Original Article

Prevalence of Autoantibodies in patient complaining of multiple joint pain in a tertiary care hospital

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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. This study was designed to determine serum level of antinuclear antibody (ANA), Rheumatoid Factor (RF) and anti dsDNA antibodies in apparently healthy population with multiple joint pain. After written informed consent, blood sample of 294 subjects was obtained by random sampling. Participantsof established autoimmune diseases were excluded. Enzyme linked immune sorbent assay (ELISA) was used to determine ANA, RF and anti-dsDNA antibody. Categorical variables were compared by using $\chi 2$ test. A p value <0.05 was considered statistically significant. Rheumatoid factor was the most frequent a-Ab (19.05%), followed by anti dsDNA (7.14%), while ANA was the lowest (3.4%) antibody detected. Only RF had a statistically significant association with gender (p=0.047). No association of these antibodies with age was detected. Rheumatoid factor auto antibody was more prevalent as compared to ANA and dsDNA antibody in healthy adults.

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Key words **: ANAs, Anti dsDNA, Anti RF, Autoantibodies, Autoimmune disease.**

Anti-nuclear antibodies (ANA) are immunoglobulin directed against autologous cell nuclear and cytoplasmic components[1-3]. The occurrence of different ANA is associated with autoimmune disease and with differences in disease severity including extent of skin involvement, internal organ manifestation and prognosis[2]. Researchers have been performing steady efforts to develop tests for detecting ANA and disease-specific auto antibodies to nuclear antigens for the diagnosis, prognostic assessment, and monitoring of patients with systemic autoimmune diseases[4]. Nowadays, measurement of ANA has been widely used to provide supporting evidence of a diagnosis of autoimmune disease such as Systemic Lupus Erythematosus (SLE), Sjögren etc[5]. SLE is a multisystem disorder that is considered as a prototype Immune Complex (IC)-mediated disease[6]. This autoimmune disease related to central or peripheral nervous system; about 17% to 75% of patients respectively[7]. Additionally, levels of antibodies against dsDNA were shown covary with SLE disease activity[8]. The aim of this study was to investigate the prevalence of ANA and anti-dsDNA in patient with suspected autoimmune disease.

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)[9] 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[10].

Materials and Methods

Serum samples were obtained from different Department of Calcutta National Medical College and Hospital. Each of these serum samples was tested for the presence of ANA, RA factor and anti-dsDNA (Aeskulisa dsDNA check, Aesku Diagnostics, Germany) by ELISA method. These tests were performed by commercial kits according to the manufacturer's instructions. First of all, results were classified as ANA positive or negative according to the definitions contained within the packages for each kit. Subsequently, anti-dsDNA results were classified as positive or negative for each patient. Borderline results were arbitrarily classified as positive.

For statistical analyses, manufacturer suggested cut-off were applied to create positive and negative values from the continuous original observations. Positivity rates,

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specificities and Spearman correlation coefficient between assays were calculated as indicated using SAS software, Version 9.2 of the SAS system for Windows. In statistical analyzes, p-value <0.05 was considered as significant.

Results

In this study, there were more female (214) as compared to male (80). Mean ±SD of age of males and females was 42.20 (3.52) and 24.54 (0.76) ranging from 13-66 and 3-65 (years) respectively. ANA was detected in 4.67% females and 0% males and RF was detected in 10% males and 22.43% females while anti-dsDNA was detected in 5% males and 7.94% females and on comparison there was no significant difference in these parameters. Frequency of ANA, RF and anti-dsDNA was 3.4%,19.04% and 7.14% respectively (Tables 1 & 2).

More females (86%) compared to males (14 %) had a-Abs. Mean age of males was 35.28 years and of females it was 22.14 years. Among the subject 36.74% were more than 35 years (55.1% males and 44.9% females), 50% were between 26-35 years (70.4%males and 29.6% females) and only 13.26% were less than 25 years of age (30.7% males and 69.3% females).

