Original Article

Interleukin 22 (IL-22) As Biomarker For Rheumatoid Arthritis In Iraqi Population

Yousif Sahib Fakher[1*], Orass Madhi Shaheed[2]

[1]College of Medicine, Department of Microbiology\ University of Al-Qadisiyah, Diwaniyah, Iraq[1]E-mail: yousifalasdy9@gmail.com
[2]College of Medicine, Department of Microbiology\ University of Al-Qadisiyah, Diwaniyah, Iraq
[2]E-mail: orass.shaheed@qu.edu.iq

***Corresponding author:-** Yousif Sahib Fakher

*College of Medicine, Department of Microbiology\ University of Al-Qadisiyah, Diwaniyah, Iraq[1]E-mail: yousifalasdy9@gmail.com
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Abstract

Objectives: To evaluate the significant importance of IL-22 as Biomarker for diagnosis of Rheumatoid Arthritis. RA is the common inflammatory polyarthritis found in medical care. RA is an autoimmune disorder of gradual onset and unclear cause affecting 1% of human, women more than men.

Material and Methods: A serum sample was collected from two groups. The first group was patients with Rheumatoid Arthritis (RA), the Second group was healthy volunteers. serum of this sample used to detect IL22 level was quantified by using ELISA.

Result: The serum IL-22 level was higher in patients with rheumatoid arthritis in compare with control group, 129.30 (33.70) versus 8.18 (3.02), respectively ($p< 0.001$). Interlukin-22 (IL-22) preferential production of interleukin22 (IL22) by T cells shows that higher cytokine level occurring chronic, T cell-mediated conditions such as RA.

Conclusion: IL22 level in this study which was bushed to using them as a biomarker for RA diagnosis.

Key words: RA, IL22, Biomarker.

INTRODUCTION

Rheumatoid Arthritis (RA) is the common inflammatory polyarthritis found in medical care. RA is an autoimmune disorder of gradual onset and unclear cause affecting 1% of human, women more than men. However, RA may impacteither joints, however it is typical to detect participation of the metacarpophalangeal (M.C.P), the interphalangeal joints of the thumbs, proximal interphalangeal(PIP) joints of the fingers, the knees, the wrists, and the metatarsophalangeal (MTP) joints of the toes. As such, the damaged joints are painful and swollen. The axial skeleton is typically spared save for the cervical spine. Weariness, morning stiffness, and malaise are prevalent (1). Tests that demonstrate increased in acute phase reactants. Anti-circulated peptide antibodies (A.C.P.A) and Rheumatoid factor(RF) are among serological abnormalities. Hand and foot radiographs indicate joint space narrowing and bone erosions. Many rheumatologists see ultrasound imaging as an extension of clinical examination, and magnetic resonance imaging (M.R.I) is also helpful in the identification of early alterations [1].

Interleukin-22, termed as IL-10-related T cell-derived inducible factors, is an IL-10 family protein (IL-TIF). However, several cells of the adaptive and innate immune systems, including CD8+ and CD4+ T lymphocytes, as well as natural killer T cells, produce IL-22[2]. Several research looking in to the interference of IL-22 in RA diseases are developing. IL-22 is largely expressed in macrophages and synovial fibroblasts. However, unlike RA macrophages, just RA synovial fibroblastscontain IL-22 receptor type I in the synovium. Furthermore, IL-22 increases the composition of monocyte chemoattractant protein 1 and the reproduction of synovial fibroblasts [3]. IL-22, in human RA, is increased in the individual's serum with developed RA. However, increase serum IL-22 provides discriminating between individuals with varied laboratory and clinical measurements and radiographic progression, referringto IL-22 has the potential to be used as an extra tool for assessing RA activity, especially in RF antibody patients and long-term illness [4] [5]. Kimetal., revealed that IL-22 increases osteoclastogenesis in human synovial fibroblasts via activation of receptor activator of nuclear factor Kappa-B ligand (R.A.N.K.L) [6].

