RESEARCH ARTICLE

Association of serum IL12 with clinical and biochemical parameters in a cohort of diagnosed rheumatoid arthritis patients on oral conventional synthetic disease modifying anti-rheumatic drugs

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Abstract

Objective: To determine the association of serum interleukin-12 levels with disease progression in active rheumatoid arthritis patients on oral conventional synthetic disease-modifying anti-rheumatic drugs.

Methods: The case-control study was conducted at the Army Medical College, Rawalpindi, in collaboration with the Pak Emirates Military Hospital, Rawalpindi, Pakistan, from January to December 2022, and comprised rheumatoid arthritis patients or either gender aged 18-75 years who were placed in group I, while group II comprised healthy controls. Demographic and clinical data was noted, and 2ml blood samples were drawn from each subject. The serum was separated and analysed using sandwich enzyme-linked immunosorbent assay to quantify serum interleukin-12 levels. Data was analysed using SPSS 22.

Results: Of the 150 subjects, 75(50%) were in group I; 27(36%) males and 48(64%) females with overall mean age 45.70±11.70 years. There were 75(50%) subjects in group II; 37(49.3%) males and 38(50.7%) females with overall mean age 31.70±7.70 years. Serum interleukin-12, erythrocyte sedimentation rate and C-reactive protein-quantitative levels were significantly higher in group I compared to group II ($p<0.05$). Smoking, positive family history of rheumatoid arthritis in a first-degree relative and history of consanguinity were identified as risk factors though they were not statistically significant ($p>0.05$). In group I (n=75), out of total study subjects, only 55(73.3%) cases belonged to the predominant castes, namely Awan, Rajput, Pathan, Araeen, Bhatti, Malik, Mughal, Sudhan, Chaudary, and Jutt. These individuals showed significantly higher mean serum interleukin-12 levels compared to patients of other castes in the same group.

Conclusion: Mean serum interleukin-12 levels were higher in rheumatoid arthritis patients despite being on oral conventional synthetic disease-modifying anti-rheumatic drugs.

Key Words: Cytokines, Interleukin-12, Arthritis, Rheumatoid, Enzyme-linked immunosorbent assay.

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Introduction

Rheumatoid arthritis (RA) is characterised as a chronic, systemic, autoimmuno-inflammatory disease that results due to the development of self-reactive T cells and consequent auto antibodies targeted against articular and extra-articular sites. It primarily affects the joints, including hands, wrists and knees. The prevalence of the disease varies greatly, depending upon geographical, environmental as well as genetic factors, and urban and rural settings of a certain global population. In a systematic review, Almutairi et al. reported mean global prevalence of RA to be 0.56%[1]. The prevalence of RA in Western Australia was observed to be 0.34-0.36% from 1995 till 2014.[2] The RA prevalence was reported to be 0.65% in Japan[3] and 0.69% in Spain[4]. The reported prevalence in Karachi, Pakistan, is 0.142%[5]. The onset of the disease primarily occurs due to genetic predisposition (60%), and prompted by environmental factors (40%)[6]. The pathophysiology of RA involves many cellular events, of which cytokines constitute a very important factor. Balanced cytokines in circulation play a crucial role in the signalling mechanisms. Dysregulation among the various cytokine levels contribute to the development of a wide spectrum of autoimmune diseases. In RA, the inception of autoimmunity, inflammation and joint destruction occurs due to an imbalance between pro-inflammatory and anti-inflammatory cytokines[7]. Interleukin-12 (IL-12) is a cytokine produced by T cells of innate immune system. IL-12 is a heterodimer (p70), comprising two covalently linked non-identical subunits. It plays a key role in maintaining balance between the inborn and adaptive sections of the immune system. It is a powerful stimulator of interferon gamma (IFNγ) by T cells and natural killer (NK) cells and promotes T helper (Th1) response, both of which result in autoimmunity[8]. Molecular analysis from

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synovial tissue revealed significantly higher expression of IL-12 p40 messenger ribonucleic acid(mRNA) among RA patients[9].

