ORIGINAL RESEARCH PAPER

Volume-7 | Issue-12 | December-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSOCIATION OF SIGNIFICANT AUTOANTIBODIES WITH RECURRENT EPISODES OF FOETAL LOSS.

Microbiology

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ABSTRACT

Recurrent Feotal loss (RFL) is a serious and potentially devastating health problem. An interest in autoimmune causes of RFL has greatly increased with the discovery of an association between presence of APA and ANA. Study was conducted to check association of significant autoantibodies in RFL in Nair Hospital, Mumbai comprising of 143 women in study group having history of RFL and 140 women in control group, clinically normal with no history of RFL. Anticardiolipin antibodies (ACA) (IgG and IgM) and total Antinuclear Antibodies (ANA) (IgG and IgM combined) and Lupus Anticoagulant (LA) giving prolonged "Activated Partial Thromboplastin Time" tests were carried out by the kits. Incidence of autoantibody was not found in control group. Highest % of autoantibodies was ACA (31.47%) followed by LA (19.35%) and ANA (15.22%) in the study group. However, none of these associations of APA and ANA in RFL were statistically significant. All these women were treated with low dose aspirin. There was no treatment offered to women who possessed LA and ANA. Out of 45(31.46%) sero-positive women for ACA, only 23 (51.11%) complied with the treatment while 22 (48.88%) did not comply with the treatment. Comparison between these two groups for their foetal outcome was found to be statistically not significant. Many issues about APS still remain poorly understood. To develop guidance on the best treatment options, well-designed randomized trials are required.

KEYWORDS

1. INTRODUCTION

Antiphospholipid antibodies (APA) are a diverse family of autoantibodies that share a common reactivity with negatively charged phospholipids. APA is a group of autoantibodies involving three clinically significant members: Anticardiolipin (ACL), Lupus anticoagulant (LA) and Biological false positive (BFP) antibodies. The two most studied APA are LA and ACL, but other important APA is those directed against phosphatidylserine and phosphatidylethanolamine 1.APA may also prevent the physiological changes that take place during pregnancy 2.There also exist a definite relationship between the gestational week of abortion and prevalence of APA. Also antiphospholipid have a well-established association with recurrent miscarriages .The incidence of APA in women with one or more 1st trimester miscarriage varies between 14% 8 and 42% 3The APA (both LA and ACA) also exist in the general obstetric population that is approximately 2% [2] Antinuclear antibodies (ANA) comprise a group of antibodies with different specificities against antigens of the cell nucleus. Total ANA of the IgG and IgM class include extractable nuclear antigen sub-populations such as anti-Ro (SS-A) anti- La (SS-B), anti-Sm, anti-Sm/RNP, anti-Scl-70, anti-Jo-1, antihistone, anti – dsDNA and anti-centromere. Thus ANA are directed towards nuclear components in human serum.4.Among recurrently spontaneously aborting women 22.7% were found to have ANA .ANA have been reported to occur in about 40% to 70% of patients with APA5.

Habitual or Recurrent Foetal Loss (RFL) is a serious and potentially devastating health problem that affects many couples who are trying to establish a family. RFL is a sequence of three or more consecutive pregnancies ending as a miscarriage before 20 weeks of gestation .6 An interest in autoimmune causes of recurrent abortion has greatly increased with the discovery of an association between the presence of APA and ANA.

Very little work is available on association of autoantibodies with recurrent episodes of foetal loss in Indian women. Detection of autoantibodies and its association may prove to be a useful guiding tool to save the likely foetal loss. Hence, the study was conducted to check the association of significant autoantibodies with recurrent episodes of foetal loss.

2. Material and methods:

2.1 Place of work:

Study was carried out over a period of three years, from February 1996 to March 1999after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai, in Department of Microbiology in association with Department of Obstetrics and Gynaecology. Part of the major study is presented in this paper.

