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INTERLEUKIN 21 AS A DIAGNOSTIC MARKER OF RHEUMATOID ARTHRITIS.

Khadija Kiran[1], Amtul Huda[2], Zuhair Bhatti[3]

1. MBBS, M Phil
Assistant Professor
Department of Physiology
Gujranwala Medical College,
Gujranwala.
2. MBBS, M Phil
Assistant Professor
Department of Physiology
Abbotabad International Medical
and Dental College.
3. MBBS, MS
Consultant Orthopedic Surgeon
Department of Orthopedics
THQ Sambrial.

Correspondence Address:
Dr. Khadija Kiran
MBBS, M Phil
Bhatti Manzil Opposite Sahi Market
Main DC Road, Gujranwala.
drkhadijakran@gmail.com

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ABSTRACT… To investigate the role of IL-21 as diagnostic marker in diagnosis of rheumatoid arthritis. **Study Design:** Cross sectional study. **Setting:** Department of Physiology and Orthopedic Gujranwala Medical College, Gujranwala. **Period:** October 2017 to October 2018 in one year duration. **Materials and Methods:** A total of 150 patients were included in the study, main variables assessed in this study were positive predictive value negative predictive value, sensitivity, specificity and accuracy of IL-21 in diagnosis of rheumatoid arthritis. SPSS version 23 was used to analyze the data. P value less than or equal to 0.05 was taken as significant. Study was started after permission from hospital ethical committee and patients were informed in detail about disease and procedure to be done. Non probability consecutive sampling was used. **Results:** The estimated sensitivity was 93.6%. The estimated specificity was 50%. Positive predictive value was 96.3% and negative predictive value was 35.7%. The overall accuracy was 90.6% for diagnosing rheumatoid arthritis. **Conclusion:** IL-21 induces MMP3 in rheumatoid arthritis patients, identification of IL-21 from synovium of patients indicates the presence of rheumatoid arthritis. We observed 90.6% diagnostic accuracy of IL-21 for rheumatoid patients taking RA factor as gold standard of diagnostic tool.

Key words: MMP3, IL-21, RA Factor, Rheumatoid Arthritis, Synovial Joints,

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INTRODUCTION

Among chronic diseases of autoimmune system Rheumatoid Arthritis is fatal one chractarised as synovial inflammation, synovial tissue hyperplasia, synovial bone and cartilage destruction.[1] Fibroblasts like synoviocytes play an important role in its development like other contributing factors or events.[2] Fibroblasts like synoviocytes (FLS) stimulate the affected synovium to migrate towards healthy and fresh synovium in arthritis patients. This shift of synovium is responsible for destruction of synovium and physiology. FLS involve the extracellular matrix and responsible for release of Metalloproteinases which are responsible for the destruction of cartilage bone and joints.[3]

In the start and progression of rheumatoid arthritis cytokines play an important role.[4] The activation of synoviocytes and development of rheumatoid arthritis involves many pro inflammatory cytokines like interleukin six and tumour necrosis factor alpha.[5] In patients of the rheumatoid arthritis development and progression of disease can be prevented by regulating the pro inflammatory cytokines.[6] Among the family of IL-2 cytokines IL-21 is an important member which binds with IL-21 receptors and form IL-21 R which is a heterodimer. Expression of IL-21 initially is found by $CD4^+$ T cells and natural killer of T cells.[7]

In the rheumatoid synovium tissues RA-FLS plays role in expression of IL-21 R. In advance Condition of rheumatoid arthritis patients increase level of IL-21 was found in plasma which is a diagnostic marker of enhanced disease activity.[8,9] Progression of arthritis and inflammatory cytokines production can be reduced and attenuated by inhibiting the IL-21 with fusion of FC proteins. In streptococcal cell wall arthritis IL-21 R deficiency protects the inflammatory joints destruction in severe cases.[10]

In a study conducted by Montleone G et al[11] reported that IL-21 enhances the MMPs expression

in fibroblasts initiation. Degradation of ECM and basement membranes are also due to the role of MMPs which are dangerous for invasion and migration of severe types of cells. Because of lack of literature available on this topic and role of IL-21 on RA-FLS invasion and migration further studies needed to investigate and prompted the potential effect of IL-21.[12,13] In our study we investigate the diagnostic role of IL-21 in diagnosis of rheumatoid arthritis and to evaluate the diagnostic accuracy of IL-21 as a diagnostic marker.

METHODOLOGY

The study was started and completed in the Department of Physiology and Orthopedic Gujranwala Medical College, Gujranwala, from October 2017 to October 2018 in one year duration. Study was started after the ethical approval from ethical board of institution and written informed consents from the patients. Non probability consecutive sampling technique was used. Patients of age limit 30 to 60 years both genders and who were suspected for rheumatoid arthritis on history and clinical examination were involved in the study. Patients having any musculoskeletal disease, diabetes, congenital anomales and comorbid disease were excluded from the study.