IL12 levels were found to be raised in both blood serum and synovial fluid of RA patients[10]. Moderately serious RA patients are initially treated with analgesics and prescribed physical activity. Later, non-steroidal anti-inflammatory drugs (NSAIDs) and steroids are included to the regime, followed by disease-modifying anti-rheumatic drugs (DMARDs), which are of two main types; conventional synthetic disease-modifying anti-rheumatic drugs(CSDMARDs), like methotrexate, hydroxyquinone, leflunomide and sulfasalazine, and relatively newer biological disease-modifying anti-rheumatic drugs(BDMARDs), like tumour necrosis factor (TNF) inhibitors, IL-12/23 inhibitors, etc[11]. Studies have been conducted previously to find association between serum IL-12 levels and RA disease progression and severity in Chinese[12], Egyptian[13], Bulgarian[14], Turkish[15], and Polish populations[16]. However, no study has been conducted to explore the phenomenon in Pakistani population. Also, most studies have revealed other cytokines' response to DMARDs, but IL-12 response to anti-rheumatic drugs in active RA patients has not been studied. The current study was planned to fill the gap by determining the association of serum IL-12 levels with disease progression in Pakistani active RA patients on oral CSDMARDs.

Patients and Methods

The case-control study was conducted from January to December 2022at the Centre for Research in Experimental and Applied Medicine (CREAM) Laboratory of the Department of Biochemistry and Molecular Biology, Army Medical College (AMC), Rawalpindi, in collaboration with the Rheumatology Department of the Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, between Jan-Dec 2022. The study was conducted after the formal approval of the institutional ethical review committee. The sample size was calculated using the World Health Organisation (WHO)-linked calculator[17] with an anticipated population proportion of 1.0%[18],with confidence level 95% and absolute precision 0.05. Using nonprobability purposive sampling technique, the sample was raised, with RA patients in group I, and healthy controls in group II. Those in the group I were diagnosed RA patients of either gender aged 18-75 years. All non-RA patients and those with chronic illnesses, like diabetes, cancers, heart diseases, stroke, hepatitis and chronic lung diseases, were excluded.

After taking written informed consent from the subjects, data on demographic and clinical parameters was noted. Blood samples (2ml) were taken from all the participants under aseptic conditions. The sample tubes were kept at room temperature for 30 minutes for coagulation to occur, followed by centrifugation at 4000 rpm for 15 minutes. For solid-phase sandwich enzyme-linked Immunosorbent assay (ELISA), human IL12p70 ELISA kit (catalogue no: E-EL-H0150) (Elabscience Taiwan Inc.) was used. The optical densities were measured spectrophotometrically at 450nm through an ELISA plate reader. A4-parameter logistic standard curve was constructed using an online calculator[19] by plotting mean optical densities of standards on Y-axis and standard concentrations on X-axis[20]. Serum IL-12 levels of unknown samples were calculated with the help of the standard curve.

Data was analysed using SPSS 22 (SPSS.V.22.0, IBM Statistic.Inc.) Categorical data was expressed as frequencies and percentages. Age and other biochemical parameters were expressed as Mean±Standard deviation (SD). For continuous scale variables, independent samples t-test, and, for categorical nominal variables, Pearson's chi-square test was used. $P<0.05$ was considered statistically significant. Baseline serum levels of biochemical parameters were not available as group I subjects were already on oral CSDMARDs at the time of the study. The disease severity and activity were assessed and calculated by DAS28, considering the assessments of tenderness and/or swelling of 28 joints, the erythrocyte sedimentation rate (ESR), and patients' global assessment of their health on a 10 cm visual analogue scale (VAS). DAS Calculator was used to obtain the disease activity values[21,22]. The scoring for determining the disease activity was adopted as; DAS28 ≥2.6 and ≤3.2, moderate disease activity: DAS28 >3.2 and ≤5.1, and high disease activity: DAS28 >5.1 as referenced values.

Results

Of the 150 subjects, 75(50%) were in group I; 27(36%) males and 48(64%) females with overall mean age 45.70±11.70 years. There were 75(50%) subjects in group II; 37(49.3%) males and 38(50.7%) females with overall mean age 31.70±7.70 years. Serum IL-12, erythrocyte sedimentation rate (ESR) and C-reactive protein-quantitative (CRP-Q) levels were significantly higher in group I compared to group II ($p<0.05$). In group I, majority cases originated from Punjab 56(74.7%), followed by Khyber Pakhtunkhwa (KP) 14(18.7%) and Azad Jammu and Kashmir (AJK) 6(8%).

