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ORIGINAL RESEARCH

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Comparison of rheumatoid factor of IgM, IgG, and IgA isotypes with disease activity score 28 in patients of rheumatoid arthritis

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ABSTRACT

Background/Objectives: For the diagnosis of rheumatoid arthritis (RA), rheumatoid factor (RF) is a commonly used serological marker. Almost two-third of patients with RA have RF in their serum and it is associated with disease activity, bone erosion, and disease outcome. RF is an autoantibody targeting the "fraction crystallizable" (Fc) region of IgG antibodies and exists as IgA, IgG, and IgM isotypes.

Subjects and Methods: The current study was aimed to compare the RF isotypes IgM, IgG, and IgA with disease activity score (DAS) 28 in RA patients. This was a descriptive study, which included 83 RA patients. Blood samples of the patients were collected from the Department of Rheumatology, Sheikh Zayed Hospital Lahore. The levels of different isotypes of RF, i.e., IgM, IgG, and IgA were determined by enzyme linked immunosorbent essay technique. Data were analyzed by SPSS 20.0.

Results: RF isotypes IgM, IgG, and IgA were detected in 83%, 10%, and 40% of RA patients, respectively. RF isotypes were compared with DAS 28. No significant association was noticed between DAS 28 and RF IgM {Odd ratio (OR) [confidence interval (CI)]} [0.5 (0.07–3.18)], RF IgG [3.0 (0.27–32.20)], and RF IgA [0.4 (0.43–0.13)]. Disease duration was also compared with RF isotypes. There was no significant association between disease duration and RF IgM [OR (CI)] [1.4 (0.46–4.86)], RF IgG [1.6 (0.50–5.0)], and RF IgA [1.1 (0.53–2.7)]. All the isotypes of RF were detected in RA patients.

Conclusion: All the isotypes of RF were detected in RA patients. IgM-RF is superior to other RF isotypes as a screening method for RA.

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KEYWORDS

Rheumatoid arthritis; rheumatoid factor; DAS28; RF isotypes

Introduction

Autoimmunity is characterized by the breakdown of self-tolerance leading to a state of abnormal humoral and cell-mediated responses against self-components [1]. Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease predominantly affecting the joints [2]. It is prevalent in almost 1% of the world population [3]. It is reported as 0.14% in Urban Sind Province [4], whereas in northern Pakistan, it's 0.55% [5]. It is mainly the disease of females, affecting approximately three times more often than male [6]. The gender differences in the prevalence of RA are diminished in older age groups [7]. The etiology of RA is unknown but production of several auto antibodies often leads to joint destruction and disability [8]. Genetic, environmental, hormonal, immunologic, and infectious factors may play important role in the pathogenesis of RA. Socioeconomic, psychological, and lifestyle factors, e.g., tobacco use, are the main environmental risk factors in the development of RA [9], whereas 50% risk for RA comes from genetic factors [10]. There is strong known genetic association of RA with polymorphic human leukocyte antigen (HLA) DRβ1 alleles [11]. HLA-DRβ1-01*15 is more common in Pakistani RA patients [12].

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In RA, an immunological response triggers an inflammatory process that ultimately manifests clinically as typical signs and symptoms of disease, such as joint swelling and tenderness [13]. Many cytokines including tumour necrosis factor TNF-α, IL-1, IL-7, IL-15, IL-17A, IL-17F, IL-18, IL21, IL-23, IL-32, and IL-33 are implicated in the pathogenesis of RA [14].

Disease activity score (DAS) is major scoring system for evaluating disease activity of RA. van der Heijde et al. [15] reported the initial development of DAS and then DAS was modified by a group of investigators from the Netherlands [16]. The use of DAS for evaluating disease activity is officially recommended by the European League Against Rheumatism (EULAR) and in clinical trials improvement in disease activity and also in daily clinical practice. For the convenience of the study, modified DAS including 28 joint count was used (DAS28) [17], instead of the original DAS based on the Ritchie articular index and 44 swollen joint count [15]. DAS28 is calculated according to the formula that is composed of number of tender joints and swollen joints, patient's global assessment of disease activity on a visual analogue scale, and erythrocyte sedimentation rate (ESR) or C reactive protein [18].

