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Full Length Research Article

ACUTE PHASE PROTEINS IN PREGNANT WOMEN WITH URINARY SCHISTOSOMIASIS IN ILIE VILLAGE, OSUN STATE, NIGERIA.

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***Background**: The acute phase proteins (APPs) are plasma proteins whose concentration rise or reduce in reaction to infection, inflammation or trauma (Baumann and Gauldie, 1990). The circulating concentration of these proteins are related to the severity of the underlying condition, thus quantification of their concentrations provide a ready means of giving valuable clinical information and extent of the disease processes (Thompson et. al., 1992).*
***Materials and Methods**: Serum levels of three acute phase proteins (transferrin, α2-macroglobulin and haptoglobin) were determined using single radial immuno-diffusion 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 USS (NP-USS) as controls.*
***Results**: The result shows that transferrin was least in P-USS group and highest in NP+USS. The highest mean value of alpha-2 macroglobulin was found in P+USS group and the least in NP+USS. Haptoglobin was significantly reduced in P+USS compared with other groups.*
***Conclusions**: The finding of this study suggests an independent effect of USS and pregnancy on serum levels of APPs, therefore APPs could be used to distinguish P+USS from P-USS*

***Keywords**: Acute phase proteins, pregnant women, urinary schistosomiasis, Nigerians.*

INTRODUCTION

The acute phase proteins (APPs) are plasma proteins whose concentrations rise (positive APPs) or reduce (negative APPs) in reaction to infection, inflammation or trauma (Baumann and Gauldie, 1990). The concentrations of C-reactive proteins, fibrinogen, alpha-2 macroglobulin, alpha-1 anti-trypsin and haptoglobin increase in response to challenge (positive APPs), while those of transferrin and albumin reduce (negative APPs). The circulating concentration of APPs is related to the severity of the underlying condition and thus useful indices of evaluating the presence and extent of the disease processes (Thompson et. al., 1992).

Mild abnormalities of liver functions are frequently seen in pregnancy but return to normal after delivery. A raised serum alkaline phosphatase is common, along with a decline in the serum albumin, but the aminotransferases remain within normal limits (Arinola et al, 2003). It has been shown that abnormal liver diseases in pregnancy result in more marked alterations in liver functions (Yip and Baker, 1985). Viral hepatitis is the most common cause of jaundice in pregnancy, and the maternal prognosis is generally good (Yip and Baker, 1985).

Studies on the serum levels of APPs during normal pregnancy are not extensive, but it has been reported that transferrin, alpha-2 macroglobulin and C-reactive protein were increased during normal pregnancy (Shimizu et al., 2002).

Schistosoma infection has been found to induce inflammatory reactions in the host leading to changes in the serum concentrations of not only the immunoglobulins but also the acute phase proteins (Arinola and Salimonu, 1998b). Moreso in the liver, schistosomule larva stage of schistosome parasite grow into adult male and female schistosomes, pair-up and mate before passing down to vesical venules to commence egg laying (Alabaranoye, 1992). It is therefore possible that the

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presence of these larvae in the liver will modulate the acute phase responses during pregnancy.

MATERIALS AND METHODS

Subjects: 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 schistosomiasis subjects were identified by the presence of terminal spined *S. haematobium* eggs in urine sediments after spinning at 1500rpm for 5 minutes (Arinola and Salimonu, 1997). 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) as controls.

Subjects with malaria, intestinal helminthes, microfilaria or history suggestive of 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 (Arinola and Salimonu, 1999).

Assays: Five milliliters of venous blood was collected from each subject into non-heparinized bottle for the measurement of serum APPs. The blood was allowed to clot, retract and the serum separated by centrifugation at room temperature (20^0C). The serum was stored at -20^oC till needed for analysis. Serum APPs (transferrin, alpha-2 macroglobulin and haptoglobin) were estimated by single radial immunodiffusion method of Fahey and McKelvey (1965) as modified by Salimonu and co-workers (1978). 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 plate of APP estimation was put in a humid chamber and incubated at room temperature (20^0C) 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 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 that mean transferrin level was least in P-USS group and highest in NP+USS. It was significantly reduced in P+USS compared with NP+USS.

TABLE 1: Values of APPs (mean ± S.D) in pregnant women, non-pregnant women with or without urinary schistosomiasis.

