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Serum IL-7 as A Diagnostic Biomarker for Rheumatoid Arthritis, Validation with EULAR 2010Classification Criteria.

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Abstract:

Background: a beam of light was thrown on IL-7 in the rheumatology community because of increased levels of expression in the RA synovium as well as the synovial Fluid. The tissue level of IL-7 is extremely accompanied by the local measures of RA activity; nevertheless, its circulating level remains controversial. **Aim of the work**: To evaluate the levels of serum IL-7 in RA cases and to compare it to disease activity. Also, to investigate IL7as a biomarker for RA diagnosis.

Patients and Methods: Serum samples from 50 RA cases and 50 age & sex-matched control subjects were tested for IL-7 via ELISA, and DAS28 was utilized for assessment of the disease activity in RA cases.

Results: The patients' mean age was 41.8 ± 12.1 y, mean disease duration was 6.5 ± 5.8 y and. The included cases were 44 women and 6 men. The mean DAS28 of cases was 3.95 ± 0.9. Serum levels of IL-7 were elevated in RA cases (105 ± 88.6 pg/ml) than in the control group (52.4 ± 35.6 pg./ml) ($p < 0.001$). Serum IL-7 levels showed significantly correlation with DAS28 ($r = 39$, $p = 0.001$). At a cutoff > 50 pg./ml, serum IL7 levels exhibited a sensitivity & specificity of 78% and 62% respectively ($p < 0.001$) in diagnosing RA.

Conclusion: Serum IL-7 levels were elevated in RA cases compared to the control group and were elevated with higher disease activity rendering it a propitious biomarker for RA. IL7 proved a great diagnostic power for RA disease that could help to understand the pathogenesis of RA and determine novel treatment options.

Key words: IL 7, Rheumatoid arthritis, Diagnostic biomarker

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease. Joint pain, swelling, and limitation of mobility represent the commonest features. The disease course shows great variations among diseased individuals. The mild course of the RA might be present in some

cases; however, the vast majority of cases have marked joint damage along with disability. In addition to the articular symptoms, RA is accompanied by extra-articular manifestations [1].

The precise cause & pathogenesis of RA is still a matter of debate. It is postulated that the imbalanced proinflammatory and anti-inflammatory cytokines activity enhances autoimmunity, chronic inflammation, and thus the destruction of joints [2].

Interleukin7 is produced by stromal cells in lymphopoietic regions that control the helper T cell's homeostasis peripherally. It induces mature Naïve as well as memory T-lymphocyte proliferation, survival, and differentiation [3], [4].

IL-7 level in joints of cases suffering RA was proved to be increased [5] and was accompanied by increased disease activity [6], on the contrary, the circulating level of IL7 in RA remains contradictory [7].

Animal models as well as human research have concluded that IL7 might be responsible for the perpetuation of inflammation, and it is now a well-recognized therapeutic target for several autoimmune diseases [8], [9].

Currently, initiation of aggressive therapy is recommended at an early phase as soon as diagnosis is established. Research interested in exploring the significance of cytokines in the pathogenesis of RA resulted in developing advanced therapeutic regimens that include the targeted cytokine-based pathways [10].

In spite of the well-documented value of the determination of ACCP as a diagnostic criterion for RA, it's still a necessity for new biomarkers to help improving early diagnosis, as the presence of ACPA is only reported in 40– 50% of patients attending with early arthritis and awaiting diagnosis [11], [5].

Hence, the aim of this study was to determine the levels of IL7 in RA cases' sera, and its relationship to disease activity, and to detect if it has a diagnostic value for early diagnosis of RA patients.

2- Patients and methods:

The study was conducted on 50 cases suffering RA diagnosed according to ACR/EULAR 2010 classification criteria of RA [12] and 50 age and sex matched control subjects. Full history taking, general and local assessment were performed for all participants. Assessment of the disease activity was done for RA patients using Disease activity score (DAS)28. while disability in patients was evaluated via Modified Health Assessment Questionnaire (MHAQ). laboratory investigations (ESR, CRP, CBC, liver function, kidney function, ACCP, RF) and Plain radiographs for both hands, feet, and chest. Serum Interleukin-7 concentration was measured in sera of the cases as well as the control subjects using (ELISA) kit following the instructions of the manufacturer (Sino Gene Clon Biotech Co., Ltd, China). Finally, all the data were collected and statistically summarized.