Smoking, positive family history of RA in a first-degree relative and history of consanguinity were identified as risk factors though they were not statistically significant

Table-1: Inter-group comparison of age, mean serum interleukin-12 levels and other biochemical parameters.

Parameters	Controls (n = 75) Mean±SD	RA Mean±SD	*p*-value
Age (years)	31.70 ± 7.70	45.70 ± 11.70	---
Serum IL-12 (pg/mL)	4.80 ±3.40	27.70±86.60	0.023
ESR (mm/hour)	12.09 ± 3.56	34.36 ± 24.80	<0.001
CRP-Q (mg/L)	5.22 ± 2.42	15.76 ± 27.00	<0.001
RF (IU/ml)	---	44.28 ± 52.49	---
ACPA (U/ml)	---	178.73 ± 280.00	---

RA: Rheumatoid arthritis, SD: Standard deviation, IL-12: Interleukin-12, ESR: Erythrocyte sedimentation rate, CRP-Q: C-reactive protein-quantitative, RF: Rheumatoid factor, ACPA: Anti-citrullinated protein antibody.P<0.05 was considered significant.

Table-2: Inter-group comparison of demographic parameters and mean serum interleukin-12 levels.

Parameters	Controls(n = 75)			RA (n = 75)		
	Frequency	Serum IL12 levels Mean± SD (pg/mL)	*p*-value	Frequency	Serum IL12 levels Mean±SD (pg/mL)	*p*-value
Gender						
Males	37	5.80±3.30	0.14	27	26.40±59.90	0.922
Females	38	3.90±3.20		48	28.50±99.10	
Smokers	12	5.30±3.20	0.566	21	29.30±13.60	0.269
+ve Family History of RA	5	6.80±4.01	0.185	29	29.50±61.00	0.888
+ve History of Consanguinity	29	5.02±3.60	0.694	53	32.40±101.00	0.471

RA: Rheumatoid arthritis, SD: Standard deviation. P<0.05 was considered significant.

Table-3: Conventional synthetic disease-modifying anti-rheumatic drugs used in rheumatoid arthritis patients.

RA patients (n = 75) CSDMARDs (As single- or multi-drug therapy regimen)	Frequency	%
Methotrexate	62	82.6%
Hydroxychloroquine	38	50.6%
Leflunomide	25	33.3%
Sulfasalazine	9	12.0%)

RA: Rheumatoid arthritis, CSDMARDs: Conventional synthetic disease-modifying anti-rheumatic drugs.

(p>0.05) (Table 2). In group I, methotrexate (MTX)was the most commonly used CSDMARD 62(82.6%) (Table 3).

In group I (n=75), majority cases originated from Punjab 52(69.3%), followed by Khyber Pakhtunkhwa (KP) 14(18.7%) and Azad Jammu and Kashmir (AJK) 6(8%), while only 2(2.7%) belonged to Gilgit/Baltistan region and only 1(1.3%) from Sindh. Besides, out of total number of study subjects in the case group, 55(73.3%) cases belonged to 4 predominant castes, namely Rajput, Pathan, Chaudary and Jutt, and they showed significantly higher mean serum IL-12 levels compared to patients of other castes in the same group. There was a statistical significance observed (p=0.007) between disease duration and the DAS score (Table 4). The average disease duration was 6.8years±5.6 and average treatment time with oral CSDMARDs was 4.8 years±1.8. There were 3(5.45%) patients in a state of remission, and 39(71%) had a Disease Activity Score (DAS) score range 3.2-5.1 corresponding to moderate disease activity Table-1.

Discussion

The current study explored the association of IL-12 with disease progression in diagnosed RA cases already on CSDMARDs. Biochemically, cytokines are small peptide molecules and include IL, IFNs, chemokines, lymphokines and TNFs. Human genome codes for about 50 ILs and related proteins. They can be broadly divided into pro-inflammatory type I cytokines and anti-inflammatory type II cytokines. The type 1 cytokines include IL-1, IL-6, IL-12, IFNγ and TNF-alpha (α). The type 2 cytokines include IL-4 and IL-10. The symptomatic or clinical progression of a disease ultimately depends upon the maintenance of balance between types 1 and 2 cytokines[23].

