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BACTERAEMIA AND ACUTE PHASE PROTEINS IN NIGERIAN WOMEN WITH SPONTANEOUS RECURRENT ABORTION

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C-reactive protein, alpha – 2 macroglobulin, transferrin and bacteraemia were studied in women with recurrent abortion and compared with the pregnant women as well as non-pregnant women with no history of abortion (controls). The results showed a significantly reduced level of transferrin but significantly raised levels of alpha-2-macroglobulin and C-reactive protein in the pregnant women with recurrent abortion (P+R) compared with pregnant women without recurrent abortion (P-R) or the controls. Four genera of bacteria (*Staphylococcus aureus, Streptococcus agalactiae, Klebsiella species* and *Clostridium perfringes*) were isolated from the blood of women with recurrent abortion while there were 2 genera of bacteria (*S. aureus* and *Strept. agalactiae*) isolated from the blood of pregnant women without recurrent abortion. This study had shown that inflammation and bacterial infection contribute to spontaneous recurrent abortion.

INTRODUCTION

Recurrent abortion is defined as a history of three or more consecutive spontaneous abortions occurring prior to the 22nd week of gestation (1). Some of the previous data suggested that recurrent abortion might be caused by failure to activate natural suppressor cells (CD8+), presence of certain auto antibodies (2), blocking factor in the serum (2) and infections (3).

In humans, there is evidence that CD8+ cells recognize the trophoblast which when activated with IL-2 elicit lyses of the trophoblastic cells. The pregnancy associated protein TJ6 when expressed on CD56+CD16- NK cells and ß cells is seen to elicit recurrent abortion and thus this expression can be used as a diagnostic tool in predicting successful pregnancy (4). In women experiencing recurrent abortion, a circulating embrotoxin has been reported, whose action involves induction of MHC I and II antigens on its targeted tissues as well as activating the macrophage to produce TNF-alpha. This in effect would cause the recognition of the induced MHC expression and thus rejection of the foetal allograft by the maternal immune system would result.

In some cases of recurrent pregnancy loss, the mother carries antiphospholipid autoantibodies against negatively charged membrane phospholipids of the placenta (5). Other immunological factors that had been found to cause recurrent spontaneous abortion are: presence of anti TLX antibodies, HLA and rhesus incompatibilities (5).

The acute phase proteins were shown to provide information on the presence of inflammatory lesions, on the prognosis of the condition and on the response to treatment (6). Studies on the levels of acute phase proteins in normal pregnancy are scanty and none had assessed the levels of acute phase proteins in Nigerian women with spontaneous recurrent abortion. A study showed that C-reactive protein (CRP) level was found not to be useful as an early predictor of clinical chorio-amnionitis and a significant difference between

maternal and neonatal CRP was demonstrated, implying a lack of transplacental transfer during labour (7). Also, serum transferrin level was low in 2nd and 3rd trimesters of normal pregnancy (8) while significantly raised C3, reduced C4 accompanied by a significant increased C4d was reported in normal pregnancy (9). This previous study concluded that pregnancy induces activation of the classical complement and that C4d concentration cannot be used to monitor disease activity in patients with connective tissue diseases during pregnancy.

Infections with bacteria had been linked with recurrent abortion and the spectrum of bacteria involved was concluded to vary (10). Bacterial infection is a potent inducer of inflammation (10) but little attention has been giving to immunological indicators of inflammation in Nigerian women with spontaneous recurrent abortion. This study will elucidate the involvement of inflammatory immunological factors and the species of bacterial in women with spontaneous recurrent abortion attending Obstetrics and Gynaecology Clinic, University College Hospital, Ibadan, Nigeria.

SUBJECTS AND METHOD

Subjects

Informed consent was obtained from eighty-four women of childbearing age before sample collection. 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. All subjects were recruited from Obstetrics and Gynaecology Clinic of the University College Hospital, Ibadan. 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 acute phase proteins was stored at –20°C till needed for analysis.

Quantitation of acute phase reactants

Acute phase proteins were quantitated by the single radial immunodifussion method as modified by Salimonu *et al* (11). 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 placed in humid box were incubated at 4°C for 18 hours. After incubation the diameters of the precipitin rings were measured with micrometer eyepiece.

Determination of bacteraemia

The method described in a standard test (12) was adopted. 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 preformed.

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

The mean age of the pregnant women without recurrent abortion (P-R) was 22.5 + 3.2yrs, the mean age for the pregnant women with recurrent abortion (P+R) was 21.0 + 5.3yrs while the mean age for the negative controls is

21.3 + 4.0yrs. There were no statistical significant differences between the ages of all subjects.

The result shows significant decrease in the mean level of transferrin in P-R or P+R compared with the controls. The Table also shows that the mean level of alpha-2-macroglobulin was significantly raised in P+R compared with the controls but was significantly reduced in P-R compared with the controls. The level of CRP was significantly higher in P+R subjects compared with the P-R or the controls.

