Immunoglobulins (Classes IgG, IgA and IgM) in Pregnant Women With Urinary Schistosomiasis in Ilie, Southwestern Nigeria.

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Abstract

Context: The occurrence of schistosome eggs or worms in the female genital tract has been reported in several clinical conditions including ectopic pregnancy and infertility. The presence of schistosome eggs in urine has been reported to correlate with female genital schistosomiasis (FGS), but the impact of schistosome eggs or adult worms on host's immune responses during pregnancy is however yet to be determined.
Aims: In view of the possible occurrence of female genital schistosomiasis without the eggs being detected in the urine except surgical biopsy. Moreso, surgical procedure may have adverse effects on both the mother and foetus. The diagnostic relevance of three immunoglobulin classes (IgG, IgA & IgM) was investigated in pregnant women with urinary schistosomiasis.
Design & Setting: Case-control study in Ilie village in Olorunda Local Government Area of Osun State, Nigeria.
Subjects: 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 non-pregnant healthy women without urinary schistosomiasis (NP-USS).
Main Outcome Measures: Serum levels of three immunoglobulin classes (IgG, IgA, IgM) were determined using single radial immuno-diffusion technique in one hundred and eight Nigerian women.
Results: All the three classes of immunoglobulin were higher in P+USS compared with other groups. Both IgA and IgG were significantly different when P+USS and other groups were compared. No significant difference existed when IgM was compared in P+USS and P-USS or NP+USS and NP-USS or P-USS and NP-USS.
Conclusions: The results shows that raised IgM during pregnancy may indicate FGS among other causes.

Key Words: Immunoglobulins, Pregnancy, Schistosomiasis [Trop J Obstet Gynaecol, 2004;21:125-127]

Introduction

Schistosomiasis, also known as bilharziasis, is a parasitic disease that leads to chronic ill health. It is one of the major communicable diseases of public health and socio-economic importance in the developing world. Despite control efforts in a number of countries, an estimated 200 million people are affected, of which 120 million are symptomatic and 20 million have severe disease. Most of these are concentrated in Africa[1].

The occurrence of *S. haematobium* eggs or worms in the female genital tract though not uncommon[2, 3], it is however not commonly diagnosed in pregnancy due to adverse effects of surgical biopsy on developing foetus and the mother. Most of the reported pregnancy-related problems of female genital schistosomiasis (FGS) were supported mainly by mechanical impacts of granuloma (infertility, ectopic pregnancy) formed by schistosome eggs[2,3,4] with scanty immunological data. Also most studies on schistosomiasis in endemic areas were concentrated on subjects of pre-puberty ages[5, 6] with little attention to the possibility of pregnant women also being infected in view of the chronic nature of the disease. The aim of this study is to measure serum immunoglobulins in pregnant women with urinary schistosomiasis (USS) and compared the results with non-pregnant subjects with and without urinary schistosomiasis to elucidate the diagnostic values of serum immunoglobulins in schistosomiasis.

Materials and Methods

Subjects: A total of 108 women aged between 15 and 30 years were recruited from Ilie village in Olorunda Local Government Area 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 schistomiasis 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[6].

Assays: Five milliliters of venous blood was collected from each subjects into non-heparinized bottle for the measurement of serum immunoglobulins. The blood

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twas 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 immunoglobulin classes (IgG, IgA and IgM) 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 immunoglobulin classes) 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 5ìl micro-dispenser, both the sera and standard were applied to the punched wells. The plate of IgG estimation was put in a humid chamber and incubated for 4 hours at 37°C while those for IgA, IgM were put in humid chamber and incubated at room temperature (20°C) for 18 hours. After incubation, the diameter of the precipitin ring was measured to the nearest 0.1mm, using precision viewer. Data were presented as means and standard deviations. One-way analysis of variance (ANOVA) was used to test for the level of significance. Student t-test was used to test the significance of differences between mean values of two groups. The probability value (p) greater than 0.05 was considered insignificant.

Results

Table 1 shows that all the three classes of immunoglobulin measured were significantly higher in pregnant subjects with USS compared to the NP-USS group.

