Serum concentrations of immunoglobulins and acute phase proteins in Nigerian women with preeclampsia

Ganiyu Arinola¹², Ayo Arowojolu³, Ayodele Bamgboye⁴, Adijat Akinwale², Adebayo Adeniyi²

¹Department of Chemical Pathology, College of Medicine, University of Ibadan; ²Department of Obstetrics and Gynaecology, University College Hospital, Ibadan; ³IAMRAT, College of Medicine, University of Ibadan, Nigeria

Received: 15 February 2006; accepted: 5 November 2006

SUMMARY

Preeclampsia is a pregnancy-specific condition that increases maternal and infant mortality and morbidity. It is diagnosed based on a triad of hypertension, significant proteinuria and rapidly increasing edema during gestation. The factors that initiate preeclampsia are unknown and still a subject of intense clinical research. The objective of this study is to provide additional immunological information about preeclampsia. To achieve this, humoral immunoochemical parameters such as three classes of immunoglobulin (IgA, IgG and IgM) and three acute phase proteins (alpha 2-macroglobulin, haptoglobin and transferrin) were measured by single radial immunodiffusion method in 32 pregnant women with preeclampsia, 36 pregnant women without preeclampsia and 24 non-pregnant women (controls). Total protein in the urine was also determined by spectrophotometric method. In women with preeclampsia, the levels of IgG, IgA, transferrin and alpha 2-macroglobulin were significantly reduced.

¹Corresponding author: Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria; E-mail: arinolaog@doctor.com

Copyright © 2006 by the Society for Biology of Reproduction
compared with subjects with normal pregnancy, but the level of haptoglobin was significantly raised in preeclampsia compared with women having normal pregnancy. Urinary total protein and IgG were significantly raised in Nigerian women with preeclampsia compared with non-pregnant controls. There were significant negative correlations between serum IgG, IgA and urinary protein. The possible involvement of immunoglobulins and acute phase proteins in preeclampsia is discussed. Reproductive Biology 2006 6 (3):265–274.

Key words: preeclampsia, immunoglobulin, acute phase proteins, pregnancy, Nigerian

INTRODUCTION

In normal pregnancy, the maternal and fetal immune systems co-exist to achieve a symbiotic relationship. This natural state of tolerance is critical for carrying pregnancy to full term [22]. Several mechanisms invoke maternal immune tolerance to the fetus expression of unique HLA surface molecule (HLA G) on extravillous cytotrophoblast and also in endothelial cells of fetal vessels in the chorionic villous including non-specific reduction of systemic immuno-reactivity and expression of complement regulator proteins in the circulation of pregnant women [23].

Preeclampsia describes a common syndrome that occurs in the second half of pregnancy and often manifests with hypertension and proteinuria [21]. Occuring in up to 10% of all pregnancies, it is one of the most serious complications of pregnancy [21]. It is the second leading cause of maternal mortality worldwide, constituting 12-18% of pregnancy-related maternal death [19]. The epidemiology of severe preeclampsia being more common in poor women mostly in developing countries [19] suggests that nutrients might be involved in the disorder.

Apart from this, many studies indicate that the syndrome is poorly defined and its pathogenesis is uncertain [1, 2]. Modern understanding of the etiopathogenesis of preeclampsia started with the exploration of immunological processes in normal pregnancy and their possible alteration in preeclampsia [8]. Petrucco [17] reviewed the evidences for the
immunological basis for the development of preeclampsia, and concluded that feto-placental antigens induced antigen-antibody complex deposition in target tissues (e.g. renal glomeruli). The immunological theory is supported by the fact that preeclampsia tends to occur more commonly in the first pregnancy or the primary pregnancy of a woman with a new spouse [5, 7, 14]. Preeclampsia is also more frequent in pregnancies arising from donor insemination [10]. These reports suggest that preeclampsia is a disease of a new father. The trophoblast antigens deposited in maternal circulation elicit maternal immune response, which increases preeclamptic pregnancies [4].

Other studies [11, 24] have reported elevated levels of granulysin (a useful marker of cell-mediated immunity), leptin, TNF-alpha, C-reactive protein and IFNγ in women with preeclampsia. The role of plasma endothelin and vascular endothelial growth factor are also evidenced in preeclampsia [13]. Preeclampsia in Nigerian women appears late in pregnancy and such a type of preeclampsia needs immediate medical attention [19]. An immunological study in preeclamptic Nigerians [22] reported raised levels of circulating immune complexes, C3c, CRP and C3 inactivator, but a depressed level of C3b inactivator. Immunoglobulins and acute phase proteins (other than C3) were not examined. It is possible that immunoglobulins with blocking effects (IgG and IgA) and some acute phase reactants may be involved in preeclampsia. To provide evidence for the involvement of immunological factors in preeclampsia we assessed the levels of IgG, IgA and IgM as well as acute phase proteins (transferrin, haptoglobin and alpha 2-macroglobulin) in Nigerian women with preeclampsia.