The diagnosis of RA is made clinically on physical examination [14] and it is associated with production of several auto antibodies such as rheumatoid factor (RF), anti-perinuclear factor antibody, anti-neutrophil cytoplasmic antibody, heterogeneous nuclear ribonucleoprotein A2/B1 (anti-RA33), anti-flaggerin, anti-keratin antibodies, and cyclic citrullinated peptide (CCP) antibodies (CCP) [19]. The first autoantibody detected in the patients of RA was RF [20]. RF is an autoantibody targeting Fc region of IgG antibodies [21] and it exists as isotypes of IgA, IgG, and IgM. RF can be detected in the majority of RA patients with established disease, and it is one of the classification criteria for RA of the American College of Rheumatology [22]. RF is the most widely used blood test for the diagnosis of RA [23]. RA primarily affects joints; however, it also affects other organs in 15%–25% of individuals [24]. The objective of this study was to compare the RF isotypes IgM, IgG, and IgA with DAS 28 in RA patients.

Subjects and Methods

This descriptive study was conducted in the Department of Immunology, University of Health Sciences (UHS) Lahore after getting approval from the Ethical Review Committee and Advance Studies & Research Board of UHS Lahore and Ethical Review Committee of Sheikh Zayed Hospital Lahore that included 83 diagnosed RA patients on EULAR criteria (2010). The RA patients included in this study were naive, sero-positive for RF, without any extra-articular symptoms and had not taken any therapy for RA. The sample size was calculated by Ahmed et al..

After an informed consent, 5-ml blood sample of patients of each gender between 18 and 65 years was collected from the Department of Rheumatology Sheikh Zayed Hospital Lahore. Patients of other autoimmune diseases like systemic lupus erythematosus, diabetes mellitus, etc. were excluded. IgM, IgG, and IgA isotypes of RF were determined using commercially available enzyme linked immunosorbent essay (ELISA) kits (Generic Assays Germany) [25] where RF IgM > 10 IU/ml, RF IgG > 20 U/ml, and RF IgA > 25 U/ml was considered positive.

Statistical analysis

The data were entered and analyzed using SPSS 20.0. For quantitative variables, mean ± standard deviation was calculated such as age and hemoglobin. Median and interquartile range (IQR) were used for quantitative variables such as disease duration and ESR. While for qualitative variables, frequencies, percentages, and graphs were drawn. Chi-square test was applied to observe association between qualitative variables. Odds ratio (OR) with 95% confidence intervals (CIs) of the association of different isotypes with DAS 28 and disease duration was estimated by logistic regression. A p value of ≤ 0.05 was considered as statistically significant.

Results

For this study, a total of 83 patients of RA were recruited that included 62 (74.7%) females and 21 (25.3%) males and on comparison there was statistically significant difference between the two groups ($p < 0.001$) as shown in Figure 1.

Table 1. Demographic data, laboratory parameters, and clinical characteristics of patients.

Age (mean ± SD) years	40.71 ± 11.59
Hemoglobin g/dl (mean ± SD)	11.57 ± 1.68
Disease duration (median and IQR)	7 (12–3)
ESR mm/1 hour (median and IQR)	35 (48–26)

Normal ranges: Hemoglobin for men (13.5–17.5 g/dl). Hemoglobin for women (11.0–15.5 g/dl).

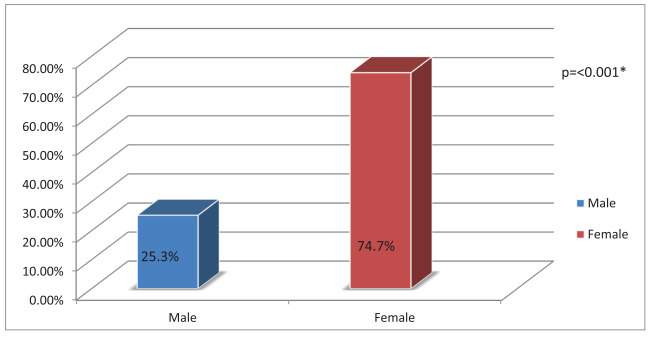

Figure 1. Frequency of males and females in study subjects.

Mean ± SD of age of the patients was 40.71 ± 11.59 years, mean ± SD of hemoglobin (Hb) of the patients was 11.57 ± 1.68 g/dl, median and IQR of disease duration was 7 (12–3) years. Median and IQR of erythrocyte sedimentation rate (ESR) was 35 (48–26) mm/1 hour (Table 1).

The mean ± SD for normally distributed variables while median and IQR for not normally distributed variables was calculated.