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	5970±29.20♦	72.80±43.00	1311.80±97.70♦
P+USS	30	59870±61.50♥♠•	98.80± 82.20♥•	1546.70± 84.90♥♠•
F, p-values		17.95,0.0001	8.45,0.009	23.50,0.00008

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
* = Significant difference between controls and NP+USS
♦ = Significant difference between controls and P-USS
♥ = Significant difference between controls and P+USS
♠ = Significant difference between P+USS and P-USS
• = Significant difference between P+USS and NP+USS

IgA was least in P-USS group and this was significant when compared with other groups. There was no significant increase when the levels of IgM in P-USS ($t = 1.17$, $p > 0.20$) and NP+USS ($t = 0.33$, $p > 0.20$) groups were compared with the NP-USS. IgG was highest in pregnant subjects with USS, followed by P-USS, NP+USS and least in NP-USS. There were significant differences in the levels of IgG when the groups were compared.

Discussion

The immunoglobulins constitute a heterogeneous group of serum proteins with diverse functions. The serum levels are influenced by diverse factors, including infections and inflammation[10]. This present study indicates elevated levels of IgG in schistosomiasis subjects (pregnant and non-pregnant) compared with NP-USS. Other workers observed similar finding[11, 12] and its level have been shown to correlate with the intensity of infection[13, 14]. The protective role of IgG against pathogens could explain the significantly high serum IgG found in USS subjects in the present study. However, a study in Nigerian children with USS reported a reduced level of serum IgG[7]. The disparity may be due to differences in the age groups of the subjects that participated in these studies. IgG was shown to increase with age especially in the females[15]. The significantly high serum level of IgG recorded in pregnant subjects without USS compared with NP-USS or NP+USS could be due to IgG being the only immunoglobulin that crosses the placenta so as to provide protective immunity to the fetus *in-utero* and in the first few months of extra-uterine life[16]. The mean serum IgA was reduced in non-pregnant subjects with USS compared NP-USS. This finding, although consistent with the findings of some workers[23] is unexpected because urinary tract infections other than USS have been found to be associated with increased serum IgA[24, 25]. However, studies have shown high serum IgA to be consistent with acute schistosomiasis and serum IgA in chronic schistosomiasis[26, 27]. The low serum IgA found in the present study could probably be an indication of chronic USS in the studied population. The mean serum IgA was also found to be significantly low in pregnant subjects without USS compared with other groups. Serum values of IgA in pregnancy are conflicting. Some investigators have reported a non-significant change in IgA in pregnancy[18, 28] while others have observed low values[19, 29]. Recent studies have however suggested that the immune system of the genital tract is unique and distinct from other human systemic tracts by producing low IgA[10]. No previous literature on IgA level in pregnant subjects with USS was available. The significantly elevated value of serum IgA in pregnant subjects with USS might have been due to IgA elevation by *S. haematobium* infection. Possible synergistic effects of pregnancy and schistosomiasis infection on IgA synthesis need to be investigated.
Raised values of serum IgM level in our subjects with USS were observed by other workers[7]; and this is

Statistically significant in pregnant subjects with USS. The continuous release of particulate antigens from adult schistosome worms that have been found to induce a greater IgM response in experimental situation[17]. This might explain the high value of IgM in subjects with USS. Previous reports on serum IgM value in pregnancy are at variance. Some studies have reported low levels of IgM in healthy pregnant women[18, 19]. The elevated level of IgM as found in pregnant subjects without schistosomiasis in the present study was observed by other workers[20]. The cause is yet to be explained. However, the physiological changes in the genitourinary system of pregnant women increased their susceptibility to sub-clinical urinary tract infections that lead to production of predominantly IgM[21, 22]. The combined effects of particulate schistosome antigens and susceptibility of pregnancy to genitourinary tract infection may explain the highest level of IgM in our P+USS subjects.

of immunoglobulins (IgG, A and M) were raised in P+USS compared with other groups, but only IgM distinguishes P+S from NP+S, P-USS and NP-USS. Thus, this study suggests the usefulness of raised serum IgM in diagnosis of P+USS of FGS.

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