SUBJECTS AND METHODS

Subjects

The protocol for this study was approved by Ibadan Ethical Review Committee in University of Ibadan/University College Hospital. Ninety-two women between 15-30 years of age were recruited for the study. Informed consent was obtained from them before sample collection. They
were divided into three groups: 32 pregnant women in 3\textsuperscript{rd} trimester with preeclampsia, 36 pregnant women in 3\textsuperscript{rd} trimester without preeclampsia and 24 non-pregnant women with no history of abortion as controls. All subjects were recruited from the Obstetrics and Gynaecology Clinic of the University College Hospital, Ibadan, Oyo State, Nigeria.

The diagnostic criteria for preeclampsia were significant proteinuria (>100 mg/day) and high blood pressure (>130/90 mm Hg) irrespective of weight of the patients or the presence of edema. The blood pressure must have manifested on at least two occasions 6 hours or more apart. Spot weight measurement was not included as one of the diagnostic criteria because the rate of weight gained signifies the rapidity of fluid accumulation in preeclampsia.

**Sample collection**

Ten milliliters of venous blood was collected from the antecubital vein into a non-heparinized bottle and spun at 1500×g for five minutes. After clot retraction the serum was separated and stored at –20\textdegree C till needed for analysis. Urine samples were collected in sterile plastic bottles.

**Quantitation of immunoglobulin classes and acute phase proteins**

Immunoglobulin classes and acute phase proteins were quantitated by the single radial immunodiffusion method [20]. 3\% noble agar was prepared in phosphate buffered saline (PBS, pH 7.2) containing 0.2\% sodium azide. One milliliter of each antisera (anti-human immunoglobulin class or acute phase protein) was mixed with 7 ml of PBS. Eight milliliters of the 3\% noble agar was thoroughly mixed with the diluted antiserum. The mixture was carefully poured on a glass plate placed on a leveler avoiding the formation of air bubble. The agar-antiserum mixture was allowed to set and wells of 3 mm in diameter were made in the agar with a circular metal punch. The punched agar was carefully removed from the plate with the smooth edge of pipette attached to a vacuum pump. Several dilutions (25\%, 50\%, 100\% and 200\%) of the standard serum were prepared in PBS. Using a 5 ml micro-dispenser the sera, urine and standards were applied to the punched wells.
The plate for IgG estimation was put into a humid chamber and incubated for four hours while those for IgA, IgM and acute phase proteins were put into a humid chamber and incubated for 18 hours. The diameter of the precipitation ring was measured along two perpendicular diagonals to the nearest 0.1 mm using eye precision viewer. The standard curves for various classes of immunoglobulin and acute phase proteins were plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

**Determination of urinary total protein**

The urine sample (100 ml) was centrifuged at 1500×g for 5 min. The Coomasie Blue protein binding procedure [25] was used to determine the urinary total protein. Briefly, 20 μl of urine sample or calibrator (BSA) was gently mixed with 1.0 ml of protein dye reagent diluted with four volumes of sterilized water in a plastic container for 20 minutes. The absorbance was read at 505 nm wavelength using Milton Roy 1001 spectrophotometer. Protein concentration was extrapolated from the absorbance values of a calibration curve.

**Statistical analysis**

Mean of two readings of the same specimen was taken as actual value. Data were presented as mean±SEM. One-way ANOVA followed by Tukey test was used to compare mean values of the three examined groups. Pearson’s correlation coefficient was used to correlate urinary protein and serum immunoglobulin levels.

**RESULTS**

Table 1 shows the mean ages and serum concentrations of IgG, IgA and IgM of the subjects. Age and IgM concentration did not differ in the examined groups. The mean level of IgG was significantly lower in subjects with preeclampsia and significantly higher in subjects with normal pregnancy
when compared with non-pregnant controls. The mean level of IgA was significantly reduced in pre-eclamptic subjects compared with non-pregnant controls.

The results presented in Table 2 shows that the mean levels of transferrin and alpha-2-macroglobulin were significantly reduced in preeclampsia compared with the non-pregnant controls or those with normal pregnancies. Haptoglobin was significantly higher in preeclamptic subjects compared with subjects with normal pregnancy but lower in both pregnant groups compared with the non-pregnant controls. Urinary total protein and urinary IgG were significantly raised in Nigerian women with preeclampsia compared with non-pregnant controls and those with normal pregnancy (tab. 3). In addition, significant negative correlations were found between serum IgG (r= -1.16) or IgA (r=-1.21) concentration and urinary protein.

**DISCUSSION**

Research on the etiopathogenesis of preeclampsia is a continuing process. This study provides additional information concerning immunological parameters for preeclampsia. Data on serum IgA during pregnancy are confusing; some investigators have reported similar levels of IgA in pregnant and non-pregnant females [16] while others reported low values [17] of IgA in pregnant females compared with non-pregnant females [27]. In the current study IgA was reduced in preeclampsia compared with non-
pregnant controls. This might have been caused by significant proteinuria in preeclampsia suggesting the possible loss of IgA.