Table 2 shows that there were four genera of bacteria (*S. aureus, Strept. agalactiae, Klebsiella spp.* and C. *perfringes*) in women with recurrent abortion while there were 2 genera of bacteria (*S. aureus* and *Strept. agalactiae)* in pregnant women without recurrent abortion. The prevalence of these bacteria was higher in P+R women compared with P-R women.

Table 1: Levels of acute phase proteins (mean ± 1sd) in pregnant women with or without recurrent abortion compared with controls

Subjects	n	Transferrin (mg/dL)	Alpha-2-Macroglobulin (mg/dL)	CRP (mg/dL)
C	24	110.3±9.0	705.5±34.9	4.3±0.9
P-R	36	100.7±25.7	609.1±27.2	7.4±0.3
P+R	24	66.8±17.1	1301.8±16.6	13.8±2.5
t, p-values[a]		2.04, <0.05	1.01,>0.2	2.65, <0.05
t, p-values[b]		6.10, <0.01	5.49,<0.01	9.00, <0.01
t, p-values[c]		22.8, <0.01	51.1,<0.01	22.9, <0.01

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)	3 (8.3)
Strept. agalactiae	3 (12.5)	1(2.8)
Klebsiella species	1(4.2)	-
C. perfringes	1(4.2)	-

Percentages are in parentheses.

DISCUSSION

CRP is a major acute phase protein, with its concentration increasing over 200 times from a low, virtually, negligible, normal level. Other recognized acute phase proteins are serum amyloid A (SAA), haptoglobin (HP), alpha 1-acid glycoprotein (AGP), fibrinogen (Fb), alpha 1-antiprotease (AP), caeruloplasmin (CP), alpha 2-macroglobulin and transferrin (13, 14). HP, AGP, Fb and AP are moderate acute phase proteins as serum concentration increases only 2-3 times during the response. Cp is a minor acute phase protein as it only increases by 60-70% in response to inflammatory lesions.

Investigators have related the presence of infection or inflammatory lesions with the acute phase protein response (13, 14, 15) and assess the diagnostic value of such biomarkers of disease but studies on the serum levels of acute phase proteins during normal pregnancy is scanty. Reduced level of alpha-2 macroglobulin was found in P-R subjects. Previous reports have associated high values of alpha- 2 macroglobulin with low birth weight (15, 16). Thus, the significantly low serum level of alpha-2 macroglobulin recorded in pregnant subjects without recurrent abortion may be of immense benefit to the outcome of the pregnancy.

Alpha-2 macroglobulin is a large plasma glycoprotein that binds many proteinases. Proteolytic enzymes released from damaged tissues as well as from phagocytic cells have their activity inhibited by being bound to alpha-2 macroglobulin (17). In addition, alpha–2 macroglobulin is also known to bind growth factors such as IL-8 (18), nerve growth factor, platelet derived growth factor-B and transforming growth factor-B (19) and transport them to their target cells where such cytokines affect cell growth and functions (20). Raised level of alpha-2 macroglobulin was observed in P+R. The possible increase in hepatic synthesis of alpha–2 macroglobulin to meet the requirement caused by tissue damage and as transport protein may account for the significantly high level of alpha–2 macroglobulin found in pregnant subjects with recurrent abortion in this study. This high level has been similarly documented in animal model (21).

Transferrin is the principal plasma protein for transport of iron. In states of iron deficiency, plasma transferrin level rises and returns to normal level upon successful treatment with iron (22.). Transferrin is a negative acute phase protein, and as such, its level is expected to reduce during inflammation, chronic liver disease, malnutrition or protein losing enteropathies (22). In view of the iron deficiency that is expected in pregnancy (23), antenatal routine iron supplement given to pregnant women affects transferrin level (22). High transferrin level observed in pregnant women without recurrent abortion could indicate a normal functioning of hepatic parenchymal cells that produces more transferrin to bind the circulation excess iron. In the present study, serum transferrin was significantly low in P+R compared to controls. During abortion, blood loose and RBC haemolysis is common (24). Overproduction of iron from periodic RBC lysis might have caused rapid consumption of transferin despite its adequate production.

CRP is a useful indicator of bacterial infection, capable of complement activation via classical pathways, platelet aggregation, inhibition of rosette formation by human T lymphocytes and inhibition of phyto-haemagglutinin induced by mitogenesis in vitro (7). The elevated levels of CRP observed in a number of our P+R subjects can only be attributed to bacterial infection that was shown to be more prevalent in P+R subjects. The elevated levels of CRP obtained in this study might also be related to state of inflammation caused by tissue damage that occurs in P+R subjects.

This study had shown that inflammation and bacterial infection contribute to spontaneous recurrent abortion in this environment.

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