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Original Research Paper

ASSESSING THE LEVELS OF ESR AND TNF-A (INFLAMMATORY MARKERS) ALONG WITH DAS 28 IN SUBJECTS WITH RA

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ABSTRACT

Background: Autoimmune diseases such as RA (rheumatoid arthritis) usually result from a persistent imbalance between pro-inflammatory and anti-inflammatory immune mechanisms which leads to chronic inflammation.

Aim: The present study aimed to assess if inflammatory markers are raised in subjects with rheumatoid arthritis and to assess the relationship between these markers and disease activity in rheumatoid arthritis measured with DAS28 (disease activity score 28).

Methods: The present study assessed 220 subjects with rheumatoid arthritis diagnosed by revised criteria for RA by ACR (American College of Rheumatology) along with 110 gender and age-matched controls. In all the subjects, serum levels of TNF-α (tumor necrosis factor-alpha), I (interleukin) 10, IL-6, and hs-CRP (highly sensitive C-reactive protein) were assessed and correlated with DAS 28. An immunoturbidimetric assay was used for assessing serum hs-CRP and a commercially available ELISA kit was used to assess the cytokine levels.

Results: The study results showed that subjects with rheumatoid arthritis had significantly higher levels of serum hs-CRP, IL-6, IL-10, and TNF-α seen in subjects with rheumatoid arthritis compared to the healthy controls with $p<0.001$. TNF-α, IL-6, and hs-CRP levels positively correlated with DAS 28 ($p<0.001$), and a negative correlation was seen in IL-10 and DAS 28 with $p<0.01$.

Conclusions: The present study concludes that significantly higher levels of inflammatory markers are seen in subjects with rheumatoid arthritis and these levels are correlated to the DAS28. These results depicted that there is a possible role of these inflammatory markers in the pathogenesis of rheumatoid arthritis. Also, these biomarkers can be used as disease activity markers in the diagnosis and management of rheumatoid arthritis.

Keywords: DAS28, IL-6, IL-10, hs-CRP, rheumatoid arthritis

INTRODUCTION

RA (rheumatoid arthritis) is a chronic inflammatory condition having characteristic polyarthritis leading to premature mortality, increased co-morbidity, systemic inflammation, immunological abnormalities, and progressive joint damage and disability affecting nearly 1% of global adult subjects. It is also reported in 1 in 1000 children and known as juvenile RA. It is more common in females compared to males and 70% of females having RA in pregnancy have remission that flares post-delivery.[1] Etiology of RA is unknown; however, it is classified under autoimmune disease. RA decreases life expectancy and is a major cause of handicaps and chronic disability which worsen with time. Existing literature data reports that advanced therapy including anti-cytokine agent introduction and early aggressive therapy might help in delayed disability, delayed joint destruction progression, eased clinical symptoms, and improved quality of life.[2]

Inflammatory processes play a vital role in RA pathogenesis. Inflammatory markers such as TNF-α, IL-6, IL-10, and CRP (C-reactive protein) have high expression in serum and synovial fluids of subjects affected with rheumatoid arthritis and have a vital role in the pathophysiology of rheumatoid arthritis. CRP is an acute-phase protein produced by hepatocytes following stimulation by cytokines such as TNF-α, IL-6, and IL-10 during acute-phase response. CRP is a general systemic inflammation marker raised in subjects with RA. Literature data reports a high increased CRP concentrations in the serum of RA subjects before the onset of RA.[3]

Various cytokines are involved in RA in almost all aspects of articular destruction and inflammation. Increase in pro-inflammatory cytokines levels lead to synovial tissue proliferation and cause articular cartilage damage along with adjacent area bone destruction. In affected joints, anti-inflammatory cytokines can also be seen, and it is hypothesized that chronic synovitis can be due to an imbalance in anti-inflammatory and pro-inflammatory cytokines produced in RA. The most abundantly expressed cytokine in RA subjects is IL-6 with biological activities that regulate the immune system, hematopoiesis, and inflammation. IL-6 stimulates immunoglobulin secretion from plasmacytes, induces bone resorption, regulates differentiation and proliferation of osteoclasts, induces synthesis of acute phase proteins such as haptoglobin, fibrinogen, CRP, and serum amyloid-A, and promotes and activates T and B cell proliferation and hence, is involved in rheumatoid factor production.[4]