Reports on serum IgM in pregnancy are also at variance. Previous studies reported lower [15] or elevated [27] levels of IgM in normal pregnant women compared with non-pregnant women. IgM is an optimal activator of complement system via the classical pathway [7]. Activation of the classical pathway of complement system is a common phenomenon during pregnancy [16]. In preeclamptic subjects, the damages to vascular walls cause intense narrowing of the blood vessels, clot formation, activation of white blood cells and complement system [11, 13].

Several studies have reported a low serum IgG level in normal pregnancy [15, 27]. However, the high serum IgG observed in women having normal pregnancy compared with non-pregnant controls might have been caused by trans-placental transfer of IgG to a developing fetus which induces more IgG synthesis. Also, pregnant women’s susceptibility to infections especially malaria [10] and immunizations against some communicable diseases that are routinely given to expectant mothers may account for the production of the excessive IgG. IgG was significantly reduced in preeclamptic subjects compared with subjects having normal pregnancy and non-pregnant controls. This might have been caused by protein loss in the urine coupled with transplacental transfer.

In studies of proteinuria, authors have used timed collection, overnight urine collection or 24 h specimen [12]. We used untimed urine specimens, because they may be conveniently collected and have been shown to give valid results [26]. There are few reports on the excretion of total protein in Nigerian pregnant women with or without preeclampsia [12]. In the present study, total protein excretion was significantly increased during normal pregnancy and more importantly in preeclampsia. The increased excretion of protein could be the result of increased filtration, reduced reabsorption or a combination of the two factors. A high glomerular filtration rate during preeclampsia probably contributed to massive proteinuria.

A previous study [13] on preeclamptic women showed that an altered maternal response to the invading throphoblast lead to placental ischaemia and a release of certain placental factors. This caused generalized maternal
endothelial cell dysfunction and release of TNF alpha and IL-6. TNF alpha inhibits in vitro hematopoiesis as well as induces acute phase reaction and expression of ICAM-1 [11, 24]. Ineffective erythropoiesis and an increase in intravascular hemolysis had been found to cause a reduction in haptoglobin levels [9]. Raised TNF alpha causes the increase in haptoglobin in preeclamptic subjects compared with women having normal pregnancy.

A reduced level of alpha 2-macroglobulin was found in preeclamptic subjects compared with subjects with normal pregnancy and non-pregnant controls. Alpha 2-macroglobulin is synthesized mainly in the liver. It is a protease inhibitor and transports Zn [9]. The reduction of alpha 2-macroglobulin may be due to its utilization by various proteases which are produced by endothelial cell dysfunction and disturbances in coagulation mechanisms.

Antioxidant enzymes primarily interfere with the production of free radicals [6] and include Cu/Zn – containing superoxide dismutase, Fe – containing catalase and Se – containing glutathione peroxidase. The three enzymes lower the potential for the formation of the highly reactive hydroxyl radical as well as other energized oxygen-containing species. A low level of alpha 2-macroglobulin may lead to reduced availability of Zn for the synthesis of superoxide dismutase and result in a raised level of free radicals in pre-eclamptic subjects [18].

A high transferrin level in pregnant women without preeclampsia could indicate its increased synthesis by hepatic parenchymal cells. Iron level in the circulation of subjects with normal pregnancy is usually higher than in non-pregnant controls because of antenatal routine iron supplementation [3]. Transferrin transports iron from cells that have transferrin receptors. Iron binding by transferrin prevents the toxic effects of Fe. The Fe-free apotransferrin has a bacteriostatic effect as it deprives the bacteria of vital Fe [9]. Reduced transferrin concentration in preeclampsia has two major effects: proliferation of bacteria in blood due to free Fe available for bacteria growth, and increased production of hydroxyl radicals (e.g. in Haber-Weiss reaction in which $H_2O_2$ interact with Fe). This may be one of the reasons for excessive free radical production in preeclampsia [6]. Reduced transferrin concentration occurs in acute phase reaction, disturbances of hemoglobin
synthesis, protein loss and latent protein deficiency (malnutrition; [19]).
Protein is lost in urine of preeclamptic subjects and preeclampsia is
predominant among poor people, therefore a low level of transferrin may
be expected in preeclampsia.

In conclusion, reduced IgG, IgA, transferrin and alpha 2-macroglobulin
concentrations in preeclamptic subjects compared with subjects with
normal pregnancy indicated disturbed humoral immune responses.

ACKNOWLEDGEMENTS

We are grateful to the participants for taking part in the study and Prof.
C.P. Muller, Department of Immunology, National Health Laboratory,
Luxembourg, Germany, for the antisera.

REFERENCES