Patient's sample of synovial tissues were obtained mechanically and send to the laboratories where it was washed in steroid cold buffered phosphate salines and digested with disphase II 150mg/ml at 37 degree centigrade for four hours and egitated gently. After that cell culture was done in Dulbecco,s modified the eagles medium. Fetal bovin serum (10%), 100mg/ml streptomycin and penicillin G 100U/ml and incubated in 5%cow enriched environment at 37 degree centigrade was used for supplimentation. Passages 4/7 used for cells culture and flow cytometric analysis. Reverse transcription polymerase chain reaction was used for extraction of RNA and enzyme linked immunosorbent assay (Elisa) was used to measure the cytokines concentration after the store of cell culture at 80 degree centigrade. Patient's blood samples were taken with all aseptic measures and stored in gel vial and send to the laboratories for RA factor.

Computer software SPSS version 23 was used for composition and analysis of the data. Numerical variables were calculated and presented as mean and standard deviation and qualitative variables were calculated and presented as frequency percentages. 2×2 contingency table was drawn for for positive and negative predictive values. Sensitivies, specificities and diagnostic accuracy was calculated.

RESULTS

One hundred and fifty patients were included in this study. It was observed that 131 patients with RA factor as well as on IL 21, known as true positive. Five patients with IL-21 are present on IL-21 but absent on RA factor, known as false positive. 9 patients had RA factor but absent on IL 21, labeled as false negative. Five patients had no on RA factor and also absent on IL 21, labeled as true negative. The difference was statistically significant (p=0.000). (Table-I).

Therefore, the estimated sensitivity was 93.6%. The estimated specificity was 50%. Positive predictive value was 96.3% and negative predictive value was 35.7%. The overall accuracy was 90.6% for diagnosing rheumatoid arthritis. (Table-II)

IL 21	RA Factor		Total	P-Value
	Yes	No		
Yes	True positive 131	False positive 5	136	0.000
No	False Negative 9	True negative 5	14	
Total	140	10	150	

Table-I. Comparison of RA factor and IL 21 the diagnosis of rheumatoid (n = 150)

Diagnostic Measures	Value
Sensitivity	93.6%
Specificity	50%
Positive Predictive Value (PPV)	96.3%
Negative Predictive Value (PPV)	35.7%
Accuracy	90.6%

Table-II. Diagnostic accuracy

DISCUSSION

In our study we observed diagnostic role of IL-

21 in patients of rheumatoid arthritism in a study conducted by Xing R et al[13] reported involvement of IL-21 in invasion and migration of fibroblasts like synoviocytes in samples of the rheumatoid arthritis patients which shows that IL-21 can be used as a diagnostic marker of rheumatoid arthritis. Huber LC et al[14] conducted a study on synovial fibroblasts and its key role in rheumatoid arthritis and reported that RA FLS have capability of invasion and migration into the bones and cartilage during progression of RA.

Liu R et al[15] conducted a study in regulatory effects of IL-21 on helper like cells in rheumatoid arthritis and concluded that IL-21 may be involved in rheumatoid arthritis pathogenesis so it can be used as a diagnostic marker of rheumatoid arthritis he also concluded that by antagonising the IL-21 treatment cause of rheumatoid arthritis can be achieved. This study goes in favour of our study. Another study by Lebre MC et al[16] conducted on this topic in 2017 and reported involvement of $CD4^{+}IL\text{-}21^{+}TNF^{+}$ T cells in destruction of synovium. Result of his study also indicates the role of IL-21 as a diagnostic marker of rheumatoid arthritis.

Xiaoyin Niu et al[17] conducted a study in 2014 on clinical biomarkers of rheumatoid arthritis and reported that along with multiple organic factors IL-21 is a contributing factor which induce the MMP3 in rheumatoid arthritis and express presence in synovial joints and fluid. By indicating the presence of IL-21 in synovium rheumatoid arthritis can be treated earlier. Magyari L et al[18] conducted a study on involvement of interleukins in rheumatoid arthritis and reported identification of disease-associated interleukin to disease onset in order to identify the pathways important for RA pathogenesis.

In 2017 Dinesh P et al[19] and reported role of IL-21 in rheumatoid arthritis pathogenesis, he also reported function of IL-21 in immune cells that promotes synovial destruction of cartilage and bone. Its involvement was also described in important signaling pathways. Cañete JD et al[20] conducted a study on role of cytokines and other soluble factors as diagnostic biomarker and shows that its presence can be confirmed by ELISA and gene expression. Cytokines were separated from joint sample of chronic disease patients of rheumatoid arthritis.

CONCLUSION

Results of our study reveal that IL-21 induces MMP3 in rheumatoid arthritis patients; identification of IL-21 from synovium of patients indicates the presence of rheumatoid arthritis. We observed 90.6% diagnostic accuracy of IL-21 for rheumatoid patients taking RA factor as gold standard of diagnostic tool.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Khadija Kiran	Conceived idea, Study design, Paper writing.	
2	Amtul Huda	Data Collection, Literature review, Data analysis.	
3	Zuhair Bhatti	Manuscript writing, Data analysis, Literature review.	