Immunoglobulin classes (IgG, IgA and IgM) and acute phase proteins in pregnant women with urinary schistosomiasis

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Summary

Background: S. haematobium **have been implicated in female genital schistosomiasis (FGS), ectopic pregnancy and infertility. The presence of schistosome eggs in urine has been reported to correlate with FGS but host immune response in FGS is yet to be determined. This gap in knowledge is addressed by this study.**

Materials and Methods: **Serum levels of three immunoglobulin classes (IgG, IgA and IgM) and three acute phase proteins (transferrin, alpha 2-macroglobulin, and haptoglobin) were determined by using single radial immunodiffusion technique in one hundred and eight Nigerian women aged between 15 and 30 years. They were made up of thirty pregnant women with urinary schistosomiasis (P+USS), thirty-six pregnant women without USS (P-USS), eighteen non-pregnant women with USS (NP+USS) and twenty-four healthy non-pregnant women without urinary schistosomiasis (NP-USS).**

Results and Conclusions: **IgG, IgA and IgM were significantly raised in pregnant women with urinary schistosomiasis compared with non-pregnant women without urinary schistosomiasis ($p<0.01$ in each case). The level of transferrin was significantly increased in non-pregnant subjects with urinary schistosomiasis compared with non-pregnant women without urinary schistosomiasis ($p<0.01$) suggesting the possibility of iron deficiency in these subjects. Alpha-2 macroglobulin was significantly elevated in pregnant subjects with urinary schistosomiasis compared with non-USS subjects, while the mean serum haptoglobin level was significantly higher in pregnant women without urinary schistosomiasis compared to pregnant subjects with urinary schistosomiasis. The results indicate that USS or pregnancy changes different aspects of humoral immunity, thus the co-existence of pregnancy and S. haematobium infection may influence each other.**

Key words: ***Serum immunoglobulins, Acute phase proteins, Pregnant women, Urinary schistosomiasis, Nigerians.***

Résumé

Introduction: S hématobium a été impliqué dans la schistose génitale féminine (SGF), grossesse ectopique et stérilité. La présence des oeufs de schistose dans l'urine a été noté d'être en corrélation avec SGF mais on n'arrive pas encore à décider la réponse d'immune dans SGF. L'objectif de cette etude est fondé sur cette omissions dans la connaissance.

Matériels et méthodes: Les niveaux de sérum de trois types différents d'immunoglobuline (IgG, IgA et IgM) et trois phases aiguës des protéines (transferrine, alpha - 2 macroglobuline, et haptoglobine) ont été décidées à travers l'utilisation de la technique immuno-diffusion radiale simple pour cent huit femmes nigériannes âgées entre 15 et 30 ans. Elles sont composées de trente femmes enceintes atteintes de la schistose urinaire (E + USS), trente six femmes enceintes sans USS (E - USS), dix-huit femmes non-enceintes avec USS (NE + USS) et vingt quatre femmes non-enceintes en très bonne santé sans schistose urinaire (NE - USS).

Résultats et conclusions: IgG, IgA et IgM étaient remarquablement élevés chez les femmes enceintes atteintes de la schistose urinaire par rapport aux femmes non enceintes sans schistose urinaire ($P < 0,01$ dans chaque cas); Le niveau de la transferrine était sensiblement augmentée chez les sujets non enceintes avec schistose urinaire par rapport aux femmes non enceintes sans schistose urinaire ($P < 0,01$) évoquant la possibilité de manque de fer chez ces sujets. Alpha - 2 macroglobuline etait sensiblement augmentée chez des sujets enceintes avec schistose urinaire en comparaison des sujets non-USS, tandis que le niveau du sérum haptoglobine était sensiblement plus élevé chez les femmes enceintes sans schistose urinaire par rapport aux sujets enceints avec schistose urinaire. A travers les résultats nous concluons que l'USS ou la grossesse change les aspects diveres d'immunité humorale, donc la coexistence de la grossesse et l'infection hématobium S pourrait influencer l'une l'autre.

Introduction

The occurrence of S. haematobium eggs or worms in the female genital tract (FGS) is common[1,2], however FGS is not commonly diagnosed in pregnancy due to adverse effects of surgical biopsy on developing foetus and mother. The effects of schistosomiasis on human reproduction are infertility, ectopic pregnancy, azoospermia[3], low birth weight[4]. Most of these reported reproduction-related problems of FGS were supported mainly by mechanical impacts of granuloma formed by schistosome eggs[1,2] with scanty immunological data. Also, most studies on schistosomiasis in endemic areas were concentrated on subjects of pre-puberty ages[5] with little attention to the possibility of co-existence of pregnancy and USS considering the chronic course of the USS. The aim of this study is to measure serum immunoglobulins and acute phase proteins in pregnant women with urinary schistosomiasis and compared the results with non-pregnant subjects with and without urinary schistosomiasis to elucidate the humoral immunological basis of FGS.