Among study participants, 83.1% were positive while 16.9% were negative for RF IgM. The comparison between positive and negative RF IgM was not statistically significant (p = 0.75). Similarly, for RF IgG, 12% were positive while 88% were negative. It was not statistically significant (p = 0.24). For RF IgA, 48.2% were positive while 51.8% were negative. It was not statistically significant (p = 0.57) (Table 2).

There was no significant association between DAS 28 and RF IgM [OR (CI)] [0.5 (0.07–3.18)], RF IgG [3.0 (0.27–32.20)], and RF IgA [0.4 (0.43–0.13)] (Table 3).

There was no significant association between disease duration and RF IgM [OR (CI)] [1.4 (0.46–4.86)], RF IgG [1.6 (0.50–5.0)], and RF IgA [1.1 (0.53–02.7)] (Table 4).

Discussion

In the current study, there were more females as compared to males, i.e., 62 (74.7%) females and 21 (25.3%) males. On comparison of gender, the difference was statistically significant (p < 0.001). It is in agreement with the study of Alam et al. [5] who studied 633 RA patients, out of which 509 (80.41%) were females and 124 (19.59%) were males.

Mean ± SD of age of the patients was 40.71 ± 11.59 years and it was in agreement with Shakiba et al. [26] documented 49.8 ± 12.3 years and Ahmed et al. [27] reported 49.5 ± 13.9 years age of their RA patients.

In the current study, median and IQR of disease duration was 7 (12–3) years, whereas Shakiba et al. [26] reported it as 9 (5–13) years. The probable reason for this difference could be the differences in population's genetics, susceptibility, or environmental factors [28,29].

In the current study, median and IQR of ESR was 35 (48–26). The current study is not agreement with Shakiba et al. [26] who determined median and IQR of ESR as 14 (18–24). The probable reason for this disagreement could be the subject having different disease intensities or the different diagnostic techniques being used for the determination of ESR in these studies.

In the current study, 83.1% patients had RF IgM, 12% had RF IgG, and 48.2% had RF IgA. Shakiba et al. reported the highest percentage of IgM isotype (46.5%) and least of IgG (21.74%) in RA patients. RF IgM and IgG were found to be raised in symptom free RA patients, while IgA is associated with severe manifestations in RA [22]. RF IgM and IgG are correlated with the symptoms of progressive erosions of joints, while extra articular manifestations are associated with RF IgA [25].

In the current study, there was no significant association between RF IgM and DAS28. Similarly, Ursum et al. [30] also could not detect significant association between IgM and DAS28. Karimifar et al. [31] suggested no correlation between DAS28 scoring and RF IgG but they observed positive correlation with IgA. The probable reason for this disagreement could be the difference in the patient cohort, severity of disease, or reference ranges used in various kits for determination of RF IgA.

Table 2. Distribution and comparison of RF IgM, IgG, and IgA isotypes in RA patients.

	RF IgM		RF IgG		RF IgA	
	Positive *n* (%)	Negative *n* (%)	Positive *n* (%)	Negative *n* (%)	Positive *n* (%)	Negative *n* (%)
	69 (83.1)	14 (16.9)	10 (12)	73 (88)	40 (48.2)	43 (51.8)
p-value	0.75		0.24		0.57	

$p \leq 0.05$ = statistically significance, p-value was calculated by using chi-square test.

Table 3. Association of DAS 28 with RF isotypes.

RF isotypes	DAS 28	*p*-value
	OR (95% CI)	
RF IgM	0.5 (0.07–3.18)	0.46
RF IgG	3.0 (0.27–32.20)	0.36
RF IgA	0.4 (0.43–0.13)	0.13

$p \leq 0.05$ = statistically significant.

Table 4. Disease duration and comparison of different isotypes of RF.

	Disease duration	*p*-value
	OR (95% CI)	
RF IgM	1.4 (0.46–4.86)	0.50
RF IgG	1.6 (0.50–5.0)	0.42
RF IgA	1.1 (0.53–2.7)	0.66

$p \leq 0.05$ = statistically significant.

Conclusion

All the isotypes of RF were detected in RA patients. IgM-RF is superior to other RF isotypes as a screening method for RA because it was found to be positive in more number of patients as compared to other isotypes but the statistical difference was not significant at all. RF titer may be valuable in estimation of disease activity and other inflammatory parameters in RA patients.

Limitations and Future Recommendations

Only serum was tested for the presence or absence of RF isotypes which could be further improved by investigating and correlating with susceptibility genes (HLA) and environmental factors. Large scale study should be carried out to measure the level of RF isotypes that could increase the reliability of findings of the current study.

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