TNF-α is one of the vital pro-inflammatory cytokines leading to joint inflammation and destruction in RA subjects. TNF-α along with its two receptors p75TNFR and p55 are widely detected in both serum and synovial fluids of subjects with RA. The disease severity is associated with TNF-α concentration in RA subjects. TNF-α is a potent mesenchymal cell stimulator and stimulates cells that release tissue-destroying MMPs (matrix metalloproteinases) including chondrocytes, osteoclasts, and fibroblasts. TNF-α inhibits tissue inhibitors of metalloproteinases by synovial fibroblast production. These dual actions are contributors to joint damage. However, IL-6 and TNF-α have synergic and overlapping actions, and few effects of these cytokines are regulated with distant mechanisms. IL-10 is a potent anti-inflammatory and immunosuppressive cytokine that is produced from a hemostatic response to inflammation and infection and plays a vital role in decreasing the intensity and duration of inflammatory and immune

reactions. As an anti-inflammatory cytokine, IL-10 is depicted to inhibit pro-inflammatory cytokine synthesis.[5] The present study aimed to assess if inflammatory markers are raised in subjects with rheumatoid arthritis and to assess the relationship between these markers and disease activity in rheumatoid arthritis measured with DAS28 (disease activity score 28).

MATERIALS AND METHODS

The present prospective clinical study was aimed to assess if inflammatory markers are raised in subjects with rheumatoid arthritis and to assess the relationship between these markers and disease activity in rheumatoid arthritis measured with DAS28 (disease activity score 28). The study was done at Department of Pathology, Shri Balaji Institute of Medical Science Mowa, Raipur, Chhattisgarh after the clearance was given by the concerned Institutional Ethical committee. The study subjects were from the Department of Medicine of the Institute. Verbal and written informed consent were taken from all the subjects before study participation.

The present study assessed 220 subjects with rheumatoid arthritis who were diagnosed using the revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) from 1987.[6] All the subjects were from the Department of Rheumatology of the Institute. All the included subjects had active RA with >3 tender and >3 swollen joints. Few of the subjects had erosive disease evidence as assessed on feet or hands X-rays. Disease activity in RA subjects was assessed using DAS 28 including 28 swollen and tender counts, patient's disease activity assessment with VAS (visual analog scale), and ESR (erythrocyte sedimentation rate). BMI (body mass index) was assessed in kg/m2 by dividing the weight (kg) by the square of the height (m2).

The study also included 110 gender and age-matched controls from the hospital staff and blood donors of the institute. The control subjects were healthy with no family or personal history of rheumatoid arthritis. After final inclusion, these controls were assessed for dyslipidemia, hypertension, and diabetes mellitus.

In all the subjects, intravenous blood samples were collected following strict aseptic and sterile protocol after overnight fasting. Blood collected was allowed for clotting at room temperature and immediately, the serum was collected using centrifugation for 10 minutes at 3500rpm. Collected serum was aliquoted in plastic tubes and stored at -27^{o}C till the assay. Serum hs-CRP levels were assessed with an immunoturbidimetric assay using calibrators and reagents. TNF-α, IL-10, and IL-6 levels were assessed using a sandwich ELISA (enzyme-linked immunosorbent assay) technique from a commercially available kit following the manufacturer's instructions. ESR and RF (rheumatoid factor) were assessed using routine procedures of the Institute.

The data gathered were analyzed statistically using SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk. NY, USA) for assessment of descriptive measures, Student t-test, ANOVA (analysis of variance), and Chi-square test. The results were expressed as mean and standard deviation and frequency and percentages. The p-value of <0.05 was considered statistically significant. Correlation in each parameter concentration and DAS28 were assessed using Pearson's correlation.

RESULTS

The present prospective clinical study was aimed to assess if inflammatory markers are raised in subjects with rheumatoid arthritis and to assess the relationship between these markers and disease activity in rheumatoid arthritis measured with DAS28 (disease activity score 28). The present study assessed 220 subjects with rheumatoid arthritis diagnosed by revised criteria for RA by ACR (American College of Rheumatology) along with 110 gender and age-matched controls. In all the subjects, serum levels of TNF-α (tumor necrosis factor-alpha), I (interleukin) 10, IL-6, and hs-CRP (highly sensitive C-reactive protein) were assessed and correlated with DAS 28. There were 60 males and 160 females in the RA group and 30 males and 80 females in the control group which was non-significant with $p>0.05$. The mean age of the study subjects was 46.69±7.10 and 47.13±7.85 years in RA and control group with $p>0.05$. The mean BMI of study subjects was 28.1±3.19 kg/m2 in RA subjects which was significantly higher compared to controls where it was26.13±2.95 with $p<0.01$. Diabetes mellitus was seen in 58 subjects with rheumatoid arthritis and 20 subjects from controls. Hypertension was seen in 52 subjects with RA and 20 controls, alcohol history was positive in 24 RA and 14 controls which was non-significant with $p>0.05$ (Table 1).

