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Occurrence of rheumatoid factor, anticardiolipin antibody and antinuclear antibody in healthy Indian adults (greater than expected in young adults)

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Abstract

Autoantibodies like Rheumatoid factor (RF), Anticardiolipin antibody (ACLA) normally used in diagnosing autoimmune diseases have also been identified in healthy individuals . The presence of such autoantibodies could alter their diagnostic and predictive value. Occurrence of these autoantibodies have been shown to vary with age, geographical location and other environmental factors. This study was done as an attempt to find the occurrence of RF,ACLA and ANA in healthy adults in South Indian population and to analyze the variations in occurrence between different adult age groups. The study was done in 150 subjects in three age groups of 50 each. Individuals between 20 to 39 years were under group 1, 40 and 59 years under group 2 and 60 years and above were under group3. Subjects were selected from health camps and blood donation camps in and around Bangalore. Rheumatoid factor was tested by latex agglutination method, Anticardiolipin antibodies by ELISA and Antinuclear antibodies by indirect immunofluorescence method. Frequency of occurrence of all three autoantibodies especially that of ACLA was greater than expected frequency in both group1 and 2 .The frequency in elderly(group3) was increased as expected. Results also show that co-occurrence of more than one autoantibody in the same individual increases as age advances. Increase in RF , ACLA, ANA in apparently healthy individuals should be assessed carefully to prevent unnecessary treatment but at the same time they may need follow up to avoid missing early diagnosis of autoimmune disease.

Key words: Autoantibodies, Rhematoid Factor, Anticardiolipin antibody, antinuclear

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Introduction

In earlier decades of the twentieth century , the term "horror autotoxicus" by paul Erlich meaning autoantibodies were somehow prevented from destroying self antigens , found broad acceptance and it was believed that autoimmune diseases was an impossibility. This Perception changed gradually when several studies demonstrated autoimmune diseases related to specific autoantibodies [1]. Since then many of these autoantibodies have been used in diagnosing autoimmune diseases.

Studies have also demonstrated that several specific surprisingly constant auto antibodies are present in all normal healthy individuals[2] . These are called as natural auto antibodies (na-Ab and have been shown to provide critical early protection against pathogens [3].

But recently autoantibodies like Rheumatoid factor(RF), Anticardiolipin antibody (ACLA) and Antinuclear antibody (ANA), long acting thyroid stimulating antibody, anticytoplasmic antibody normally used in diagnosing autoimmune diseases have also been identified in healthy individuals [2]. The presence of such autoantibodies in healthy individuals could alter their diagnostic and predictive value.

Occurrence of these autoantibodies vary with age, geographical location and other environmental factors [4]. Rheumatoid factor, Anticardiolipin antibody and Antinuclear antibody are widely used in India to aid diagnosis

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of rheumatoid arthritis , thrombotic conditions and SLE respectively but its occurrence in healthy individuals is not much explored. This study was done as an attempt to find the occurrence of RF,ACLA and ANA in healthy adults in South Indian population. It was also done to analyze the variations in occurrence between different adult age groups.

Materials and Methods

The study was done in 150 subjects (68 males and 82 females) in three age groups of 50 each. Individuals from 20 to 39 years were under group 1, those between 40 and 59 years were under group 2 and elderly subjects of 60 years and above were under group3. A comparable male : female ratio of 48 : 52 % was seen in younger age groups (group 1 and 2) but in the elderly group (group 3), females were higher by 20% . Health camps and blood donation camps were conducted in different semi urban areas in and around Bangalore to include a wider representative population. A proforma was used to collect their sociodemographic status and medical history of all the subjects attending the camps. Exclusion of predisposing risk factors (autoimmune diseases, diabetes, chronic infection and malignancies) was by history taking and general physical examination. Informed consent was obtained from all individuals. Blood samples were collected from all apparently healthy individuals and were investigated for the presence of autoantibodies. The blood samples were stored at minus twenty degrees Celsius and tests were done for all samples at the same time in the immunology department of Ramaiah medical college Bangalore. The study was approved by the Institutional review board.

Rheumatoid factor was tested by latex agglutination method(LAT) (using labkit code no 40121-100 test lot no 04). In routine practice, and particularly in developing countries such as India, LAT can still be the preferred method for RF determination when compared to nephelometry.[5] In this method polystyrene latex particles were coated with IgG acts as the antigen and binds with the rheumatoid factor in the test sample which acts as the antibody. This binding results in an agglutination reaction which can be visualized through the naked eyes The latex reagent sensitivity was adjusted to detect a minimum of 8 IU/ml of rheumatoid factors according with the WHO international standard without previous sample dilution.