2.2 Participants, Sample Collection and serological analysis:

A special proforma was designed for the present study and accordingly the obstetric history of each woman was recorded. All the women in this study were between the reproductive age group and were pregnant at the time of screening. Those women who history of RFL due to any had known genetic or endocrine defects or haematological disorders were excluded from present study. A total of 283 subjects were studied, comprising of 143 in the study group and 140 in the control group. Study group comprised of all those women who had a history of RFL and Control group comprised of clinically normal women with at least one previous full term normal delivery and no history of RFL Blood was collected from the subjects and serum was separated. Serum samples were stored at -20oC and thawed when the tests were conducted. Anticardiolipin antibodies (ACA) (IgG and IgM) BINDAZYME, Birmingham)7and total Antinuclear Antibodies (ANA) (IgG and IgM combined) (DIASTAT ELISA) 8and Lupus Anticoagulant giving prolonged "Activated Partial Thromboplastin Time" (APTT) (CEPHOTEST) 9tests were carried out by the kits as per the manufactures instructions. Observation and results were noted and appropriate statistical analysis was carried out wherever necessary.

3. Results:

Table 1: Incidence of autoantibodies in study and control group:

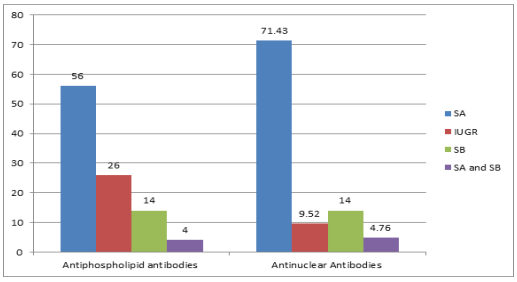
Types of total autoantibody	Study Group	Control group

Anticardiolipin antibodies	45/143(31.47 %)	0/140(0%)
Antinuclear Antibodies	21/138(15.22%)	0/77(0%)
Lupus Anticoagulant	6/31(19.35%)	0/43 (0%)

Incidence of autoantibodies in study group was observed, but it was not found in control group . Highest % of autoantibodies was ACA (31.47%) followed by LA (19.35%) and ANA (15.22%) in the study group.

Graph1: Association of significant autoantibodies with recurrent episodes of foetal loss (%)

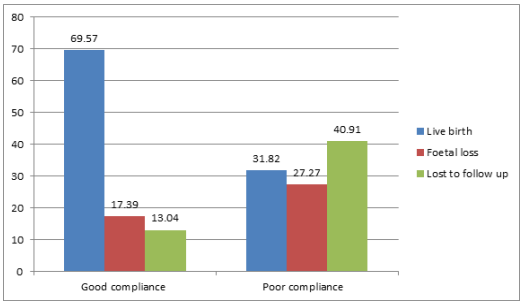
n 1 (APA) =50 ; n 2(ANA) = 21



Autoantibodies like APA and ANA were most commonly associated with spontaneous abortion (SA). But none of these associations were statistically significant. Chisquare X2 (DF=3) = 2.5 ; p=0.47(Not Significant)

Graph 2: Effect of treatment on foetal outcome in women sero-positive for Anticardiolipin antibodies. (%)

n1 (Good compliance) =23 ; n2 (Poor compliance) =22



Graph showed effect of treatment on the outcome of pregnancy in women sero-positive for ACA. All these women were treated with low dose aspirin. There was no treatment offered to women who possessed LA and ANA. And out of 45(31.46%) sero-positive women for ACA, only 23 (51.11%) complied with the treatment while 22 (48.88%) did not comply with the treatment. Comparison between these two groups for their foetal outcome was found to be statistically not significant.