Statistical Analysis: coding of the data was done to be analyzed via the SPSS version 20. We presented the data in the form of frequency and % or mean ± SD. Statistical tests employed are independent sample *t*-test and Chi Square test for comparison. Correlation coefficient (r) (Pearsons' correlation) test was done to detect correlation, and ROC curve analysis to determine cutoff value of IL-7. P-values of less than 0.05 were detected as significant.

3- Results

The 50 RA cases mean age was 41.8 ± 12.1 years (20–64 years) and disease duration 6.5 ± 5.8 y (2 months– 21 y). The 50 controls were of matched age and sex (20–64 years) ($p = 0.999$), 6 males and 44 females ($p = 0.999$). The criteria of the cases are presented in Table 1. serum IL7 was significantly elevated in RA patients compared to the control subjects (105 ± 88.6 vs. 52.4 ± 35.6 respectively; $p < 0.001$) (Table 2) (figure 1). Positive correlation was found between serum IL7 and DAS 28, disease duration and ESR with P- value (0.003, 0.037, 0.001) respectively (Table 3)(figures 2,3,4). No differences were proved in the level of S.IL7 among patients with positive and negative

ACPA nor patients with positive and negative RF (P-value > 0.775, P-value > 0.687) respectively (Table 4). The ROC curve was used to evaluate the efficacy of plasma IL7 as an effective diagnostic biomarker for RA cases. At a cutoff value > 53 ng/ml, plasma IL7 exhibited a sensitivity of 78% and a specificity of 62 % for the diagnosis of RA with AUC 0.731 ($p < 0.001$), the PPV was 67.2, whereas the NPV was 73.8 (Table 5) (figure5).

4 - Discussion

In the current study, serum level of IL7 was higher in cases with RA (105 ± 88.6 pg./ml) than in controls (52.4 ± 35.6 pg./ml) with P value ($p < 0.001$).

This result matched with various studies that demonstrated increased plasma IL7 in RA cases in comparison to control subjects, ***van Roon et al in 2003***, measured the plasma levels of IL7 in 34 RA cases and 32 healthy volunteers and revealed that the plasma level of IL7 in RA patients was fivefold higher, they also found that CRP level in RA cases has a positive correlation with IL7[6].

In 2004, Stabler et al found that RA patients showed increased cytokines assessed, with the highest level for IL 2&4 &5 &6 &7, &10 and IL-13 [14]. Another multi-cytokine profile study done by ***Hitchon et al in 2004***, showed higher levels of serum IL-7 (~ 300 pg/ml) in their RA patients [15].

Pickens et al in 2011, concluded that IL7 and the receptor expression were highly increased in RA synovial fluids and macrophage of peripheral blood [16]. ***In 2022, Abdel Baki et al*** tested sixty-six rheumatic arthritis cases and twenty control subjects for Gal-3 and IL7 and observed that plasma Gal-3 & IL7 were elevated in RA cases in comparison with control subjects [17].

On the other side, other studies revealed a decreased concentration of circulating IL7 in cases with Rheumatoid arthritis. ***Churchman and Ponchel in 2008, Ponchel and et al in 2005,*** and ***Burska et al in 2018*** [13],[18],[5].

The discrepancies in the results of IL7 expression in RA patients might result from the usage of various ELISAs. IL7 level markedly depends upon the status of serum collection, particularly, the tubes used for collecting the blood samples, as recommended by instructions of ELISA manufacturers. The blood IL7 value revealed for control subjects in these studies varied as well [13].

The available particular binding sites on T-cells might be denoted by the level of circulating IL-7. Various kinds of cytokines (like IL-1&-2, TGF-β, TNF-α, and IFN-c) produce upregulation or downregulation of IL7 expression in various tissues, and further studies are crucial to determine the mechanism that modulates circulating levels of IL7 [19].

In our study, serum IL-7 exhibited a significant correlation with disease activity in RA cases presented by DAS28 score (P value = 0.003), and with ESR (P value < 0.001). These results matched with many studies, ***Van Roon et al in 2003***, documented elevated blood IL7 in RA cases that correlated with CRP [6]. The level of IL7 in blood in juvenile RA cases in the study done by ***Benedetti and his colleagues in 1995***, showed a significant elevation in comparison with control subjects, and they have a correlation with the inflammatory markers (serum level of IL6, CRP) as well as with the systemic criteria [20].

Also, ***Van Roon et al in 2007***, concluded that serum levels of IL7 were related to the disease activity indicators (such as the erythrocyte sedimentation rate and DAS 28) [21]. ***Chen et al in 2013***, documented those cases with elevated DAS28 scores showed increased IL7 levels in RA [22]. ***In 2022, Abdel Baki et al*** found that IL-7 was higher in RA cases and was correlated with DAS28 [17].