MATERIAL AND METHODS

This study included Iraqi people (as patients) with RA who visit Rheumatology department in Al-Sadr Medical City in Najaf. This study has been conducted on 50 RA patients which were diagnosed with Rheumatoid arthritis (according to rheumatologist physicians in accordance with ACR/EULAR 2010 Criteria and serological tests). They were 1 male and 49 females, and the patients age was 20-70 years. All patients were questioned by rheumatologist and researcher about name, age, gender, DM, HT, smoking, RA-family history and other questions as mentioned in questionnaire. Patient disease duration at that minimum one month and maximum 10 years and according to the data in rheumatologist question

from RApatient's researcher done the DAS-28-ESR for patients to classify RA patients depending on the DAS28-ESR to mild, moderate and sever. The blood sample was drawing and put in sterile gel tube, centrifuged for separation of serum after allowing to clot at the room temperature, after that serum sample was put in Eppendorf tube for each one patient and kept at -20 to -45 °C until used. In addition to that about 50 healthy volunteers were included as a control group.
Human Interleukin 22 Assay done by Enzyme Linked Immunosorbents Assay (E.L.I.S.A), (Bioassay technology laboratory (BT LAB)/China). Human IL-22 antibody has been pre-coated on the plate. When IL-22 from the sample is introduced, it binds to the coated antibodies on the wells. However, human IL-22 antibody that has been biotinylated is then added, and it binds to the sample's IL-22. The biotinylated IL-22 antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is extracted during a washing step after incubation. Immediately after the substrate solution has been added, color changes according to the concentration of human IL-22. By adding an acidic stop solution, the process is stopped, and absorbance is then measured at 450 nm.

RESULTS

Table 1 lists the demographic details of both the study's patients and the control group. The man ages of patients were 45.44 ±11.39 years and it ranged from 20 to 70 years and that of control group was 33.53 ±8.35 years and it ranged from 22 to 56 years; patients were therefore significantly older than control group ($p < 0.001$). likewise, there wasn't any obvious variationin gender distribution between patients and control groups ($p = 0.307$).
The study included 50 patients with RA and the duration of disease was ranging from one month to 10 years with a mean of 3.58 ±2.99 years. The disease severity was assessed according to DAS-28 scoring system which ranged from 2.57 to 5.59 and the mean was 4.65 ±0.85 and patients were accordingly classified into 3 patients (6 %) with mild disease, 27 patients (54 %) with moderate disease and 20 cases (40 %) with severe disease shown in figure1.
As shown in table 2 and figure 2, patients with rheumatoid arthritis had considerably greater blood levels of IL-22 than the control group, 129.30 (33.70) versus 8.18 (3.02), respectively ($p< 0.001$). Correlation of serum cytokine level to other characteristics is shown in table 3, Serum IL-22 showed significant positive correlation to age, CRP, RF, ESR and anti-CCP.

Table1: Demographic features of control and patient's participants included in this research

Characteristic	**Patients** ***n* = 50**	**Control** ***n* = 50**	***p***
Age (years)			
Mean ±SD	45.44 ±11.39	33.53 ±8.35	<0.001 I ***
Range	20 -70	22 -56	
Gender			
Male, *n* (%)	1 (2.0 %)	3 (6.0 %)	0.307 Y NS
Female, *n* (%)	49 (98.0 %)	47 (93.0 %)	

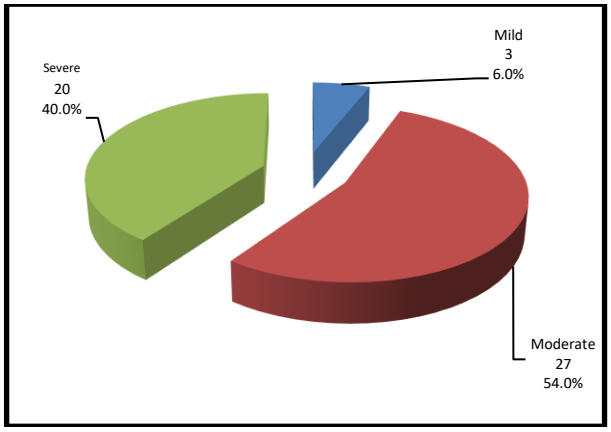

Figure1: Pie chart showing classification of patients with rheumatoid arthritis according to severity of disease based on DAS-28