Chisquare X^2 y = 1.5(p=0.23) ; p=0.11 (by Fishers test))

4. DISCUSSION:

The magnitude of the problem caused by APA is largely unknown, especially in developing countries. One reason for this is that the diagnosis of APA is problematic. Laboratory tests for APA are expensive and not easily available. Also, there is a lack of uniformity in the criteria used by different centres for diagnosing the APA syndrome. Since diagnostic criteria include foetal loss, the syndrome can be diagnosed only after foetal loss has occurred. Moreover, since some form of therapy is initiated in most women with APA and pregnancy loss, it is nearly impossible to assess the effects of these antibodies on pregnancy outcome (if APA were left untreated). One study from India reported a 40% prevalence of ACA among women with recurrent pregnancy loss. Authors had found 7% of normal pregnant women had ACA compared with 15% with recurrent pregnancy loss.[10]

All three APA (ACA, LA, BFP) were originally thought to be different manifestations of the same antibody. However, a recent report of the chromatographic separation suggests that they are different from each other and are independently associated with foetal loss. However, it is possible that some antibodies could express all three APA activities 2. The two most widely studied antibodies of APA are ACA and LA. In study group of present study, the incidence of ACA found was 31.47%, LA 19.35% and biologically false positive 29.37%. In present study, the incidence of total ACA in the study group was found to be increased in 42% women with habitual abortion. Autoantibodies like APA and ANA were most commonly associated with spontaneous abortion. But none of these associations were statistically significant in our study. All these women were treated with low dose aspirin. There was no treatment offered to women who possessed LA and ANA. In addition, out of 45(31.46%) sero-positive women for ACA only 23 (51.11%) complied with the treatment while 22 (48.88%) did not comply with the treatment. Comparison between these two groups for their foetal outcome was found to be statistically not significant.

The mechanisms of pregnancy loss in APA are still unknown, and different hypotheses have been proposed as possible causes. Different histological examinations of the placenta have demonstrated thrombosis, infarction, and even inflammatory changes11. Several treatment reports on prevention of pregnancy loss, including corticosteroids, low-dose heparin, heparin, and immunoglobulins, have been used either as single agents or in combination. Also, several studies have also suggested that decreased APA antibody levels were associated with a successful outcome, and few studies have followed the ANAs and APA levels in pregnant women with these diseases12 .

The AntiPhosphoLipid Syndrome (APLS) is recurrent pregnancy loss and thrombosis in the presence of APA. Experimental data shows that passive transfer of APA result in clinical manifestation of APLS, that is, fetal loss and thrombocytopenia.13 APA are a heterogeneous group of autoantibodies that are detected by both immunoassays and functional coagulation tests. The antigenic targets are negatively charged phospholipids and serum phospholipid-binding proteins. Despite the strong association between APA and thrombosis, the pathogenic role of APA in the development of thrombosis has not been fully elucidated. The most frequently utilized serologic markers for APLS are LA, ACA and recently anti-beta-2-glycoprotein 1 antibodies.13 Interestingly, the trophoblast can be targeted by APA, especially by anti-phosphatidylserine antibody(APS). Cardiolipin is not present in the trophoblast plasma membrane; nonetheless, anticardiolipin (ACL) has been implicated in trophoblast pathology. Perhaps the cross reactivity between APS and ACL may have contributed to this pathology.[14]

Previous author shad reported that women with recurrent pregnancy losses demonstrated significantly higher prevalence of ACL, APS and APE (anti-phosphatidylethanolamine antibody) than those of normal fertile controls. 15 It is noteworthy that the current rheumatological definition of APS does not include APS or APE evaluation. 16In addition, current therapy for pregnant women with APA, which is focused on preventing thrombosis by anticoagulation, is only partially successful in averting miscarriage.[17]

5. CONCLUSION:

Many issues about APS remain poorly understood. This includes basic concepts in pathogenesis, diagnostic criteria and optimum modalities of therapy for maximum benefits. To develop guidance on the best treatment options, well-designed randomized trials are required.13As new technologies emerge and a better understanding of how the many components of the immune system interact to aid in the growth of the fetus, new treatments will be available to help women with recurrent spontaneous abortions or multiple implantation failures with or without any history of pregnancy loss. Meanwhile, it is clear that local and systemic immunological differences can be found in these women compared to women with normal pregnancies. [10]

6 Acknowledgement:

Authors are grateful to Nair Golden Jubilee Research Foundation for providing financial help to carry out this study and staff of the Microbiology Department of T. N. Medical College and B. Y. L. Nair Charitable Hospital for their moral support.

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