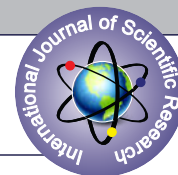


EVALUATION OF TNF- α AND IL-6 IN PATIENTS WITH RHEUMATOID ARTHRITIS & ITS CORRELATION WITH DISEASE ACTIVITY

Rheumatology

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ABSTRACT

Background:- Rheumatoid arthritis (RA) is a chronic autoimmune systemic inflammatory multi-system disease of unknown origin that can affect many parts of the body, but mainly attacks synovial joints.¹ The diagnosis of patients with established Rheumatoid Arthritis (RA) is important to approach the disease activity & to initiate the treatment. It depends upon symmetrical polyarthritis characteristically involving small joints of the hands and measurement of objective biomarkers. The “gold standard” index used to assess disease activity both in trials and in the clinic has been the Disease Activity Score-28 (DAS-28), but the calculation of DAS28 requires the use of a formula involving square roots and logarithms which can only be done with a specific calculator.^{2,3,4} Pro-inflammatory cytokines are thought to play a major role in chronic inflammation and joint destruction in RA in which TNF- α and IL-6 are central mediators, and they are highly sensitive markers of disease activity in these patients. As no such study has been done in the Indian population, therefore we undertook this study to understand the role of TNF- α and IL-6 in assessing disease activity in RA and to see if there was any correlation in disease activity as assessed by DAS28 with TNF- α and IL-6.^{5,6}

Materials & Methods:- This current study was conducted in the Department of Medicine, Postgraduate Institute of Medical Education & Research, Dr. Ram Manohar Lohia Hospital, New Delhi. The study was conducted from November 2016 to March 2018 & the total number of patients included in the study were 60 with RA diagnosed according to the 2010 ACR-EULAR criteria.⁷ Disease activity was calculated using ESR, DAS 28 & Health assessment Questionnaire (HAQ).⁸ TNF- α and IL-6 were correlated with disease activity as measured by DAS28 and with a greater functional disability as assessed by HAQ score.

Conclusion:- In patients with rheumatoid arthritis there is a statistically significant correlation between TNF- α and IL-6 with disease activity as measured by DAS28 score. In our study, out of TNF- α and IL-6, TNF- α is a better biomarker of disease activity as compared to IL-6. Functional disability, as assessed by HAQ scores, is directly proportional to TNF- α and IL-6 with patients with higher titers having a greater functional disability.

KEYWORDS

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune systemic inflammatory multi-system disease of unknown origin that may affect many parts of the body but mainly attacks synovial joints. RA affects nearly 1% of the adult general population.¹ The impact of RA is not restricted to the affected joints but is associated with several systemic complications linked to the underlying disease process. Multiple organs and organ systems are likely involved in RA, especially in severe disease.⁹ It seems that the persistence of inflammatory mediators likely contributes to this extra-articular involvement.¹⁰

The diagnosis of patients with established RA is based upon symmetrical polyarthritis characteristically involving small joints of the hands with/without deformities along with measurement of objective biomarkers. Initially, ESR and CRP were correlated with active disease but were discordant in one-quarter of patients irrespective of disease activity.¹¹ This leads to the creation of composite indices which consider multiple variables. The “gold standard” index used to assess disease activity both in trials and in the clinic has been the Disease Activity Score-28 (DAS-28), but the calculation of DAS28 requires the use of a formula involving square roots and logarithms which can only be done with a specific calculator.^{2,3,4} Hence came the need for biomarkers that could be measured to assess disease activity.

Pro-inflammatory cytokines are thought to play a major role in chronic inflammation and joint destruction in RA in which TNF- α and IL-6 are central mediators, and they are highly sensitive markers of disease activity in these patients. Hence, we undertook this study to understand the role of TNF- α and IL-6 in assessing disease activity in RA and to see if there is any correlation in disease activity as assessed by DAS28 with TNF- α and IL-6.^{5,6}

MATERIALS & METHODS

This current study was hospital-based cross-sectional Observational study, which was conducted in the Department of Medicine, Postgraduate Institute of Medical Education & Research, Dr. Ram Manohar Lohia Hospital, New Delhi. The study was conducted from November 2016 to March 2018. The total number of patients included

in the study were 60 and all patients seen in the OPD or admitted in IPD were enrolled and investigated as per study design. Each participant gave written, informed consent to participate in the study. Consecutive patients, who satisfied the inclusion criteria, were enrolled after ruling out the exclusion criteria. The study protocol was approved by the institutional review board and the institutional ethics committee of Post Graduate Institute of Medical Education & Research, Dr RML Hospital, New Delhi, Room No.414,^{4th} floor, Administration Block with notification no. of TP(MD/MS) (58/2016)/IEC/PGIMER/RMLH 7762/16.

