Original article

Prevalence of Autoantibodies in Healthy Adults in Pakistani Population

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Abstract

Introduction: Autoimmune phenomenon is attributed to a number of diseases which were once considered idiopathic. In humans, production of auto antibodies (a-Abs) against self-antigens is quite frequent but earlier their presence was associated with autoimmune diseases, however a-Abs have been documented in non-autoimmune disorders i.e. complicated pregnancy, cancer, stroke etc. In Pakistan, limited data is available on frequency of a-Abs, therefore this study was designed to determine serum level of antinuclear antibody (ANA), Rheumatoid factor (RF) and anti dsDNA antibodies in apparently healthy population. ***Materials and methods:*** After written informed consent, blood sample of 256 subjects was obtained by random sampling. Participants of established autoimmune diseases were excluded. Enzyme linked immunosorbent assay (ELISA) was used to determine ANA, RF and anti-dsDNA antibody. Categorical variables were compared by using χ^2 test. A p value <0.05 was considered statistically significant. ***Results:*** Rheumatoid factor was the most frequent a-Ab (18%), followed by anti dsDNA (7.4%), while ANA was the lowest (0.4%) antibody detected. Only RF had a statistically significant association with gender (p=0.047). No association of these antibodies with age was detected. ***Conclusion*:** Rheumatoid factor auto antibody was more prevalent as compared to ANA and dsDNA antibody in healthy adults.

Keywords: ANAs; Anti dsDNA; Anti RF; Autoantibodies; Autoimmune diseases

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Introduction

Autoimmune phenomenon is attributed to a number of diseases which were once considered idiopathic. Weak immune reaction against self is needed to maintain homeostasis but whenever antigenic pressure is high or altered, disruption of homeostasis leads to autoimmunity[1].

In humans, production of autoantibodies (a-Abs) against self-antigens is frequent and it was thought that a-Abs are associated with autoimmune diseases but increased level of serum a-Abs have been documented in non-autoimmune disorders i.e. complicated pregnancy, cancer, stroke etc[2,3,4]. Low level of natural a-Abs are present in healthy individuals[5] but their level varies with age, physical loads, and stress caused by various unfavorable factors. Generation and level of a-Abs are associated with changes at molecular level in certain cells that may leads to autoimmune damage in some organs or whole body[6].

Regarding development of a-Abs, first phase is asymptomatic and without a-Abs, second phase is benign, asymptomatic but have a-Abs whereas third phase is symptomatic with a-Abs. Disruption of

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activating and inhibitory signals causes production of pathogenic a-Abs[7]. In autoimmunity, there are abnormal changes in the rate of apoptotic or necrotic events and abnormalities in expression/secretion of multiple autoantigens [1].

Antinuclear antibody (ANA) have central role in autoimmunity and it is directed against variety of nuclear antigens. It is found in different autoimmune disorders and in healthy individuals[3,9] but it enables clinicians to predict diagnosis and severity of disease[7,8]. Some members of ANA family of a-Abs may present years before the diagnosis of disease.

a-Abs against double stranded DNA (ds-DNA) are specific for systemic lupus erythematosus (SLE) and 70% of SLE patients have these antibodies whereas <0.5% of healthy individuals may have these antibodies[10].

Rheumatoid factor (RF) is heterogeneous antibody of IgM class; directed against Fc fragment of IgG. It is used as a disease marker of rheumatoid arthritis (RA)[11] but it can be detected in other connective tissue and inflammatory disorders. About 1-5% of healthy individuals may have this antibody and they are at increased risk to develop RA[12].

Most of the a-Abs belongs to IgG class but IgM and IgA classes have also been detected. Upon significant rise in the level of a-Abs, autoimmunity starts and autoimmune disorders have different male to female ratio e.g. SLE 9:1, RA 2.5:1, myasthenia gravis 2:1, Hoshimotos thyroiditis 10:1, multiple sclerosis 2:1, etc [13,14].

Presence of a-Abs supports the diagnosis of autoimmune disorders but do not confirm it, therefore clinical symptoms and / tissue damage should be present for clinical diagnosis [15]. Blood biomarkers are needed for early diagnosis of autoimmune diseases and for identification of those who are at risk to have these disorders. The data on the frequency of a-Abs in healthy individuals is important for the diagnosis of autoimmune disorders [15,16,17,18]. Studies have been performed in healthy individuals in various countries [19] but limited data is available about the frequency of a-Abs in Pakistan, therefore, this study was designed to determine serum level of ANA, RA and anti dsDNA antibodies in apparently healthy population.