IL-7 is a member of the IL-2/IL-15 family that's synthesized by stroma cells (that include the thymus gland, BM, and soft tissue), GIT& hepatic epithelium, fibroblast cells, smooth muscles, endothelium, and keratinocyte cells, and DCs [23].

In our study, serum IL7 has diagnostic sensitivity (78%) and specificity (62%) for RA disease, its PPV was 67.2, whereas the NPV was 73.8. So IL7 can be used as a biomarker for RA diagnosis.

Similar to our result, ***Goëb et al in 2013***, concluded the capacity of serum IL7 to detect cases having very early inflammatory joint manifestations who might develop RA within 2 years later,

irrespective to positive or negative ACCP status. They postulated that, following ACCP, IL7 might serve as the 2nd-best diagnostic marker for RA [11].

Burska et al in 2018, demonstrated that IL7 was the 2nd best predictive marker following swollen joints count and that it's an appropriate biomarker for ACPA-negative rheumatoid arthritis with a good model fit [5].

In 2022, Abdel Baki et al found that serum levels of IL-7 revealed a sensitivity of 92.4 percent and a specificity of 95 percent ($p < 0.001$) in diagnosing RA [17].

To conclude, the serum level of IL7 was considerably greater in RA patients in comparison with control and was elevated in patients with increased disease activity making it a promising diagnostic marker for RA that could help to understand the pathogenesis of RA and determine novel treatment options.

Table 1. Characteristics of RA cases.

Parameter (mean ± SD) or No. (%)	**RA cases (no =50)**
Age (years)	41.8 ± 12.1
Duration (years)	6.5 ± 5.8
Modified HAQ score	0.7 ± 0.3
DAS 28	3.95 ± 0.9
VAS	40.7 ± 10.9
ESR (mm3/1st hr)	47 ± 27.5
Positive RF	54 (100)
Positive ACPA	48(100)

SD: standard deviation, MHAQ: modified health assessment Questionnaire, VAS: visual analogue scale, DAS 28: 28 joints disease activity score.

Table 2. serum level of interleukin 7 in RA patients and control.

S.IL7	**RA cases (n = 50)**	**Control (n = 50)**	**p**
mean ± SD	105 ± 88.6	52.4 ± 35.6	0.001
Median (min-max)	78.5(22-476.5)	43.7(0.5-123)	

IL7: interleukin 7, *P-value is significant

Table 3. Correlation between serum levels of interleukin 7 and characteristics of RA cases.

Variable r (p)	**RA patients (n = 50)**
Age 0.088	0.543
DAS28 0.410	0.003
Duration 0.297	0.037
Vas 0.052	0.722
M HAQ 0.235	0.101
ESR 0.542	<0.001

MHAQ: modified health assessment Questionnaire, VAS: visual analogue scale, DAS 28: disease activity score 28.

Table 4. Association between IL7, ACPA, and RF in the RA group:

RA cases	**mean ± SD**	**P value**

(n = 50)			
ACPA	**Negative (26)**	101.211± 81.74034	0.775
	Positive (24)	109.166 ± 97.09326	
RF	**Negative (27)**	110.354 ± 92.6417	0.687
	Positive (23)	100.115 ± 85.6635	

RF: rheumatoid factor, ACCP: anti cyclic citrullinated peptide.

Table 5. Cut off, sensitivity, specificity, PPV and NPV of IL7 in detection of RA:

Items	**Values**
Cut off	>53
P-value	<0.001*
Area under curve	0.731
Sensitivity	78.00(64.0 - 88.5)
Specificity	62.00(47.2 - 75.3)
positive predictive value	67.2(58.3 - 75.1)
negative predictive value	73.8(61.6 - 83.2)

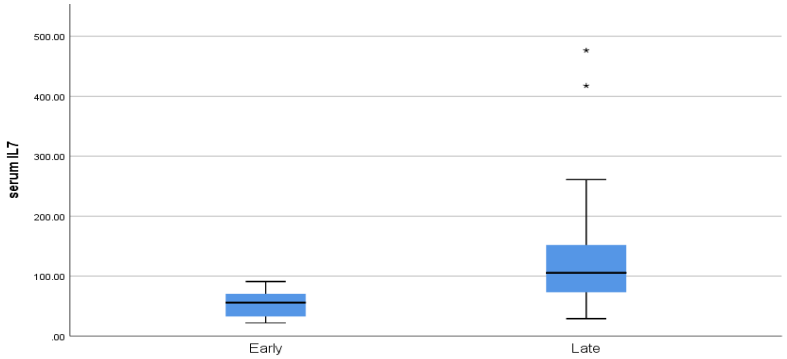

Figure 1. Comparison between early and late RA regarding the IL7 level.