Materials and methods

Subjects: 108 women aged between 15 and 30 years were recruited from Ilie village in Olorunda Local Government Area

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of Osun State, Nigeria, into the study. Informed consent was obtained from them before sample collection and the need for the study was explained in local language when necessary. Urinary schistosomiasis subjects were identified by the presence of terminal spined *S. haematobium* eggs in urine sediments after spinning at 1500rpm for 5 minutes[6]. The subjects were grouped into thirty pregnant women with urinary schistosomiasis (P+USS), thirty-six pregnant women without urinary schistosomiasis (P-USS), eighteen non-pregnant women with schistosomiasis (NP+USS) and twenty-four non-pregnant women without schistosomiasis (NP-USS).

Subjects with malaria, intestinal helminthes, microfilaria or history suggestive liver disease, were excluded from the study. Microfilaria was examined in thick blood films stained with Giemsa while intestinal helminthes eggs were examined in normal saline preparation of faecal samples stained with Dobell iodine[7].

Assays: Five milliliters of venous blood was collected from each subjects into non-heparinized bottle for the measurement of serum immunoglobulin classes (IgG, IgA and IgM) and APPs (transferrin, alpha 2-macroglobulin and haptoglobin). The blood was allowed to clot, retract and the serum separated by centrifugation at room temperature (20°C). The serum was stored at –20°C till needed for analysis. Serum APPs and immunoglobulin classes were estimated by single radial immunodiffusion method of Fahey and McKelvey[8] as modified by Salimonu and co-workers[9]. One milliliter of each of the appropriate antiserum (anti-human APPs) was mixed with 7ml of phosphate-buffered saline (PBS) in a clean glass tube. Eight milliliters of the prepared 3% noble agar was measured into a long glass tube and thoroughly mixed with the diluted antiserum. The mixture was carefully poured on a glass plate placed on a leveler, avoiding formation of air bubble. The agar-antiserum mixture was allowed to set and wells of 3mm 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 pasture pipette attached to a vacuum pump, taking care not to damage the sides of the wells.

Several dilutions of the standard serum were prepared in PBS. Using a 5μl micro-dispenser, both the sera and standard were applied to the punched wells. The plates of APPs and immunoglobulin classes (IgA and IgM) estimation were put in a humid chamber and incubated at room temperature (20°C) for 18 hours while the plate for IgG estimation was incubated for 4hrs at 4°C. After incubation, the diameter of the precipitin ring was measured to the nearest 0.1mm, using precision viewer. Data were presented as mean and standard deviation. Student's 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 that the highest serum levels of all the three immunoglobulins were found in pregnant subjects with USS. The lowest value of IgA was found in pregnant subjects without USS, while the non-pregnant without USS subjects recorded the lowest value of IgM and IgG. Pregnant subjects with USS had statistically significantly elevated serum levels of all the three immunoglobulins compared to the non-pregnant subjects with USS (IgA: t=18.70, p<0.01; IgM: t=2.59, p<0.01; IgG: t=30.10, p<0.01), and non-pregnant without USS subjects (IgA: t=14.10, p<0.01; IgM: t=2.25, p<0.05; IgG: t=52.00, p<0.01). Although the serum levels of the three immunoglobulins were also found to be higher in pregnant subjects with USS than in pregnant subjects without USS, this was not statistically significant with IgM (t=1.56, p>0.10). The high serum values of IgM and IgG recorded in non-pregnant subjects with USS compared with non-pregnant without USS subjects were only statistically significant with

Table 1 Immunoglobulin values (mean ± S. D) in pregnant women, non-pregnant women with or without urinary schistosomiasis.

Subjects	n	IgA (mg/dL)	IgM (mg/dL)	IgG (mg/dL)
NP - USS	24	348.80 ± 66.80	62.30 ± 26.70	678.70 ± 30.90
NP + USS	18	273.40 ± 56.20	65.20 ± 29.60	855.10 ± 73.60
P - USS	36	59.70 ± 29.20	72.80 ± 43.00	1311.80 ± 97.70
P + USS	30	598.70 ± 61.50	98.80 ± 82.20	1546.70 ± 84.90
t, p-value[a]		3.97, < 0.01	0.33, > 0.20	9.54, > 0.01
t, p-value[b]		20.10, < 0.01	1.17, > 0.20	36.20, < 0.01
t, p-value[c]		14.10, < 0.01	2.28, < 0.05	52.00, < 0.01
t, p-value[d]		18.70, <0.01	2.59, < 0.01	30.10 < 0.01
t, p-value[e]		41.50, < 0.01	1.56, > 0.10	10.30, < 0.01

Values are in mg/dL
NP - USS = non-pregnant subjects without urinary schistosomiasis
NP + USS = non-pregnant subjects with urinary schistosomiasis
P + USS = pregnant subjects with urinary schistosomiasis
P-USS = pregnant women without urinary schistosomiasis
a = NP-USS compared with NP + USS
b = NP -USS compared with P-USS
c = NP-USS compared with P + USS
d = NP + USS compared with P + USS
e = P-USS compared with P+USS

Table 2 Values of APPs (mean ± S. D) in pregnant women, non-pregnant women with or without urinary schistosomiasis.