There were more females among RA patients in the current study, which is in agreement with studies done in Taiwan[24], Greece[25,] and the United Kingdom[26]

A Turkish study reported median ESR 19.0mm/hour and median CRP 5.6mg/L27, while the current study had median ESR 12.09mm/hour and CRP 5.2mg/L.

There were 16% smokers in the current study's RA cases, while in one study there were 33% smokers among RA patients.[28] There were only 5% cases of RA having positive family history, while a study reported 44.8% such cases.[29]

Under pathological conditions, IL-12 present at the active sites of inflammation induces the development, survival and cell division of Th1-committed cells, producing a powerful Th1 response. It also causes the T regulatory cells (Tregs) to secrete IFNγ, thereby causing sustenance of organ-specific autoimmunity in RA and other autoimmune diseases[30]. The first anti-IL-12/23

Table-4: Mean serum interleukin-12levels and disease outcome in the predominant castes (n-55) of rheumatoid arthritis patients.

Caste-wise Predominant RA subjects (n = 55) CSDMARDs (As single or multi drug therapy regimen)								
				Disease outcome as per *DAS score				Significance of
Pre-dominant Castes (Frequency)	Serum IL12 (pg/mL)	Average Disease Duration (Years)	Average Duration of Treatment (Years)	Remission (<2.6)	Low Disease activity (2.6 – 3.2)	Moderate disease activity (3.2 – 5.1)	High disease activity (>5.1)	DAS Score with relevance to Disease (p=<0.05)
(n=55)		Mean±SD (6.8yrs±5.6)	4.8±1.8 years	3	9	39	4	
Awaan 14	10.50 ± 13.00	7	5	1	2	10	1	
Rajput 11	32.00 ±57.00	7	5	1	Nil	10	Nil	
Pathan 9	45.00 ± 90.01	7	5	Nil	3	4	2	
Araeen 5	5.41 ± 5.02	8	5	1	Nil	4	Nil	
Bhatti 3	6.63 ± 2.62	5	3	Nil	1	1	1	p=<0.007
Malik 3	8.51 ± 0.8.02	5	3	Nil	1	2	Nil	
Mughal 3	11.11 ± 14.03	9	5	Nil	2	1	Nil	
Sudhan 3	8.05 ± 6.00	5	3	Nil	Nil	3	Nil	
Chaudary 2	22.61 ± 31.00	13	7	Nil	Nil	2	Nil	
Jutt 2	337.01 ± 473.03	8	5	Nil	Nil	2	Nil	

*DAS: Disease Activity Score.

monoclonal antibody, 'ustekinumab', was formally approved by the Food and Drug Administration (FDA) for use in rheumatic diseases in 2007. Since then, it has been successfully used in patients of psoriatic arthritis, ulcerative colitis, and Crohn's disease, but its response in RA has not been encouraging[18]. The most used CSDMARDs for RA treatment in the current cohort were MTX, sulfasalazine, hydroxychloroquine and leflunomide. To date, MTX remains the first line of treatment for RA patients,[31] and its use in the current cohort was 82.6%.

Consanguinity was one of the issues found involved with RA disease in the current study. Chronic diseases with a defined genetic causal component not only tend to remain confined within specific castes and ethnic groups, they increase over successive generations. Therapeutic strategies should be devised considering the ethnic disparity of a certain population.[32] Ethnic disparity has been reported to be one of the crucial factors to cater to drug therapy's response in RA incidence.[33]

The current study has two major limitations: a relatively small sample size, and ethnic diversity. The first might have affected the statistical power of the study, while the latter may have introduced confounding variables and limited the generalizability of the findings.

Conclusion

The Pakistani cohort with active RA exhibited ethnic diversity and demonstrated a higher level of mean serum IL-12 despite being on oral CSDMARDs.

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Author's Contributions

IM and **IR:** Data collection, analysis and drafting.
AM and **IR:** Study design, data interpretation, review for intellectual content and final approval.
ZAB and **AJ:** Acquisition of data