It was also seen that there were significantly higher smokers in the RA group with no smokers in controls with $p<0.01$. Morning stiffness was 85±41.73 in RA subjects and no stiffness was seen in controls. Duration of RA was 58.1±19.86 months in RA subjects. The rheumatoid arthritis factor was positive in 168 subjects from RA groups. The mean ESR was 49.80±18.54 mm/hour in the RA group. A family history of rheumatoid arthritis was positive in 30 subjects with RA. Former fracture was positive in 32 subjects with RA and 6 controls which was statistically non-significant with $p>0.05$. DAS 28 scores in the RA group were 4.11±1.75 (Table 1).

The study results showed that for assessment of laboratory values in RA and control study subjects, TNF-α levels were 8.56±5.97 and 1.16±0.69 pg/ml in RA and control group subjects which was significantly higher in RA group with $p<0.001$. Similar significantly higher levels of IL-10, IL-6, and hs-CRP were seen in subjects with rheumatoid arthritis compared to control group subjects with $p<0.001$ (Table 2).

On assessing the correlation of different parameters in study subjects with rheumatoid arthritis, a highly significant positive correlation was seen of DAS28 scores to TNF-α, IL-6, and hs-CRP $p<0.001$. A significantly negative correlation was seen between DAS28 and IL-10 levels. In subjects with rheumatoid arthritis, a highly significant positive correlation was seen in TNF-α and hs-CRP levels with $p<0.001$ and IL-6 and hs-CRP levels with $p<0.001$, whereas, a negative correlation was seen in IL-10 and hs-CRP levels with $p<0.001$. A positive correlation was seen in IL-6 and TNF-αwith $p<0.001$ and a negative correlation in TNF-α and IL-10 with $p<0.05$. No statistically significant association was seen in IL-10 and TNF-α (Table 3).

DISCUSSION

The present study assessed 220 subjects with rheumatoid arthritis diagnosed by revised criteria for RA by ACR (American College of Rheumatology) along with 110 gender and age-matched controls. In all the subjects, serum levels of TNF-α (tumor necrosis factor-alpha), I (interleukin) 10, IL-6, and hs-CRP (highly sensitive C-reactive protein) were assessed and correlated with DAS 28. There were 60 males and 160 females in the RA group and 30 males and 80 females in the control group which was non-

significant with p>0.05. The mean age of the study subjects was 46.69±7.10 and 47.13±7.85 years in RA and control group with p>0.05. The mean BMI of study subjects was 28.1±3.19 kg/m2 in RA subjects which was significantly higher compared to controls where it was26.13±2.95 with p<0.01. Diabetes mellitus was seen in 58 subjects with rheumatoid arthritis and 20 subjects from controls. Hypertension was seen in 52 subjects with RA and 20 controls, alcohol history was positive in 24 RA and 14 controls which was non-significant with p>0.05. These demographics were comparable to the studies of Milman M et al[7] in 2010 and Aletaha D et al[8] in 2006 where authors assessed subjects with demographic data comparable to the present study in their respective studies.

The study results showed that there were significantly higher smokers in the RA group with no smokers in controls with p<0.01. Morning stiffness was 85±41.73 in RA subjects and no stiffness was seen in controls. Duration of RA was 58.1±19.86 months in RA subjects. The rheumatoid arthritis factor was positive in 168 subjects from RA groups. The mean ESR was 49.80±18.54 mm/hour in the RA group. A family history of rheumatoid arthritis was positive in 30 subjects with RA. Former fracture was positive in 32 subjects with RA and 6 controls which was statistically non-significant with p>0.05. DAS 28 scores in the RA group were 4.11±1.75. These results were consistent with the studies of Mittal GA et al[9] in 2002 and Murakami M et al[10] in 2012 where disease data in rheumatoid arthritis subjects reported by the authors in their studies was similar to the present study.