Anticardiolipin antibodies were tested by ELISA after house standardization [6,7]. Polystyrene microplate strips were coated with 50 µg/ml of lyophilized cardiolipin in ethanol which bind to the plate and used as the solid phase containing bound antigens. The unbound free antigens were washed away. 300µl of 10%FCS in PBS was added as blocking solution and this binds to any excess protein binding site in the microtitre plate after the binding of the antigen. To this the diluted test sample was added and antibodies in the sample attach to the antigens coupled to the solid phase. The unbound proteins were washed off. In the next step, the attached antibodies were detected by binding with enzyme labeled Anti Human IgG peroxidase (diluted in FCS) antibodies. 50µl of TMB was added as substrate in each well and incubated at 37 ^{0}C for 15mts. 50µl of 0.1N H2SO4 was added to stop the reaction and the optical density(OD) reading was taken at 490 nm using an Elisa reader. Values above 20GPL for IgG and above 10GPL for IgM were taken as positive

Antinuclear antibodies were tested by indirect immunofluorescence method[8] (using UI507 ANA HEP-2 BIODIAGNOSTICA LTD). Slides made from human epithelial cell line were used as the antigen substrate. Diluted human sera (test sample) was incubated with the substrate. Antibodies present in the sera bind to the antigenic nuclei in the substrate forming antigen-antibody complexes. The unreacted antibodies were washed off. The substrate was then incubated with Human anti IgG rabbit globulins coupled with fluorescein isothiocyanate which binds with these complexes and the nuclear pattern appears apple green when viewed under a fluorescent microscope. Slides showing patterns in greater than 40% with 1: 40 dilution were taken as positive.

Results

The total number of subjects positive for one or more autoantibodies in each age group (n=50) were 18(36%) in group 1, 18 (36%) in group 2 and 25 (50%) in group 3. The overall occurrence appeared to be the same (36%) in both Group1 and Group2. But when each autoantibody was considered separately the results were slightly higher in group 2 than that of group 1(table 1). This difference was due to the co occurrence of any two autoantibodies in the same individual which was 8% in Group 2 where there was no co –occurrence in the 20-39 yrs Group(gp1). The co occurrence in the elderly group was 16% (gp3).

In group 1 and 2 (male : female ratio of 48 : 52) the frequency of RF, ACLA & ANA was 9, 22 and 9% in males and 7, 24 and 11% in females which shows that the increased occurrence in the younger age groups is not gender biased.

Among the autoantibodies studied IgG -ACLA was found to occur more frequently in all three groups with a total frequency of 28% (n=39/150) in comparison to 11%

(n=17/150)of both RF and ANA . IgM ACLA was not much raised with an occurrence of only 5% (n=6/150)

Statistical Analysis was done to assess the variations in frequency between the three age groups. ANOVA and Chi square test were done using SPSS -ender 16 Package. Significance was assessed at 5% level of significance. Table 2 shows the non significance between all three groups. Even though results showed a comparatively higher frequency in the elderly group it was not statistically significant. This gives an important clinical implication as it can be attributed to the increased occurrence in the younger Indian adults.

***Table 1**. Number of individuals positive for each autoantibody separately in each age group*

	Group1 (20-39yrs) (n=50)	**Group2 (40-59yrs)** (n=50)	**Group 3 >/=60yrs)** (n=50)
Rheumatoid factor (RF)	3(6%)	5(10%)	9(18%)
Antinuclear antibody (ANA)	4(8%)	6(12%)	7(14%)
Anticardiolipin antibody (ACLA)	11(22%)	12(24%)	16(32%)
Total	18(36%)	18(36%)	25(50%)

Table 2. p values between all three groups showing statistical non significance(indicating clinical significance)

	Group 2 (40-59 yrs)			**Group 3 (60yrs and above)**		
	RF	ACLA	ANA	RF	ACLA	ANA
Group1 20-39yrs	0.35	0.81	0.34	0.07	0.26	0.33
Group 2	—	—	—	0.25	0.38	0.77

***Table 3.** Results of 3 other studies [12,13,14] on RF,ACLA and ANA done on younger and elderly age groups in comparison with the results of this study*