The detailed history including the mode of onset, the joints involved, duration of early morning stiffness, family history of rheumatoid arthritis, extra-articular symptoms with the general physical and systemic examination was performed in each participant. Each patient completed a **Health Assessment Questionnaire (HAQ)**.⁸ The health assessment questionnaire is an instrument to measure the impact of the disease on a patient's life. It assesses the ability of the person to carry out various activities of daily living and provides a measure of the disability caused by the disease. Joint involvement was assessed using a tender joint count and swollen joint count. These data were combined with an erythrocyte sedimentation rate (ESR) and a patient global assessment (PGA) of disease activity to obtain a Disease Activity Score (DAS 28). DAS 28 is a validated measure of disease activity in patients with rheumatoid arthritis, with higher scores corresponding to greater disease activity.¹² In order to calculate the score, 28 joints are assessed for swelling and tenderness. These include 10 proximal interphalangeal joints, 10 meta-carpal-phalangeal joints, two wrist joints, two elbow joints, two shoulder joints and two knee joints.

DAS 28 score = $0.56 \times \sqrt{\text{tender joint count}} + 0.28 \times \sqrt{\text{swollen joint count}} + 0.70 \times \ln [\text{ESR}] + 0.014 \times (\text{patient's global assessment on a scale of 1-100, measured using Visual analogue scale})$. The **cut-off values of DAS 28 for disease activity** are: >5.1-High disease activity, >3.2 – ≤ 5.1-Moderate disease activity, ≤ 3.2-Low disease activity, < 2.6-Remission.

Interleukin-6 and TNF- α were calculated for each patient by using the

Diaclone IL-6 and TNF- α ELISA Kits respectively, which is a solid-phase sandwich ELISA. A capture antibody highly specific for IL-6 and TNF- α respectively has been coated to the wells of the microtiter strip plate provided during manufacture. The binding of IL-6 and TNF- α samples with known standards to capture antibodies and subsequent binding of the biotinylated anti-IL-6 and anti-TNF- α respectively, secondary antibody to the analytic is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the respective concentration of IL-6 and TNF- α present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentrated forming a standard curve. This standard curve can then be used to accurately determine the respective concentration of IL-6 and TNF- α in any sample tested. **Erythrocyte sedimentation rate (ESR)** was calculated for each patient by using the Westergren method. Values are expressed as mm in the first hour.

Categorical variables were presented in number and percentage and continuous variables were presented as mean $\pm$ SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then nonparametric test was used. Statistical tests were applied as follows-

1. Spearman rank correlation coefficient was used to assess the association of DAS 28 with IL-6 and TNF- α .
2. Univariate and multivariate linear regression was used to find out significant parameters affecting DAS-28.

A p-value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

OBSERVATIONS AND RESULTS

Our study included 60 patients with rheumatoid arthritis whose ages ranged from 18 to 45 years. The mean age of the study population was 36.92 years. There were 12 men and 48 women. Erythrocyte sedimentation rate ranged from 5 to 90 mm in the first hour, with a mean $\pm$ SD of 30.58 ± 18.21 mm in the first hour, also 44 patients were CRP positive and 16 were CRP negative. In our study population, 44 patients were RF positive and 16 were RF negative. The minimum value of TNF- α levels detected was 1.43pg/ml and a maximum value detected was 457.6pg/ml . The characteristics of TNF- α levels in the study population and by gender are shown in Table 1

Table 1: -TNF- α Levels In The Study Population & By Gender

TNF- α	STUDY POPULATION	F	M	
Sample size	60	48	12	0.542
Mean $\pm$ St dev	18.82 ± 58.29	20.15 ± 64.9	13.51 ± 13.76	
Median	10.2	9.71	11.1	
Min-Max	1.43-457.6	1.43-457.6	2.35-55.14	
Inter quartile Range	6.395 - 12.710	6.395 - 12.710	7.610 - 12.810	

The minimum value of IL-6 levels detected was 0.35pg/ml and a maximum value detected was 342.5pg/ml . The characteristics of IL-6 levels in the study population and by gender are shown in Table 2

