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Original article

Relationship between tumor necrosis factor-alpha (TNF) profile and urinary tract pathology in rural Nigerians with *Schistosoma haematobium* infection

Onyebiguwa Patrick Goddey Nmorsi[1*], Clement Isaac[1], Nnamdi Callistus Deguelle Ukwandu[2], Emmanuel Momoh[1], Maria Ihenata Ekozien[3], Afe Omolola Ekundayo[3] and Stanley Ogoinja[4].

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Abstract

Background: Schistosomiasis is estimated to affect more than 200 million people especially in rural and agricultural areas. *Schistosoma haematobium* causes significant urinary tract diseases and is mediated by T cell dependent granulomatous responses to the schistosome eggs. Since tumor necrosis factor alpha (TNF) is elicited by Th 1 responses and implicated in granulomatous responses to the ova trapped in the bladder wall of *Schistosoma haematobium* infected persons, it is important to ascertain the relationship between intensity of infection and urinary tract pathology in our locality.

Methodology: The urine samples from volunteers were subjected to parasitological investigations to ascertain the presence of *S. haematobium* ova in their urine. The TNF profile was ascertained using standard enzyme-linked immunosorbent assay (ELISA). The ultrasonographical investigation was carried out on the *S. haematobium* infected participants using transabdominal ultrasonography.

Results: Nineteen out of 40 rural Nigerians infected with *S. haematobium* showed severe infection while the remaining 21 individuals had light infection. Males (26) were more severely infected than females (14). Children (30) were more infected than adults (10). The serum TNF concentration correlated positively with the intensity of infection (r^2 = 0.97). Serum TNF was negatively correlated with the age of the volunteers (r^2 = -0.36). The mean TNF concentration among subjects with heavy infection (535.7.4±415.5 pg/ml) was significantly higher than that among those with light infection (93.8±40.9pg/ml) at (χ^2 = 341.0, $p<0.05$). Also the concentration of TNF in the sera of children (448.2±140.2pg/ml) was significantly higher than that in adults (180.0±152.1 pg/ml) at (χ^2 = 114.6, $p<0.05$). The ultrasonographic investigation revealed eight types of urinary tract pathology, namely, abnormal wall thickness (70%), irregular bladder wall (55%), echogenic particles (75%), calcification (60%), pseudopolyp (12.5%), masses (10%), residual volume (30%) and hydroureter (7.5%) among 28 subjects. These participants with urinary tract pathology had relatively high serum TNF ranging from 190.6±15.6 pg/ml in abnormal wall thickness to 630.6±15.6 pg/ml among individuals with masses.

Conclusion: The bladder and kidney pathology revealed in this investigation as well as the intensity of infection correlated with the levels of serum TNF among *S. haematobium* infected participants in Ihieve-Ogben, Nigeria. We observed an association between high level TNF with heavy infection and urinary tract pathology.

Keywords: Schistosoma haematobium, Tumor necrosis factor, Urinary tract pathology, Light infection, Heavy infection, Transabdominal ultrasonography, rural Nigerians.

INTRODUCTION

Schistosomiasis is estimated to affect more than 200 million people, especially in rural and agricultural areas, and between 500 and 600 million are said to be at risk of infection globally [1]. *S. haematobium* causes significant urinary tract diseases [2 - 6]. These urinary tract diseases range from mild symptomatic heamaturia, irregular bladder wall to hydronephrosis, hydroureter, kidney failure and squamous cell carcinoma [1, 3]. Among these features, Keita *et al.,* documented irregular bladder wall as the most frequent abnormal urinary tract pathology [7]. In many en-

1. Tropical Diseases Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
2. Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
3. Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
4. Department of Radiology, University of Benin Teaching Hospital, University of Benin, Benin City, Nigeria.
*Corresponding author:
E-mail: nmorsiopg@yahoo.com

demic zones, variability among individuals to the development of this disease is observed along with the manifestation of infection progressing into heavy infection [8, 9].

The development of urinary tract pathology in *S. haematobium* infection is due to delayed hypersensitivity [10]. It is mediated by T cell dependent granulomatous responses to the schistosome eggs [11, 12]. Tumor necrosis factor - alpha elicited by Th1 responses has been implicated in the development of granulomatous responses to ova trapped in the bladder wall of subjects infected with urinary schistosomiasis [3]. Furthermore, the production of cytokines such as TNF is a key event in inflammation related to human infectious disease and malignancy such as bladder cancer [13].

