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Study of autoantibodies in recurrent foetal loss in tertiary care hospital

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Abstract
Recurrent foetal loss (RFL) is a serious and potentially devastating health problem. It appears that autoantibodies serve as major risk factor, leading to foetal loss but not much work is available on role of autoantibodies in Indian women. Hence, study was carried out to ascertain roleof increased autoantibodies levels to RFL in Nair Hospital, Mumbai. Study comprised of 143 women in study group having history of RFL and 140 women in control group. Anticardiolipin antibodies (ACA) and Antinuclear Antibodies (ANA) and Lupus Anticoagulant (LA) giving prolonged "Activated Partial Thromboplastin Time" tests were carried out by the kits. Incidence of autoantibodies was found only in study group, as compared to control group. The incidence of ACA (31.47%), ANA (15.22%) and LA (19.33%) in study group was found to be highly significant. (p<0.001). The data shows a high percentage of ACA IgG (30.07%) as compared to ACA IgM (22.38%), Out of 143 women investigated for ACA, 45 (31.46%) women had ACA IgG and /or IgM antibodies, while 20.98% women showed ACA IgG and IgM both. The incidence of total APA (34.96%) was higher as compared to ANA (15.22%), while the occurrence of both APA and ANA was relatively low (8.69%).A statistically significant incidence of elevated autoantibody level was found only in the women with a history of RFL as compared to clinically normal women. Thus, elevated levels of autoantibodies played a significant role in failure of pregnancy.

Keywords: Autoantibodies, antinuclear antibodies, recurrent foetal loss

1. Introduction
Recurrent foetal loss (RFL) is a serious and potentially devastating health problem that affects 3% of all couples. It is defined as two or more abortion before 20 week sof pregnancy. [1] Current diagnostic procedures identify etiological factors, such as translocations, immunologic factors, endocrine disorders and uterine abnormalities in 50% of these couples. The other 50% are diagnosed as couples with unexplained RFL [2].
Abnormal immune status of a mother has long been thought to result in an increased risk of pregnancy loss. These thinking have been confirmed by the various studies done indicating significant higher abortion rate in-patients with autoantibodies [3]. An interest in autoimmune causes of recurrent abortion has greatly increased with the discovery of an association between the presence of Anti Phospholipid Antibodies (APA) and Anti-Nuclear Antibodies (ANA). APA comprise a group of autoantibodies like Anti Cardiolipin antibodies (ACA), Lupus Anticoagulant (LA) [4], while Anti-Nuclear antibodies comprise a group of antibodies with different specificities against antigens of the cell nucleus [5].
The commonest clinical scenario in women with APA is recurrent miscarriages. Similarly even ANA plays a role in pregnancy wastage. In recurrent spontaneous abortion, the incidence of APA has been reported to be 41.26% and ANA 22.7% [6]. Not only in foetal losses, have these APA also been described in association with a variety of medical disorders characterized by thrombocytopenia and or thrombosis and neurologic sequelae [4].
APA appears to be directed at negatively charged phospholipid in plasma membranes and therefore they increase the risk of vascular thromboses. It has also been postulated that they may exert direct pathogenic effects *in vivo* by interfering with homeostatic processes that take place on the phospholipid membranes such as platelets or endothelium. APA may also prevent the physiological changes that take place during pregnancy [4].

There also exist a definite relationship between the gestational week of abortion and prevalence of APA. The incidence of APA in women with one or more 1st trimester miscarriage varies between 14% and 42% [7]. Toyoshine and colleagues in 1991 also reported an incidence of 42.8% of APA in late foetal losses. The APA (both LA and ACA) also exist in the general obstetric population that is approximately 2% [4].

It appears that autoantibodies serve as major risk factor, leading to foetal loss but not much work is available on role of autoantibodies in Indian women. Hence, the study was carried out to ascertain the role of increased autoantibodies levels to RFL.

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 had history of RFL due to any 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

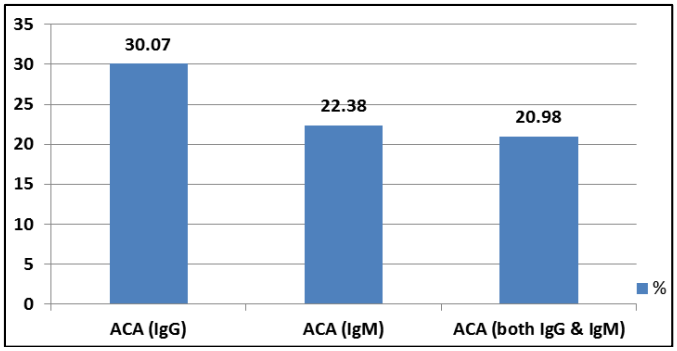
Blood was collected from the subjects and serum was separated. Serum samples were stored at -20^{o}c and thawed when the tests were conducted. Anticardiolipin antibodies (ACA) (IgG and IgM) BINDAZYME, Birmingham) [8] and total AntiNuclear Antibodies (ANA) (IgG and IgM combined) (DIASTAT ELISA) [9] and Lupus Anticoagulant giving prolonged “Activated Partial Thromboplastin Time” (APTT) (CEPHOTEST) [10] tests were carried outby the kits as per the manufacturer’s 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