Materials and Methods

This cross sectional study was carried out in the Department of Immunology, University of Health Sciences Lahore (UHS) Pakistan. It was approved by the 'Ethical Review Committee' and 'Advanced Studies Research Board' of UHS Lahore. After written informed consent, serum from 256 subjects was obtained by random sampling. Participants with established autoimmune disease were excluded. ELISA technique was used to determine ANA (GA, Germany), dsDNA (Inova USA) and RF antibody (Glory Science, USA). Subjects with ANA >1.0, dsDNA >75 IU/ml and RF >40 U/ml binding index were considered positive. SPSS-20.0 (IBM-SPSS, Inc, Armonk, New York) was used for statistical calculations. Categorical variables were compared by using χ^2 test. $p \leq 0.05$ was considered statistically significant.

Results

In this study, there were more male (133) as compared to female (123). Mean ±SD of age of males and females was 25.9 (3.52) and 24.54 (0.76) ranging from 18-37 and 16-45 (yrs) respectively.

ANA was detected in 0.8% males and 0% females and RF was detected in 22.6% males and 13% females while anti-dsDNA was detected in 4.5% males and 10.6% females and on comparison there was no significant difference in these parameters. Frequency of ANA, RF and anti-dsDNA was 0.4%, 18% and 7.4% respectively (Table 1 & 2).

Table 1: Comparison of autoantibodies based on gender

Variable	Male (n=133) (n,%)	Female (n=123) (n,%)	Total (n=256)	*p*-values	OR (95% CI)
dsDNA Positive	6 (4.5)	13 (10.6)	19 (7.4)	0.065	0.4 (0.147-1.087)
ANA Positive	1 (0.8)	0	1 (0.4)	1.00	1
RF Positive	30 (22.6)	16 (13.0)	46 (18)	0.047	1.948 (1.004-3.785)

More males (52%) compared to females (48 %) had a-Abs. Mean age of males was 25.98 years and of females it was 24.54 years. Among the subject 57% were less than 25 years (45.9% males and 54.1% females), 40% were between 26-35 years (60.6% males and 39.4% females) and only 3% were more than 35 years of age (50% males and 50% females).

Among the subjects 1 (0.4%) had ANA, 46 (18%) had RF and 19 (7.4%) had dsDNA. On comparison of gender, out of 133 males, 22.6 % had RF, 4.5% had dsDNA and 0.8% had ANA whereas out of 123 females, 13% had RF, 10.6% had dsDNA and none of the female had ANA. RF was significantly associated with gender (Odds ratio 1.948, 95% confidence interval, (1.004-3.785) while ANA and dsDNA were not associated with gender. It was observed that more males than females (22.6% vs 13%) had RF. Further, none of the a-Abs was associated with age (Table 1and 2).

Table 2: Comparison of autoantibodies based on age

Variable	Age group		p value	OR (95% CI)
	≤25 years (n=146)	>25 years (n=110)		
	Positivity (n)%	Positivity (n)%		
Anti-dsDNA	11 (7.5)	8 (7.3)	.937	1.039 (0.40 - 2.67)
ANA	1 (0.7)	0	1.000	1
RF	26 (17.8)	20 (18.2)	0.939	0.975 (0.51-1.86)

Discussion

In the present study, ANA was detected in 0.4% of healthy individuals which is lower than already documented i.e. 4% to 13% [20, 21, 22, 23]. ANA has been reported as 7.6% in Omani population [24] and 4.2% in Saudi Arabian population[20]. Baig and Shere (1989) also suggested ANA as four-fold more prevalent in females than in males in Saudi Arabian population, whereas in the current study, there was no difference of ANA between healthy male and females. Different geographic location, age of the subjects and different ethnicity could be the probable reasons for this difference in the prevalence of ANA in healthy individuals [20].

In the current study, there was high prevalence of RF in males (22.2%) than females (13%) and the prevalence of RF was 18% that is higher than 5.58% reported by Mordvinov (2000) in Moldova Republic population. Further, they reported RF as more prevalent in females as compared to males that is contradictory to the current study. Different ethnicity and environmental factors could be the probable reason for this difference [25]. It should be noted that RF a-Abs are frequently present in healthy subjects and can be detected in chronic infections. It have been suggested that RF may have a protective or regulatory role in the immune system [24].

In the current study, 7.4% subjects had anti dsDNA that is higher than 3.3% documented in the Omani population[26]. Difference in the study population and higher sample size in the current study could be probable reasons for this difference as Adel et al. (2007) included only 30 healthy individuals. Anti-Abs to DNA have been associated with drug induced SLE, mixed connective tissue disease, RA, scleroderma and Sjögren's syndrome. The presence of a-Abs in healthy subjects may be a prognostic signal of a disease in future or it may be a normal phenomenon linked to the environmental/geographic conditions and ethnicity [27].

Conclusion:

Rheumatoid factor auto antibodies were more prevalent as compared to dsDNA and ANA in healthy adults. Further, RF was associated with gender as it was prevalent more in males compared to females. ANA and anti dsDNA were not associated with age and gender.

Conflict of interest

None of the researcher has any financial or other interest in the products that were used for this study.

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