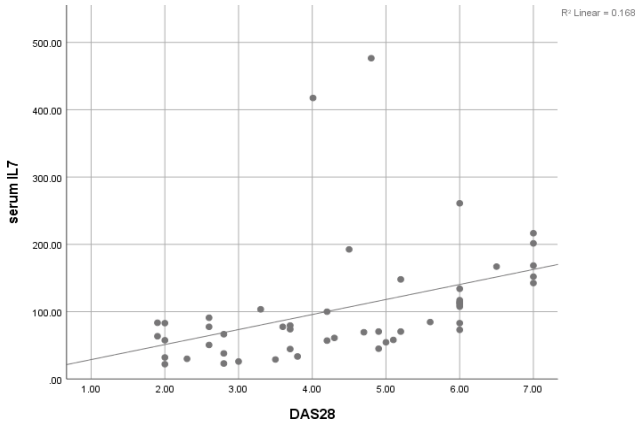

Figure 2. Correlation between IL7 and DAS28 in RA

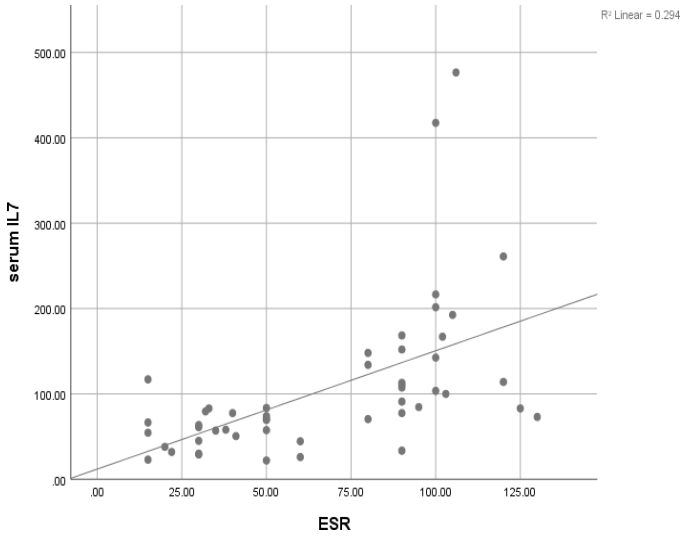

Figure 3. Correlation between IL7 and ESR in RA

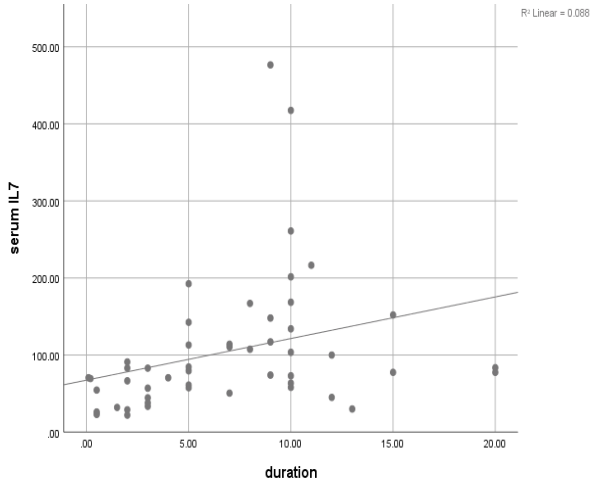

Figure 4. Correlation between IL7 and disease duration in RA

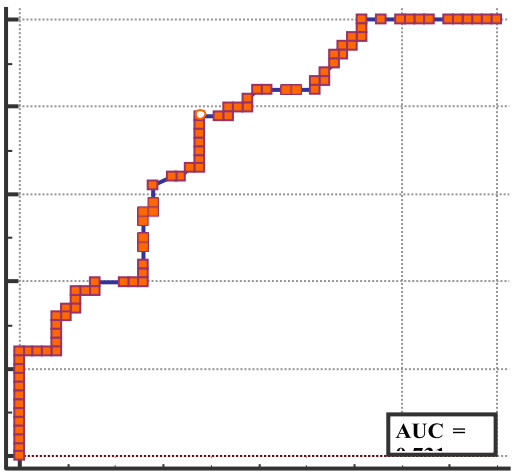

Figure 5. ROC curve for prediction of RA from IL7 level

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