Bacteremia And Immunoglobulin Classes In Nigerian Women With Recurrent Abortion

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Abstract

Apart from antiphospholipid- and thyroid- autoantibodies which were reported as underlying causes of recurrent pregnancy loss, specific IgG to patermal MHC and Rh was proposed. This raises the possibility of other classes of immunoglobulin in recurrent abortion.

Twenty-four pregnant women with recurrent abortion (P+R), thirty-six pregnant women without recurrent abortion (P-R) and twenty-four non-pregnant women with no history of abortion as controls were recruited from Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria.

Immunoglobulin classes (IgA, IgG and IgM) and prevalence of bacteria type in the blood measured by single radial immunodifussion and standard microbiology method respectively. The mean levels of IgA, IgG and IgM were raised in both P-R and P+R subjects compared with the controls. Four genera (*S. aureus, Strept. agalactice, Kleibsiella species* and C. *perfringes*) of bacteria were detected in P+R subjects while 2 genera (*Kleibsiella species* and C. *perfringes*) of bacteria were found in P-R group.

Bacteremia and raised immunoglobulin levels are occurrences in subjects with recurrent abortion.

Keywords: Immunoglobulins, Bacteria, pregnancy, recurrent abortion.

Introduction

Recurrent abortion is defined as the loss of three or more consecutive pregnancies and it is a significant health problem affecting 2-5% of reproducing couples worldwide¹.

Genetic, anatomic, hormonal and environmental factors² have been implicated in the etiology of recurrent abortion, but a sizeable proportion remains unexplained³.

Antiphospholipid- and thyroid- autoantibodies were reported to be underlying process that causes recurrent pregnancy loss². Blocking factors found in the mother's blood are generally associated with IgG anti-paternal MHC and this is suggested to prevent maternal cells from attacking the foetus.

Correspondence to: Dr. O.G. Arinola Department of Chemical Pathology University College Hospital Ibadan Ibadan, Nigeria. By binding to receptors that cause the activation of NK cells, IgG is seen to block the NK cell cytotoxic effects⁴. Thus, loss of these blocking factors may lead to recurrent abortion.

Immunological mechanisms in pregnancy loss were also reported to occur when Rh-negative mothers produce IgG antibodies specific for the Rh or Rh-negative blood group antigen expressed on the red blood cells of her foetus⁵. It is known that the maternal IgG passes into the foetus and protect newborn when presented with a potential infection. However, IgG anti-Rh antibodies attack the foetal red blood cells that cause haemolytic anaemia in the foetus and newborn. ABO blood group incompatibility was shown sometimes to be pathogenic to pregnancy, however it is not clear as to whether it is a competent cause of recurrent abortion⁵. A large number of bacterial and viral agents have been impli-

A large number of bacterial and viral agents have been implicated as possible causes of abortion. Syphilis is a maternal infection, which can spread across placenta to cause intrauterine death and abortion⁶. Of the 5 known immunoglobulin classes, only IgG specific to Rh and paternal MHC has been implicated in recurrent abortion. Thus raising possible involvement of other classes in recurrent abortion. In this study, the levels of IgG, IgA and IgM were determined Nigeria women with recurrent abortion. Moreso, prevalence of bacteria in the blood of the subjects was also assessed.

Materials and Methods

All subjects were recruited from Obstetrics and Gynaecology Clinic of the University College Hospital, Ibadan. Informed consent was obtained from the participants before sample collection and the motive behind the study was explained. They were divided into three groups viz: twenty-four pregnant women with recurrent abortion (P+R), thirty-six pregnant women without recurrent abortion (P-R) and twenty-four non-pregnant women with no history of abortion as controls.

Excluded from the study were those with history of diabetes, cervical incompetence, history of uterine fibroids and abnormalities in the shape of the uterus. Determination of diabetes status was carried out by measuring the plasma glucose of the patients, which was compared with the standard⁷ while the Consultant Gynaecologists carried out other exclusion criteria.

Sample collection

Ten milliliters of venous blood was collected into non-heparinized bottle and spun at 1500 x g for five minutes. This was allowed to retract and the serum separated for the measurement of serum immunoglobulins and acute phase proteins. The serum was stored at -20° C till needed for analysis.