Subjects	n	Age (years)	Transferrin	Alpha-2 Macroglobulin	Haptoglobin
NP-USS	24	21.30±4.00	110.30±90.00	705.50±34.90	549.00±30.70
NP+USS	18	21.00±4.00	139.90±18.50	582.40 ±24.50	770.40 ± 32.10
P-USS	36	22.50±3.20	100.70±25.70	609.10±27.20	273.40±26.10
P+USS	30	22.40±4.20	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]		0.77, >0.20	15.78, <0.01	33.17, <0.01
	t, p-value[e]		4.68, <0.01	17.5, <0.01	82.65, <0.01

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 subjects without urinary schistosomiasis
a = Controls (NP-USS) compared with NP+USS; b = Controls compared with P-USS; c = Controls compared with P+USS; d = P+S compared with P-USS; e = P+S compared with NP+USS

There was no significant difference in the mean values of transferrin when P+USS was compared with P-USS or when NP-USS was compared with P+USS. The highest mean value of alpha-2 macroglobulin was found in P+USS group and the least in NP+USS.

Significant difference existed when all the groups were compared with the NP-USS or P+USS. NP+USS had the highest mean value of haptoglobin and the least in P+USS group. Haptoglobin was significantly reduced in P+USS compared with other groups.

DISCUSSION

We recorded a significant high mean serum transferrin in non-pregnant subjects with USS. This could be as a result of iron deficiency from blood loss in the urine of USS subjects (Prual et. al., 1992). Red cell haemolysis and blood loss that is associated with urinary schistosomiasis results in iron depletion thus reduced stimulation of transferrin formation (Silverman and Christenson, 1996). However in pregnant subjects with USS, the serum transferrin was further reduced. Extractions by the foetus (Akinsooto et. al., 2001; Breymann, 2002) and loss to haematuria are possible causes of transferrin deficiency in pregnancy with USS. The routine ante-natal iron supplementation is expected to normalise transferrin level, as successful treatment of iron deficiency anaemia with iron has been known to returns plasma transferrin level to normal (Silverman and Christenson, 1996). It is likely that iron loss to haematuria as a result of USS superceedes the replacement by supplementation.

Low serum haptoglobin was recorded in pregnant subjects with and without USS. Low value of haptoglobin is associated with a syndrome of haemolysis, elevated liver enzymes and low platelets [HELLP syndrome] (Rath et. al., 2000), which was not detected in any of the subjects evaluated. However, Gatzka and co-workers (2002) 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, the differential diagnosis of a reduced haptoglobin during pregnancy, aside from HELLP syndrome needs to be investigated. One possible advantage of the reduced haptoglobin in pregnant women is the expected removal of its inhibitory effect on Th-2 cytokines needed to maintain the later part of pregnancy. Haptoglobin was found to inhibit the functions and levels of Th 2 cytokines (Delassus, 1994). The significantly high serum haptoglobin level observed in non-pregnant subjects with USS is in line with earlier observations by Arinola and Salimonu (1998b) whose study considered primary school children with USS.

The observation of elevated haptoglobulin in NP+USS is consistent with its role as scavenger of free haemoglobin. Free haemoglobulin in the blood of USS subjects might have releases from RBCs lysed by adult or eggs of schistosome parasites.

Proteolytic enzymes released from damaged tissues as well as from phagocytic cells have their activity inhibited by being bound by alpha-2 macroglobulin (James, 1997). The α-2M is also known to bind growth factors such as IL-8 (Kurdowska et. al., 2000), nerve growth factor, platelet derived growth factor-beta and transforming growth factor-β (Gonias et. al., 2000) and transport them to their target cells where such cytokines affect cell growth and functions (Tiggelman, 1996; Rand et. al. 2000). The significantly low value of alpha-2M found in non-pregnant subjects with USS and in pregnant subjects without USS could probably 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-2M 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 (Shimizu et. al., 2002). Our current finding shows that pregnancy alone reduces the three APPs while urinary schistosomiasis alone increases the levels of transferrin and haptoglobin but reduces the level of alpha-2 macroglobulin while co-existence of both pregnancy and urinary schistosomiasis reduces transferrin and haptoglobin but raises alpha-2 macroglobulin, therefore raised alpha-2 macroglobulin during pregnancy may suggest the presence of *Schistosoma haematobium* infection in pregnant women.

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