Table2: Evaluation of IL22 levels in patients with RA compared to control group

Characteristic	**Patients** ***n* = 50**	**Control** ***n* = 50**	***p***
IL-22			
Median (IQR)	129.30 (33.70)	8.18 (3.02)	< 0.001 M ***
Range	43.85 -1420.46	5.96 -48.54	

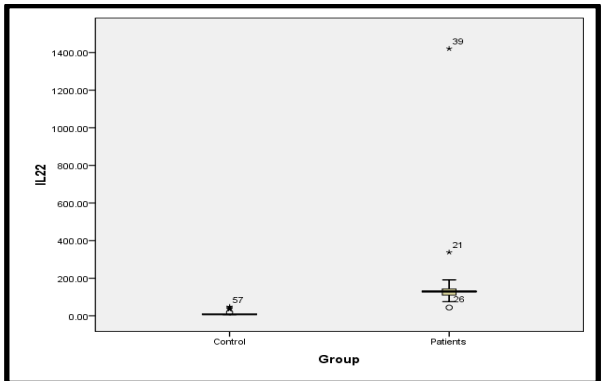

Figure 2: Box plot showing comparison of median IL-22 between control group and patients with rheumatoid arthritis.

Table 3: Correlation of serum IL22 level to other characteristics

Characteristic	IL-22	
	r	*p*
Gender	0.038	0.735
Age	0.394	< 0.001***
CRP	0.319	0.004**
RF	0.306	0.006**
ESR	0.537	< 0.001***
Anti-CCP	0.302	0.033*
DAS28	-0.015	0.919

DISCUSSION

The present study agreement with Al-Rubaye et al., (2017), Cope (2019) and Sura et al., (2021). which demonstrated age at onset is usually between 30 – 70, (7), high incidence of RA among age groups 45- 75 years (8) and majority of RA patients btween40- 59(9), respectively. In regards of the mean age of patients, the present study shows that the mean age is 45.44±11.39 years found in patients with RA. This result is compatible with Fathi et al. (2018), (10), that indicated the mean age in years was 49.1 ± 13. The higher frequency of RA in females implies that female's hormonal variables contribute to the disease's development. The high of (RA) incidence occurs in the fifth decade of life, around the time of menopause in women. Estrogen, for example, have been proposed to have a pro-inflammatory effect (11).

Interlukin-22 (IL-22) preferential production of interleukin22 (IL22) by T cells shows that higher cytokine level occurring chronic, T cell-mediated conditions such as RA and also that IL-22 has an involvement in the pathophysiology of these disorders(12).According to the present research results, the serum IL22 level was considerably greater in patients with rheumatoid arthritis compared to the control group, 129.30 (33.70) versus 8.18 (3.02), respectively (p<0.001), and this result agreement with study done by Laila Mohamed at al.,2018in Egypt that indicated to IL-22 levels were elevated in RA patients compared with controls(12).Also, the result of current study agreement with study conducted by Zhao L et al.,2013 that indicated to the levels of IL-22 were higher in all RA patients than in healthy control(13).The present study disagrees with Wei Zhong et al.,(2017) that indicated to the level of IL22 was significantly reduced Following treatment only in patients responsive to treatment(14),while in our study the patients with positive history for treatment make up 88% from the total patients with R.A and level of IL22 still elevated.In regarded Correlation of serum IL22 level to other characteristics the current study showed significant positive correlation between serum level of IL-22 with age, CRP, RF, ESR and anti-CCP.The current findings in line with result of Laila Mohamed at al.,2018 that found there is positive correlation between IL-22 serum level and C-Reactive protein(CRP), DAS 28 score and Rheumatoid factor (RF)(12).Another study conducted by laurindo Ferreira et al.,2012 also agreement with our study that indicated to there is positive correlation between level of IL-22 and Rheumatoid Factor (RF)(15).

CONCLUSION

The current study findings indicate that the serum IL22level was higher in patients with rheumatoid arthritis, which could be a very important predictive marker for rheumatoid arthritis.

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