Quantitation of immunoglobulin classes

Ten (10ml) of blood was collected from each subject by venepuncture and allowed to clot. After the clot had retracted at room temperature (20°C) the serum was separated by centrifugation. Immunoglobulin classes (IgG, IgA and IgM) were estimated by single radial immunodiffusion technique of Fahey and Mckelvey⁸. A volume of an optimally diluted nonspecific anti-serum was mixed with noble agar and poured on glass plate. Wells of equal diameter were cut in the antibody agar mixture. The wells were filled with test or standard serum. The plates for IgG measurements were incubated at 37°C for 3 hours. Those of IgA and IgM were placed at room temperature (20°C) for 18 hours. After incubation the diameters of the precipitin rings were measured with micrometer eyepiece.

Determination of bacteremia:

The method described in a standard test was adopted⁹. Five (5) ml of blood was collected into 10-15 ml of broth. The content was thoroughly mixed and incubated at 37°C for 7 days. Each day the content was examined for turbidity. The turbid culture was inoculated into blood agar plate, chocolate agar and MacConkey plate and incubated at 37°C overnight. The characteristic appearance of the organism grown on each plate was noted and necessary biochemical tests were preformed⁹.

Data Analysis

Data were presented as mean and standard deviation. Student t-test was used to test the significance of differences between mean values. The probability value (p) greater than 0.05 was considered insignificant.

Results

Table 1 shows the mean concentrations of IgG, IgA and IgM of the subjects. There were no statistical significant differences between the ages of all subjects. The result shows that IgM was higher in P-R or P+R subjects when compared with the controls but P+R subjects had highest mean values of IgM. In contrast, the mean level of IgA was lower in P-R or P+R when compared with controls. Significant difference in the mean values of IgM (p.>0.05), but shows a significant difference in the values of IgG and IgA

Subjects	n	Ages (Years)	IgA(mmg/ dl)	IgG (mg/dl)	IgM (mg/dl)
С	24	21.30±4.0	348.8±66.8	678.7±30.9	62.3±26.7
P-R	36	22.5±3.2	59.7±29.2	1311±97.7	72.8±43
P+R	24	21.0±5.3	255.6±58.9	1450±29.3	92.3±39.1
T ₁ P- values ^a			19.9, < 0.01	26.6, <0.01	0.82, > 0.2
T ₁ -P- values ^b			6.20, < 0.01	49.8, <0.01	1.91, >0.05
T ₁ -P- values ^c			5.18, <0.01	25.4,<0.01	1.03, > 0.2

Keys

P-R= Pregnant subjects without recurrent abortion

P+R= Pregnant subjects with recurrent abortion

(a) = Controls compared with P-R

(b) = P-R compared with P+R

(c) = Controls compared with P+R

Table 2 : Percentage distribution of different isolates of bacteria
in the blood of women with recurrent abortion (P+R) and without
recurrent abortion (P-R)

Organisms isolated	P+R	P-R
S aureus	8(33.0)	3(8 3)
Strept. Agalactice	3(12.5)	1(2.8)
Kleibsiella species	1(4.2)	-
C. perfringes	1(4.2)	-
Percentages are in pa	rentheses	•

(p<0.05) between the subjects and the control group. Table 2 shows that there were four genera of bacteria (*S. aureus, Strept. agalactice, Kleibsiella species and* C. *per-fringes*) in women with recurrent abortion while there were 2 genera of bacteria (*S. aureus and Strept. agalactice*) in pregnant women without recurrent abortion. The prevalence of these bacteria was higher in P+R women compared with P-R women.

Discussion

The level of immunoglobulin is a balance between the rate of antibody synthesis and catabolism that depends on antigenic challenge¹⁰. The immunoglobulins protect both

Table 1: Immunoglobulin levls (Means± s.d) in pregnant women
with or without recurrent abortion compared with the controls

the foetus and the mother against bacterial, viral or parasitic infections, in spite of genetic differences. Maternal and foetal cells are close in contact over the whole course of pregnancy with no evidence of either humoral and/or cellular immunologic response of mother against foetus. The likely involvement of immunoglobulin classes in foetal rejection experienced by certain mothers is proposed by this study, since previous studies implicated autoantibodies, "blocking factors" and anti-Rh IgG^{2, 4,5} in recurrent abortion.