Among the subjects 10 (3.4%) had ANA, 56 (19.05%) had RF and 21 (7.14%) had dsDNA. On comparison of gender, out of 80 males, 8 % had RF, 5% had dsDNA and none of the male had ANA whereas out of 214 females, 22.42% had RF, 7.94% had dsDNA and 4.67% 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 females than males (22.42% *versus* 8%) had RF. Further, none of the a-Abs was associated with age (Figs 1 & 2).

Discussion

In the present study, ANA was detected in 3.4% of healthy individuals with multiple joint pain which is lower than already documented ie, 4% to 13%[11-14]. In the current study, there was high prevalence of RF in females (22.43%) than males (10%)[15]. It should be noted that RF a-Abs are frequently present in healthy subjects and can be detected in chronic infections. ANA positivity rate found in our female patients is a consistent result with the knowledge of the autoimmune diseases are more frequent in women[16, 17]. This predominancy was researched by Leo and et al. According to their study, the hormone profile, fetal microchimerism and some strategic genes which are on the sex chromosomes are playing role on this relationship[18]. Our three years' experience of testing autoantibodies was shared in this study. Reliable test results are very important for the health of the patients with autoimmune disorders. For being a dependable laboratory, having enough knowledge and experience about the chosen methods of autoantibody tests is mandatory. A good relationship with the clinicians is also an indispensable component of confidential analysis and reporting.

Table 1 — *Comparison of autoantibodies based on gender*

Variable	Male (n=80)	Female (n=214)	Total (n=294)	p- values
ANA Positive	0	10	10	0.068
RF Positive	8	48	56	0.044
Ani-dsDNA Positive	4	17	21	0.6097

Table 2 — *Comparison of autoantibodies based on age*

Vaiable	Age group		p value
	<25 (n=39) Positivity (n)%	>25 (n=255) Positivity (n)%	
ANA Positive	1	9	1
RF Positive	12	44	0.1456
Anti-dsDNA Positive	5	16	0.1906

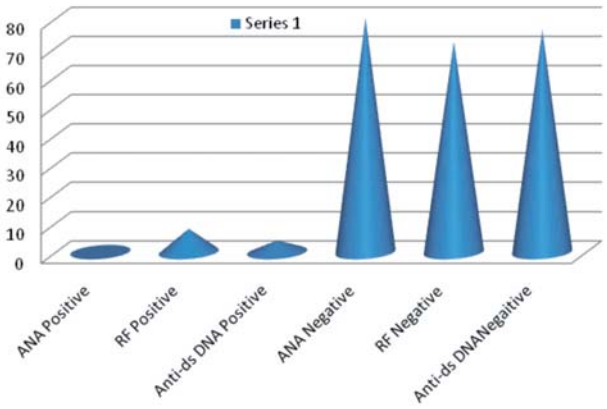

Fig 1 — Male ANA, RF and Anti-dsDNA test result Comparison Chart

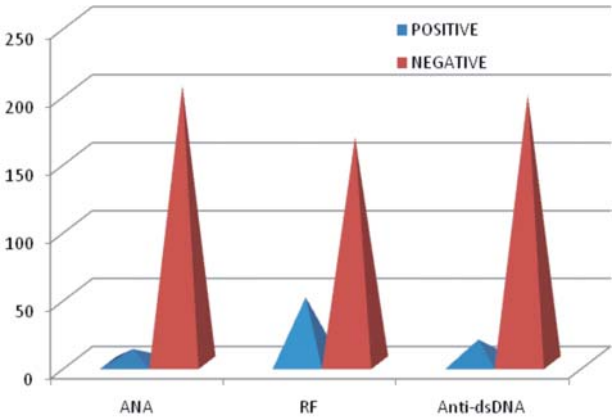

Fig 2 — Female ANA, RF and Anti-ds DNA test result Comparison Chart

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 researchers has any financial or other interest in the products that were used for this study.

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