Subjects	n	Transferrin	a-2 Macroglobulin	Haptoglobin
NP - USS	24	110.30 ± 90.00	705.50 ± 34.90	549.00 ± 30.70
NP + USS	18	139.90 ± 18.50	582.40 ± 24.50	770.40 ± 32.10
P - USS	36	100.70 ± 25.70	609.10 ± 27.20	273.40 ± 26.10
P + USS	30	105.70 ± 26.60	725.90 ± 31.90	100.90 ± 15.30
t, p-value[a]		7.22, < 0.01	13.40, < 0.01	22.60, < 0.01
t, p-value[b]		2.09, < 0.05	11.30, < 0.01	36.30, < 0.01
t, p-value[c]		0.88, > 0.20	2.23, < 0.05	64.90, < 0.01
t, p-value[d]		5.26, < 0.01	17.50, < 0.01	83.70, < 0.01
t, p-value[e]		0.77, > 0.20	15.80, < 0.01	33.20, < 0.01

Values are in mg/dL
NP - USS = non-pregnant subjects without urinary schistosomiasis
NP + USS = non-pregnant subjects with urinary schistosomiasis
P + USS = pregnant subjects with urinary schistosomiasis
P-USS = pregnant women without urinary schistosomiasis
a = NP-USS compared with NP + USS
b = NP -USS compared with P-USS
c = NP-USS compared with P + USS
d = NP + USS compared with P + USS
e = P-USS compared with P+USS

IgG (t=9.54, p>0.10) not with IgM (t=0.33, p>0.20).

Non-pregnant subjects with USS recorded the highest value for transferrin while the lowest values were found in pregnant subjects without USS. This high serum transferrin was statistically significant when compared with those found in pregnant subjects with USS (t=5.26, p<0.01) and NP-USS (t= 7.22, p<0.01). The highest serum level of haptoglobin was similarly recorded in non-pregnant subjects with USS, and was statistically significant when compared with serum levels recorded for pregnant subjects with USS (t =83.70, p<0.01) or the non-pregnant without USS subjects (t= 22.60, p<0.01). However, unlike transferrin and haptoglobin, the highest value of alpha-2 macroglobulin was recoded in pregnant subjects with USS; and was statistically significant when compared to values found with the controls (t=2.23, p<0.05), non-pregnant subjects with USS (t=17.50, p<0.01) and pregnant subjects without USS (t=15.80, p<0.01).

Discussion

This present study indicates elevated levels of IgG in schistosomiasis subjects, both pregnant and non-pregnant females. Others have documented similar observations[10, 11]; and its level has been shown to correlate with the intensity of infection[12]. The protective role of IgG against pathogens could explain the significantly high serum IgG found in USS subjects in the present study since it is the main immunoglobulin secreted during secondary immune response. The significantly high serum level of IgG recorded in pregnant subjects without USS could be due to the fact that IgG is the only immunoglobulin that can cross the placenta which provide protective immunity to the foetus *in-utero* and in the first few months of extra-uterine life[13]. During pregnancy, the synthesis of IgG is increased to allow for foetal and maternal requirements[13], thus explaining the rise in IgG of pregnant women without USS.

Raised values of serum IgM level as recorded in subjects with USS; have been similarly observed by other workers[7], and this is statistically significant in pregnant subjects with USS. The continuous release of particulate antigens from adult worms that have been found to induce a greater IgM response in experimental situation[13] may explain the high IgM value in pregnant subjects with USS. Reports on serum IgM value in pregnancy are at variance. Some studies have reported low levels of IgM in healthy pregnant women[14]. The elevated level of IgM as found in pregnant subjects without schistosomiasis in the present study, have been similarly observed by other workers[15]. It may be due to increased susceptibility of genitourinary system of pregnant women to sub-clinical urinary tract infections[16], which leads to predominant IgM production.

The mean serum IgA was reduced in non-pregnant subjects with USS compared NP-USS subjects. This finding, although consistent with the findings of some workers[17] (Mutapi et. al., 1997), is unexpected because other urinary tract infections have been found to be associated with increased serum IgA[18]. The mean serum IgA was also found to be significantly low in pregnant subjects without USS. Serum values of IgA in normal pregnancy are conflicting. Some investigators have reported a non-significant change in IgA in pregnancy[14], while others have observed low values[19]. Recent studies have however suggested that normal female genital tract secretes low IgA[20]. Although studies on IgA level in pregnant subjects with USS were not encountered, the significantly elevated value of serum IgA in pregnant subjects with USS will require further investigation.