IgG constitutes the predominant antibody in the serum, about 80 percent and the principal antibody in the secondary antibody response¹¹. IgG is the only antibody that crosses the placenta from the mother to the foetus, which is due to its Fc fragment and is protective to the mother and offspring in the first few months of life. In pregnancy, several studies have reported a low serum IgG level^{12, 13}. This observation was related to haemodilution¹⁴. However, a significantly high serum IgG was observed in this study may be caused by trans-placental transfer of IgG to developing foetus that induces more synthesis. Also, proness of pregnant women to infections especially malaria which is endemic in the tropics¹⁵ may account for the production of the excessive IgG.

Immunizations against some communicable diseases that are routinely given to expectant mothers could also stimulate more IgG production, being the predominant antibody synthesized in the secondary immune response. The significantly high serum IgG observed in pregnant subjects with recurrent abortion may be due to its protective role both to the offspring and the mother. Although, it had been established by Kalra et al¹⁶ in their investigations that there was an increase in the IgG serum levels in women with recurrent abortions that had been attributed to first trimester recurrent abortions.

Immunoglobulin A is the predominant antibody class in sero-mucus secretions such as saliva, tears, colostrums, respiratory, gastro and genitourinary secretions. It could be said that this antibody has little or no effect in pregnancy as it is found mainly in mucous secretions. Serum IgA level was lower in pregnant women without recurrent abortion compared to controls. Serum values of IgA in pregnancy are conflicting. Some investigators have reported a non-significant change in IgA during pregnancy^{12,} ¹⁷ while others reported low values^{13, 18}. The role of haemodilution during pregnancy cannot be ruled out in explaining the cause of its low level. Similarly, serum IgA levels were lower in pregnant women with recurrent abortion as compared to the control group. The finding in this present study was in accordance with some researchers¹³ who reported reduction in serum IgA levels in women with recurrent pregnancy loss.

Immunoglobulin M constitutes about 10 percent of the total serum immunoglobulins and is the first antibody to be produced in immune response and activates the complement system¹⁹. Lockwood et al²⁰ established that recurrent abortions, stillbirths or unexplained fetal growth were associated with IgM. This was also in agreement with Cowchock et al²¹ that patients with unexplained recurrent abortions had the prevalence of IgM. Thus, high production of this antibody in pregnancy is destructive which may lead to fetal loss that may be due to defective implantation and subsequently causing defective placentation²².

Report on serum IgM in pregnancy are at variance. Some studies have reported low levels of IgM in pregnant women^{12, 13}. The elevated level of IgM as found in pregnant subjects without recurrent abortion in the present study has been similarly observed by other workers¹⁸. However the physiological changes in the genitourinary system of pregnant women increased their susceptibility to urinary tract infection^{23, 24}, which the body responds to by producing IgM along with IgA isotypes. The mean serum level of IgM in pregnant subjects with recurrent abortion was found to be higher than in controls but lower than the pregnant women without recurrent pregnancy loss. The observation in this study was in concordance with some investigators.

Lockwood et al²⁰ and Cowchock et al²¹ had earlier established in their investigations that there was an increase in the serum IgM levels in subjects with recurrent abortion. The reason for the differences in the level of serum IgM in subjects with recurrent abortion and pregnant subjects without recurrent abortion remain unpredicted, however, Lockwood et al²⁵ reported that increase in serum IgM of women with recurrent abortion could be due to the nonspecific binding of IgM which suggests polyclonal IgM elevations. Unander et al²⁶ also established that increase in the IgM serum levels in patients with recurrent abortion might be due to infections.

It was shown that women with septic abortion might develop bacteraemic shock and renal failure²⁷. *S. aureus* produces enterotoxins (toxic shock syndrome toxin). *C. perfringes* also produce enterotoxins, aggressin and hyaluronidase (which breaks down intercellular cement substances and promotes the spread of infection along tissue spaces), collagenase and other protenases breaks down tissues and liquefy muscles²⁸. It was reported that staphylococcal enterotoxins initiate cascade of events involving tumour necrosis factor and interleukin 2²⁹. These are Th 1 cytokines that favours only early pregnancy whose actions

are detrimental to late pregnancy and pregnant outcome³⁰. These previous finding suggests possible involvement of bacteria in recurrent abortion as shown by the present study where *S. aureus, S. agalactice, Klebsiella* and *C. perfringes* were isolated from the blood of some of women with recurrent abortion.

The observation of raised levels of three immunoglobulin classes (IgG, IgA and IgM) and isolation of four genera of bacteria in some of women with recurrent abortion supports involvement of abnormal humoral immune responses and bacterial infection in recurrent abortion.

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