It was seen that for assessment of laboratory values in RA and control study subjects, TNF-α levels were 8.56±5.97 and 1.16±0.69 pg/ml in RA and control group subjects which was significantly higher in RA group with p<0.001. Similar significantly higher levels of IL-10, IL-6, and hs-CRP were seen in subjects with rheumatoid arthritis compared to control group subjects with p<0.001. These findings were in agreement with the results of Masi AT et al[11] in 2001 and Edwards III CK et al[12] in 2012 where similar to the present study, authors also reported significantly higher levels of TNF-α, IL-10, IL-6, and hs-CRP in their study subjects.

Concerning the assessment of the correlation of different parameters in study subjects with rheumatoid arthritis, a highly significant positive correlation was seen between DAS28 scores to TNF-α, IL-6, and hs-CRP p<0.001. A significantly negative correlation was seen between DAS28 and IL-10 levels. In subjects with rheumatoid arthritis, a highly significant positive correlation was seen in TNF-α and hs-CRP levels with p<0.001 and IL-6 and hs-CRP levels with p<0.001, whereas, a negative correlation was seen in IL-10 and hs-CRP levels with p<0.001. A positive correlation was seen in IL-6 and TNF-αwith p<0.001 and a negative correlation in TNF-α and IL-10 with p<0.05. No statistically significant association was seen in IL-10 and TNF-α. These results were in line with the findings of Rahman EMA et al in 2005 and Fox DA in 2000 where the correlation of different parameters to DAS scores reported by the authors in their studies correlated to the results of the present study.

CONCLUSIONS

The present study, considering its limitations, concludes that significantly higher levels of inflammatory markers are seen in subjects with rheumatoid arthritis and these levels are correlated to the DAS28. These results depicted that there is a possible role of these inflammatory markers in the pathogenesis of rheumatoid arthritis. Also, these biomarkers can be used as disease activity markers in the diagnosis and

management of rheumatoid arthritis. Further awareness in the population for early screening, diagnosis, and management of RA might help in controlling mortality and morbidity associated with RA.

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TABLES

S. No	Characteristics	RA subjects (n=220)	Controls (n=110)	p-value
1.	**Gender**			
a)	Males	60	30	>0.05
b)	Females	160	80	
2.	**Age (years)**	46.69±7.10	47.13±7.85	>0.05
3.	**Mean BMI (kg/m2)**	28.1±3.19	26.13±2.95	**<0.01**
4.	**Diabetes mellitus**			
a)	Yes	58	20	>0.05
b)	No	162	90	
5.	**Hypertension**			
a)	Yes	52	20	>0.05
b)	No	168	90	
6.	**Alcohol**			
a)	Yes	24	14	>0.05
b)	No	196	96	
7.	**Smoker**			
a)	Yes	34	-	<0.01
b)	No	186	-	
8.	**Morning stiffness (min)**	85±41.73	-	**-**
9.	**RA duration (months)**	58.1±19.86	-	**-**
10.	**RF**			
a)	Positive	168	-	**-**
b)	Negative	52	-	**-**
11.	**Mean ESR (mm/h)**	49.80±18.54	-	**-**
12.	**Family RA history**			
a)	Yes	30	-	**-**
b)	No	190	-	**-**
13.	**Former fracture**			
a)	Yes	32	6	>0.05
b)	No	188	104	
14.	**DAS28**	4.11±1.75	-	**-**

Table 1: Demographic data of the study subjects

S. No	Parameters	RA subjects (n=220)	Controls (n=110)	p-value
1.	**TNF-α (pg/ml)**	8.56±5.97	1.16±0.69	**<0.001**
2.	**IL-10 (pg/ml)**	20.49±12.80	13.49±11.54	**<0.01**
3.	**IL-6 (pg/ml)**	54.76±28.29	12.57±8.40	**<0.001**
4.	**Hs-CRP (mg/L)**	8.56±5.97	1.16±0.69	**<0.001**

Table 2: Laboratory values in RA and control study subjects

S. No	Parameters	Hs-CRP	IL-6	IL-10	TNF-α	DAS28
1.	**Hs-CRP**	-				
2.	**IL-6**	0.734	-			
3.	**IL-10**	-0.352	-0.241	-		

4.	**TNF-α**	0.471	0.559	0.076	-	
5.	**DAS28**	0.870	0.605	-0.267	0.406	-

Table 3: Correlation of different parameters in study subjects with rheumatoid arthritis