Table 2: - IL-6 Levels In The Study Population & By Gender

	Study Population	Sex		P value
		F	M	
IL-6				0.147
Sample size	60	48	12	
Mean $\pm$ St dev	26.74 ± 63.28	23.71 ± 62.81	38.84 ± 66.5	
Median	7.3	6.66	10.01	
Min-Max	0.35-342.5	0.35-342.5	0.67-188.6	
Inter quartile Range	4.100 - 12.350	4.037 - 10.455	6.120 - 21.930	

Most patients had moderate disease activity by DAS28. Scores ranged from 1.18 to 6.10, with a mean of 3.05 and a standard deviation of 1.17. The distribution of patients by DAS28 is shown in Table 3

Table 3: - Distribution Of Patients By DAS28

DAS28 Categories	Number of patients
Remission (DAS28 < 2.6)	25
Low Disease Activity (DAS28: $2.6 - \leq 3.2$)	11
Moderate Disease Activity (DAS28: $> 3.2 - \leq 5.1$)	20
High Disease Activity (DAS28 > 5.1)	4

HAQ score ranged from 0.5 to 2.68 with a mean of 1.69 and a standard deviation of 0.57. The distribution of patients by DAS28 is shown in Figure 1

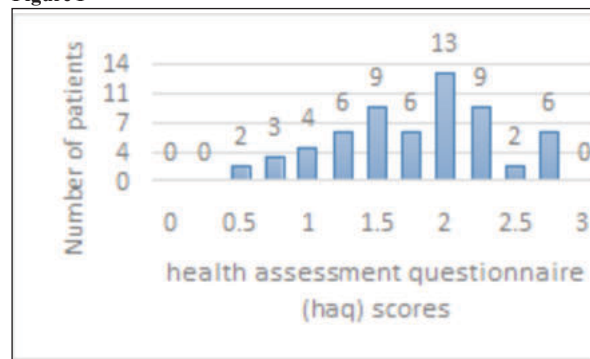


Figure 1. Distribution Of Patients According To HAQ Scores

Analytical statistics were done to assess the correlation between different variables. For this Regression Analysis was done- first univariate and then multivariate regression.

Univariate linear regressions were performed to assess the relationship between DAS28 as an outcome measure with variables like ESR, TNF- α and IL-6 as predictor variables, significant relationship found between DAS-28, ESR, TNF- α and IL-6 (Table 4).

Table 4. Univariate Linear Regression For DAS28 On ESR, TNF- α And IL-6

DAS-28	Unstandardized Coefficients	Standardized Coefficients	P value	95.0% Confidence Interval for B	
	B	Std. Error Beta		Lower Bound	Upper Bound
ESR	.046	.006	.719	<.0001	.034 .058
IL-6	.007	.002	.388	.002	.003 .012
TNF- α	1.896	.352	.578	<.0001	1.192 2.600

The relationship between TNF- α & IL-6 with DAS-28 is also shown in Figures 2 & 3.

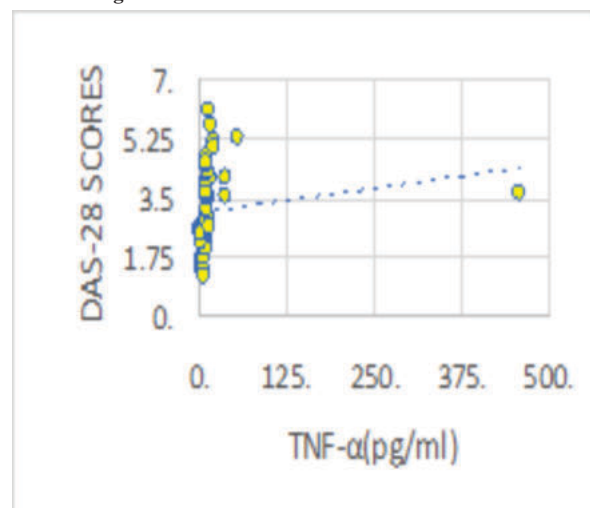


Figure 2. Scatter Plot Showing A Relationship Between TNF- α And DAS28.