	20 to 60 yrs vs (60yrs and above)			
	Study no 1	**Study no 2**	**Study no 3**	**present study**
ACLA	2.3% (51%)	2% (12%)	1.2% (0%)	23% (32%)
ANA	0% (31%)	1% (09%)	1.6% (08%)	10% (14%)
RF	1.6% (14%)	1.5% (12%)	- (03%)	8% (18%)

Discussion

B lymphocytes which are responsible for humoral immunity have two opposite requirements. One is immune responsiveness (ability to react against virtually any foreign substance or antigen) and the other is immunological tolerance or self tolerance (should not react against self antigens) which prevents autoimmune reaction. Some of the self tolerance mechanisms are central tolerance in bone marrow itself by apoptosis (clonal deletion) and peripheral tolerance mechanisms like clonal ignorance, effect of T suppressor cells on B cells and sequestration of antigens. Failure of these self tolerance mechanisms such as decrease in T cell suppression[9], defective apoptosis[10], release of sequestered antigens , molecular mimicry[11] result in the formation of autoantibodies .

These autoantibodies can be natural autoantibodies or can be pathogenic autoantibodies. A substantial proportion of circulating autoantibodies in healthy individuals exhibit polyreactivity and self-reactivity and these are referred to as natural autoantibodies (NAAs). NAAs play an important role in eliminating pathogens. These natural autoantibodies are subjected to regulatory mechanisms that pre-

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vent them from causing autoimmunity[3] The general network of na-Ab has been named Immunculus. Pathologic changes at the molecular level, (Immunculus distortions) result in autoantibodies which precede or accompany different diseases and many of these autoantibodies like Rheumatoid factor(RF), Anticardiolipin antibody (ACLA) and Antinuclear antibody (ANA), long acting thyroid stimulating antibody, anticytoplasmic antibody have been used in diagnosing autoimmune diseases. Recent evidences show that some of the pathogenic autoantibodies used in diagnosing autoimmune diseases have also been identified in healthy individuals[2]. The presence of such autoantibodies in healthy individuals could alter their diagnostic and predictive value.

A comparative analysis of the results of this study on the occurrence of RF,ACLA and ANA in healthy individuals with reports of other similar studies [12,13,14](table 3) show that the frequency of occurrence of autoantibodies in the elderly group > 60 years in other studies are almost the same as in this study. But , the frequency of occurrence between 20 and 60 years is 8- 23% in this study whereas in other studies it is less than 3 %.

The reporting of increased autoantibodies with ageing has been consistent and one possible explanation for the increase is that naive T cell population decreases with aging, and there is a shift from T helper 1 to T helper 2 cytokine profiles with stimulation, which could augment B cell-mediated autoimmune disorders. As the immune system shifts to a Th2 response, tolerance mechanisms fail and autoreactive antibodies increase [15]

The rationale for the increase in autoantobodies in the younger age groups is not well defined. One study suggests that exposure to infection in early life may produce distinct effects on different autoantibody systems. In particular, diarrhoeal illness may have a particular role in the development of autoantibodies[16]. These could be considered as natural autoantibodies thereby enhancing host immune reaction to foreign agents [17] As diarrhea is commonly seen in India this could be a possible explanation for the increase in young adults. A review in Lancet states that autoantibodies associated with rheumatoid arthritis may predict disease in healthy individuals in whom hormonal changes might be a trigger for autoimmune disease. [18]

Two different retrospective studies on ANA [19] and RF [20] have shown that autoantibodies may be present many years before diagnosis of autoimmune disease , while patients are still asymptomatic. Another study reports that Populations such as doctors and relatives of patients with autoimmune disease tend to presents increased ANA titers. [21]

Thus the increased occurrence of RF,ACLA and ANA in Indian young adults could either be natural autoantibodies developing due to early exposure to environmental factors or it could be an indicator of preclinical stage of autoimmune disease.

Another study has shown that homosexuals may have higher levels of autoantibodies[22]but this has not been considered in the present study.

Conclusion

RF , ACLA & ANA are widely used as diagnostic purpose and an increase in apparently healthy individuals should be assessed carefully to prevent unnecessary treatment but at the same time they may need follow up to avoid missing early diagnosis of an autoimmune disease . Autoantibodies may act as markers of future disease in presently healthy individuals.

Limitations

The trend in the occurrence of autoantibodies in Indian population can be seen by this study but the sample size is small and may not be sufficient to form a conclusive opinion. Long-term large studies of outcome are needed to assess the use of assaying autoantibodies.

Also the proportion of females in the elderly group is slightly higher but this does not affect the outcome of the study.

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