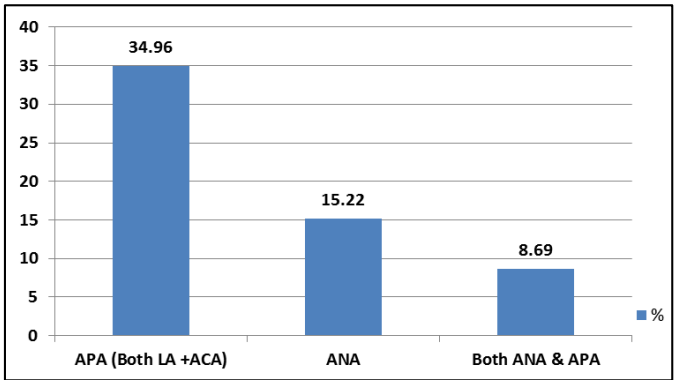
Types of total autoantibody	Study Group	Control group
Anticardiolipin antibodies	45/143(31.47%)	0/140(0%)
Antinuclear Antibodies	21/138(15.22%)	0/77 (0%)
Lupus Anticoagulant	6/31(19.35%)	0/43 (0%)

The total number of women investigated for autoantibodies was 143 and 140 in study and control group respectively. Incidence of autoantibodies was found only in study group, as compared to control group. The incidence of ACA (31.47%), ANA (15.22%) and LA (19.33%) in study group was found to be highly significant after applying Fisher Exact probability test (FP) (one tailed) for the above analysis.(p<0.001)



Graph 1: Serological profile of Anti Cardiolipin Antibodies in study group. n=143

The data shows a high percentage of ACA IgG (30.07%) as compared to ACA IgM (22.38%), Out of 143 women investigated for Anti Cardiolipin Antibodies, 45 (31.46%) women had ACA IgG and /or IgM antibodies, while 20.98% women showed ACA IgG and IgM both.



Graph 2: Comparison of APA with ANA in study group

The incidence of total APA (34.96%) was higher as compared to ANA (15.22%), while the occurrence of both APA and ANA was relatively low (8.69%) in the study group.

4. Discussion

Basic maternal health care has been grossly neglected in a developing country like India [11]. Women seek medical help only after undergoing series of foetal loss. Hence gross negligence, ignorance and hesitant approach to the clinician accounts for scanty data on recurrent foetal wastage in India. The part of the present study estimated the incidence of immunological factors (such as APA and ANA) in women with a history of recurrent foetal loss.

Previous works indicated that autoantibodies may explain a considerable percentage of “sine causa” miscarriages [12]. These autoantibodies directed to membrane phospholipids (APA) and nuclear antigens (ANA) were found in 34% of women with more than two recurrent foetal losses [6]. This incidence was found to reach 50% by the end of fifth pregnancy loss. Hence there occurred a 15% increase in the incidence with each successive pregnancy that was lost. This suggested that the failing foeto-placental unit may release phospholipid antigens that were auto antigens to the woman [6]. Autoantibodies were found to occur in 18-43% of patients with recurrent pregnancy loss. The antibodies

commonly identified include APA (14%) and ANA (7%) [13]. In present study, the incidence of autoantibodies (APA and ANA) found in more than three recurrently aborting women was 41.25%.

Earlier literature suggested that women with Anti Phospholipid Antibodies have a significant risk of reproductive failure and adverse pregnancy outcomes [7]. Cow hock *et al.* and Unander al had reported a prevalence of 13% to 40% in women with history of recurrent foetal loss [14]. Kwak *et al.* had estimated an incidence of 41.26% of APA at the time of third pregnancy loss [15]. In present study, a statistically significant incidence of 34.96% of APA was found in study group. Thus APA was commonly found etiology in women suffering from RFL. In present study, we found an incidence of 15.22% of ANA in study group. ANA were not detected in our healthy control group. While Kwak *et al.* had reported an incidence of 22.7% of ANA among recurrently aborting women. ANA had been reported to occur in about 40% to 70% of patients with APA [15].

The two most widely studied antibodies of APA are ACA and LA. In present study, the incidence of total ACA in the study group was found increased in 42% women with habitual abortion [7]. Birds *et al.* had reported an incidence of 41% [90]. While Reece *et al.* had reported 14% ACA [16] and Barbui *et al.* had [17] reported 8% ACA. As previously reported this fluctuation from one study to the other may be due to the difference in the methods used to detect autoantibodies in different laboratories. Another possibility might be that present selection criteria could have given us a higher percentage of 31.46% of ACA in study group.

LA found in study group of present study was 19.35%. Barbui *et al* had reported 14% LA. Reece *et al.* had reported 8% LA. Anitha *et al.* reported an incidence of 20.6% of LA in Indian women suffering from adverse foetal outcome [18]. Our findings of LA are similar to Anitha *et al.* study.

In healthy pregnant women, the prevalence of ACA and LA was very low. Lockwood *et al.* had reported an incidence of LA 0.27% and ACA 2.2% [14]. Brown *et al.* had documented ACA IgG to be 1.25% [7]. While Kwak *et al.* had reported absence of ACA or LA in healthy multiparous women. Our findings were similar to Kwak *et al*. as we found no incidence of increased APA level in the control group. As suggested previously [19], APA may be rarely found in the general obstetric population.

The role that maternal autoimmune response to placental antigens play in success or failure of human pregnancy is widely debated. APA levels in women with recurrent spontaneous abortions of unknown cause may predict the success or failure of the pregnancy. Rai and Regan *et al* had reported a range of 50-75% foetal loss rate in women with APA [1]. While Pattison *et al* had given a predictive range of 48% to 76% for a subsequent pregnancy loss or thrombotic episode in women with APA [4]. Haywood brown *et al* reported that the pregnancy loss rate among untreated women with elevated ACA was significantly increased. The frequency of recurrent abortion and foetal death in the presence of LA was greater than 90% in untreated women [7].

5. Conclusion

A statistically significant incidence of elevated autoantibody level, ACA (p<0.000), antinuclear (p<0.000) and Lupus anticoagulant) (p<0.001) was found only in the women with a history of RFL as compared to clinically normal women. Thus, elevated levels of autoantibodies play a significant role in failure of pregnancy.

6. Acknowledgement

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