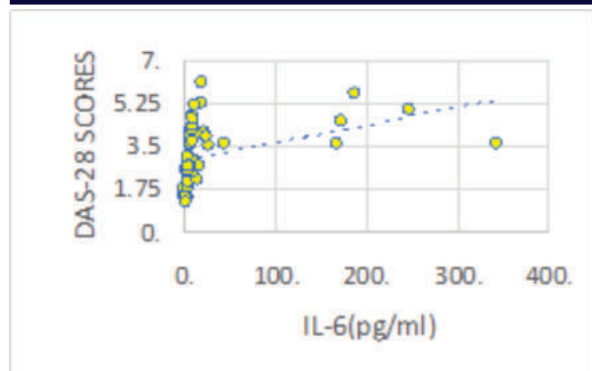


Figure 3. Scatter Plot Showing A Relationship Between IL-6 And DAS28

After Univariate regression analysis, multivariate linear regression analysis was done to assess ESR, TNF- α and IL-6 as predictor variables as independent predictors of DAS28. After adjusting for confounding factors, ESR and TNF- α were the significant factors affecting DAS-28 with p value <0.0001, ESR was the most significant factor with standardized beta as followed by TNF- α with standard error of 0.313 (Table 5).

Table 5. Multivariate Linear Regression For DAS28 On ESR, TNF- α and IL-6

DAS-28	Unstandardized Coefficients		Standardized Coefficients	P value	95.0% Confidence Interval for B	
	B	Std. Error			Lower Bound	Upper Bound
ESR	.038	.005	.598	<.0001	.028	.049
IL-6	.001	.002	.053	.569	-.002	.004
TNF- α	1.217	.313	.371	<.0001	.590	1.844

Univariate linear regression analysis was also done to compare HAQ with various patient parameters using HAQ as the dependent variable. A significant correlation was seen between HAQ with TNF- α and IL-6 (Table 6 and Figure 4 & 5).

Table 6. Univariate Linear Regression For HAQ On TNF- α And IL-6

MD-HAQ	Unstandardized Coefficients		Standardized Coefficients	P value	95.0% Confidence Interval for B	
	B	Std. Error			Lower Bound	Upper Bound
IL-6	.003	.001	.338	.008	.001	.005
TNF- α	.404	.203	.253	.051	-.002	.809

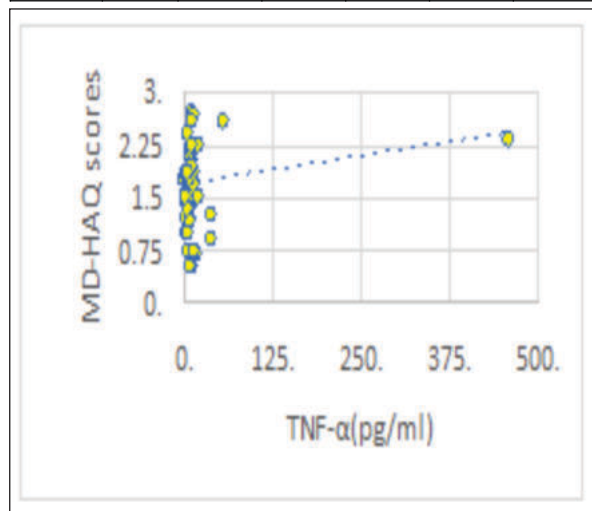


Figure 4. Scatter Plot Showing Relationship Between TNF- α And MD-HAQ Scores.

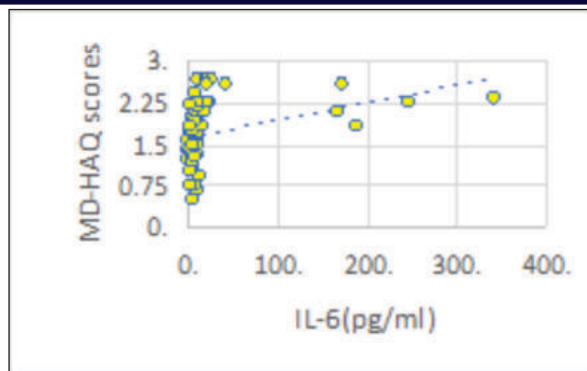


Figure 5. Scatter Plot Showing Relationship Between IL-6 And MD-HAQ Scores

DISCUSSION

Therapy for rheumatoid arthritis has seen great progress over the past 10 years, including the approval of new drugs and the implementation of new strategies. Owing to newer therapies now available for the treatment of RA, the goal of therapy is directed at minimizing disease activity to prevent long-term complications. Routine assessment of disease activity is required for tailoring the therapy for individual patients. DAS28 is the most widely accepted measure for disease activity in patients with RA. However, due to the complex formula involved in the calculation, its use in clinical practice is limited. Hence, biomarkers are required which can assess disease activity in RA in a more precise and simplified manner. TNF- α and IL-6 are pro-inflammatory cytokines that are thought to play a major role in chronic inflammation and joint destruction in RA patients and they are highly sensitive markers of disease activity in these patients.

