation and bone erosion in RA [15, 16]. Receiver operating characteristic (ROC) curve analysis revealed that IL-13 and IL-17 concentration indicated RA presence with, 1.000 accuracy using the concentration of IL-13 and IL-17 was >231.66 pg/mL. >255, 93 pg/ml respectively as an optimal cut-off value for discrimination between patients with RA and control (95% CI, 0.995-1.000 p <0.001) with sensitivity and specificity equal to 100 and 100% respectively as figure (1.2). We can demonstrated that from the present study IL-13 and IL-17 have cut off value that yield the high specificity and sensitivity 100%. thus we can speculate that these markers might have important prognostic and predictive value. Regarding DAS 28 score the mean value was 4.77 ±1.41 and it ranged from 2.72 to 7.82. Both IL-13 and IL-17 showed significant positive correlation with DAS 28 score as shown in table 3. DAS 28 is one of systems that have been extensively establish and accept by (EULAR) for disease activity measurement in clinical trials and are often considered the gold standard to measure disease activity[17]. The results of current study show that IL-13 and IL-17 have significantly correlation with DAS28 (p=0.034, and 0.044) respectively. This Association improve the importance of the interleukin in pathology of disease and disease progression this significant association between expression level of these studied cytokine and DAS which shade alight on prognostic significance of these cytokine in RA disease pave the way to be used as a new biomarker for Disease severity. The present study was performance to investigate the Association of IL-23R gene rs11209026 SNP in Iraqi RA patients. Allele and genotype frequency revealed that the frequency of the AA, AG, GG genotype reagent, substrate solution, D, ISCU enzyme reagent, substrate solution, D, ISCU, D, ISCU enzyme reagent, substrate solution, D, ISCU, D, ISCU enzyme reagent, substrate solution, D, ISCU.
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4. CONCLUSION:
The present finding indicated that both IL-13 and IL-17 concentrations elevated in patient with RA than the control which confirms that IL-13 and IL-17 play acute role of the pathogenesis of RA by making the inflammation consistent and lead to joint destruction. Moreover, the concentrations of IL-13 and IL-17 have significant positive correlation with DAS 28 and disease severity that reflect aprognostic value of this cytokine in RA patient. Finally current data demonstrated that there was no significant association between IL-23R rs11209026 gene polymorphism and susceptibility to RA in Iraqi population so no considered as risk factor for RA.

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