We recorded a significant high mean serum transferrin in non-pregnant subjects with USS. This could be as a result of iron deficiency induced by chronic haematuria of USS[21]. During USS infection, there is blood loss due to piercing of urinary bladder by terminal spin of S. haematobium eggs and lysis of red blood cells by adult schistosome[5, 7] both of which lead to reduced circulating iron. The consequent of the reduced body iron is increased transferrin since there is no iron to bind. In pregnant subjects with and without USS, the serum transferrin was reduced. During pregnancy iron deficiency caused by foetal extraction[22] necessitates the routine antenatal iron supplementation, which could have affected the transferrin level.

Low serum haptoglobin was recorded in pregnant subjects with and without USS. Such low values are associated with a syndrome of haemolysis, elevated liver enzymes and low platelets [HELLP syndrome][23], which are not detected in any of the subjects evaluated. However, Gatzka and co-workers[24] reported a case of low haptoglobin in a pregnant woman without any clinical signs of pre-eclampsia or abnormal laboratory results. In view of this report and current findings, the differential diagnosis of a reduced haptoglobin during pregnancy, aside from HELLP syndrome need to be investigated. One possible advantage of the reduced haptoglobin in pregnant women is to remove its inhibitory effect on Th-2 cytokines needed to maintain the later part of pregnancy[25]. The significantly high serum haptoglobin level observed in non-pregnant subjects with USS is in line with earlier observations by Arinola and Salimonu[7], and is consistent with its role as scavenger of free haemoglobin. Proteolytic enzymes released from damaged tissues as well as from phagocytic cells have their activity inhibited by being bound by a-2 macroglobulin[26]; and is also known to bind growth factors such as IL-8[27] (Kurdowska et. al., 2000), nerve growth factor, platelet derived growth factor-ß and transforming growth factor-ß[28] and transport them to their target cells where such cytokines affect cell growth and functions[29].

The significantly low value of alpha-2 macroglobulin found in non-pregnant subjects with USS and in pregnant subjects without USS could be due to its utilization in binding proteolytic enzymes released from damaged tissue in schistosomiasis, and as transport protein for cytokines to placenta for cell growth and function in pregnancy. The possible increase in hepatic synthesis of alpha-2 macroglobulin to meet the requirement in proteolytic enzymes released from damaged tissues and as transport protein in pregnancy could have accounted for the significantly high values found in pregnant subjects with concomitant USS. Such high values have also been documented in animal model[30]. Proteinases released from damaged tissues and phagocytic cells bind alpha-2 macroglobulin[29, 30]. Therefore, low level of alpha-2 macroglobulin in NP-USS may be the result of its consumption by proteolytic enzymes released during tissue damage,

which is caused by migrating spiny eggs, larva and adult worms of schistosome parasites.

Maternal immune responses in pregnancy are biased towards humoral and away from cell-mediated immune response[31]. There is evidence that Th 1 cytokines can be harmful to pregnancy, whereas Th 2 cytokines are protective. IFN-gamma and IL-2 induce foetal loss[31]. Positive correlations have been reported between resorption and the expression of IFN-gamma, IL-2 and TNF[32]. In contrast to these deleterious effects of Th 1 cytokines, the Th 2 cytokine IL-10 protects against foetal death in murine model of spontaneous resorption, and there is a negative correlation between resportions and IL-10 expression[32].

In human schistosomiasis, Th 1 cells produced high level of interferon (IFN) at the pre-acute stage of infection but subsequently diminish[33]. Th 1-specific IL-2 and IFN peak at acute stage and concurrently decreased at the chronic stage when Th 2 specific IL-4, IL-5, IL-6 and IL-8 are more predominant than IL-2[33].

IL-1 (Th 1 cytokine) is a potent immune regulator that is involved in the production of acute phase proteins. Production of IgG and IgE are stimulated by IL-4 (Th 2 cytokine) while the level of IgA and polyclonal immunoglobulin production are controlled by IL-5 and IL-6 respectively[34]. Based on this, the present study showed that Th 2 responses predominate in P-USS or P+USS subjects but Th 1 responses predominate in NP+USS subjects. This is an indication that immune responses, which are protective to pregnancy supercede immune responses to schistosomes in pregnant women with USS. The implication is that since Th 2 cytokines favour successful pregnancy, the presence of S. haematobium infection during pregnancy (FGS) may have no negative impact on pregnant outcome but pregnancy may aggravate the course S. haematobium infection which is controlled by Th 1 cytokines at acute stage.

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