Hence, we undertook this study in the Indian population from the period of November 2016 to March 2018 to understand the role of TNF- α and IL-6 in assessing disease activity in RA and to see if there was any correlation in disease activity as assessed by DAS28 with TNF- α and IL-6. In our study, 60 patients (48 women and 12 men) were included. A detailed history was taken, and a clinical examination performed. Laboratory parameters including RF, Anti-CCP titers and ESR, TNF- α and IL-6 were performed in all patients. DAS28 and HAQ scores were calculated for all patients. The mean age of the study population was 36.82 ± 5.47 years and the median value was 38 years. ESR was done in all patients, the value of which ranged from 5 to 90 mm in the first hour, with a mean ESR of 30.58 ± 18.21 mm in the first hour and a median of 29. Thus, our study population consisted largely of women most of whom were in their third and fourth decade of life. Anti-CCP titers in our patients ranged from 5.35 to 337 IU/ml with mean levels of 116.96 ± 103.46 IU/ml. In our study mean TNF- α and IL-6 were 18.82 ± 58.29 and 26.74 ± 63.28 , respectively. Most of our patients had moderate disease activity with DAS28 ranging from 1.18 to 6.1 with a mean DAS28 of 3.05 ± 1.17 and a median DAS28 of 2.7. HAQ scores ranged from 0.58 to 2.68 with a median HAQ of 1.76 and a mean of 1.69 ± 0.57 . So, the values of HAQ score were also high showing greater functional disability.

Univariate linear regression analysis was used to assess the relationship between DAS28 as an outcome variable and TNF- α and IL-6 as a predictor variable. This showed a correlation coefficient of 0.82, 0.754 and a p-value of <0.0001, <0.0001 respectively which is a significant correlation between the DAS28 and TNF- α , IL-6. Multivariate linear regression analysis was done to assess the independent predictive value ESR, TNF- α and IL-6 on DAS28.

It was found that ESR ($p = <0.0001$), TNF- α ($p = <0.0001$) are independent predictors of disease activity while IL-6 ($p = 0.569$) was not independent predictors of disease activity. The correlation of TNF- α and IL-6 levels with disease activity in this study is concordant with the results of some previous studies in the literature like Takeuchi et al¹³, Petrovic-Rackov et al¹⁴, Kang SY et al¹⁵ and Van Leeuwen et al¹⁶. We also studied the effect of various parameters on the HAQ score. Univariate linear regression analysis was done to look for any correlation between HAQ with TNF- α and IL-6 which showed correlation coefficient=0.35, 0.592 and p-value=0.0061, <0.0001 respectively, as this is also concordant with the studies done by Milman et al¹⁷ and Takeuchi et al¹³.

In our study, TNF- α and IL-6 have been found to have a significant correlation with disease activity as measured by DAS28 and with a greater functional disability as assessed by HAQ score. So, in patients with RA, serum levels TNF- α and IL-6 can be used as a marker of higher disease activity and greater functional disability. The limitation of our study is that our study was a cross-sectional study, but ideally to look for such predictors one would also want to look at within-patient variability in cytokines expression in relation to disease activity through a longitudinal study, preferably with multiple assessments before as well as after initiation of therapy. Secondly, our study had a small sample size of 60 cases due to which our statistical analysis was limited to basic correlation and comparison. So further studies should be done in larger number of patients with RA.

CONCLUSIONS

In patients with rheumatoid arthritis, there is a statistically significant correlation between TNF- α and IL-6 with disease activity as measured by DAS28 score. In our study, out of TNF- α and IL-6, TNF- α is a better biomarker of disease activity as compared to IL-6. Functional disability, as assessed by HAQ scores, is directly proportional to TNF- α and IL-6 with patients with higher titers having a greater functional disability. If confirmed in larger studies, our results suggest that TNF- α and IL-6 could supplement clinical assessment in the assessment of disease activity in patients with rheumatoid arthritis.

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