Schistosome immuno-epidemiology studies have shown that the development of antigen responses is related to cumulative exposure to parasite antigen [14, 15] and that the rate of development of different components of these responses, presents distinct profiles across the host age range. Cytokine responses to *S. haematobium* infection have been reported to show contrasting profiles with age [16]. A study in Kenya indicated the association of schistosomiasis morbidity with production of a high level of TNF [17].

There is little of information on the role of cytokines such as TNF in pathology of urinary schistosomiasis in this part of the globe, despite the relevance of the disease. This paper therefore attempts to evaluate the relationship between intensity of infection, age, TNF and urinary tract pathology in *S. haematobium* infection among rural Nigerians

MATERIAL AND METHODS

This study was carried out in Ihieve-Ogben, a rural community in the Owan East local government area of Edo State. The study area is located within the guinea savanna region of the state at latitude 6 °N and longitude 6 °E. The majority of people are engaged in Agriculture especially farming and hunting, while a few, mostly women, are traders. The village has a stream which the inhabitants use as a source of water and place for recreational activities. There are about 1,000 inhabitants in this community.

The present investigation started with a community mobilization campaign. This involved educating the inhabitants regarding the significance of the study as well as seeking their consent. Ethical permission was obtained from FaithDome Medical Center, Ekpoma, Edo State, Nigeria and the State Ministry of Health, Benin City, Edo State, Nigeria. Mid stream urine samples were collected from infected individuals between 11:00 and 13:00 GMT after slight physical exercise. The specimen was kept in a wide-mouthed screw capped 50 ml size container. These bottles containing the urine samples were immediately transported to our parasitological laboratory for detection of the ova of *S. haematobium.* The ova were quantified and classified as light infection (>50 ova/10 ml) and heavy infection (>50 ova/10 ml) according to WHO standards [18].

Whole venous blood (3 ml) of individuals positive to *S. haematobium* was collected from a peripheral vein by venipuncture and kept in a sterile EDTA bottle. The blood was processed by centrifugation, and the serum was immediately subjected to cytokine assays. The serum TNF concentration was determined by standard enzyme-linked immunosorbent assay (ELISA) using kits obtained from Abcam plc, Cambridge, United Kingdom. From the information supplied by the manufacturer, the normal serum TNF concentration was defined as 8 pg/ml.

Ultrasonographical investigations were carried out on the 40 infected volunteers by transabdominal ultrasonography at the Radiology Unit of FaithDome Medical Center, Ekpoma, Edo State, Nigeria. The investigations were carried out as described by Nmorsi *et al.,* using a Fukuda Denshi UF 4000 (Japan) ultrasound machine with a 3.5 MH_2 frequency curulinear real time probe [19]. The urinary tract abnormalities were catagorised according to WHO standards [20].

The data obtained in this study were subjected to statistical analysis, namely, correlation and chi-square tests using Microsoft Excel statistical package.

RESULTS

Table 1 presents the mean serum TNF profile of the volunteers by age and sex. The overall mean TNF level for the 40 infected children was 314.9±140.3 pg/ml. The lowest mean serum TNF (113.3±152.1 pg/ml) was reported among the volunteers above 26 years, while the highest (630.1 pg/ml) occurred among children 6 - 10 years old. The highest egg burden (618.1 ± 42.2) occurred among these children. The serum TNF of the infected participants correlated negatively with age (r = -0.2). Also the concentration of TNF in the sera of the children (448.2±140.2pg/ml) was significantly higher that in adults (180.0±152.1 pg /ml) at (χ^2 = 114.6, p<0.05).

The serum TNF concentration according to the intensity of infection and age among the 40 *S. haematobium* infected volunteers is presented in Table 2. A significant difference was observed between the mean serum TNF concentration of 21 participants with light infection (93.7± 76.9pg/ml) and that of 19 subjects with heavy infection (535.7.4±415.5 pg/ml) at (χ^2 = 341.0, p<0.05). The mean egg burden and the serum TNF were positively correlated (r = 0.97). The egg burden correlated with the mean serum

Table 1: The serum TNF concentration of *S. haematobium* infected participants by age and sex.

Age group in years	Sex Male	Female	Mean TNF concentration (pg/ml)	Mean egg burden/ 10ml of urine
1-5	2	1	115±60.4	42.5±38.9
6-10	10	6	630.1±450.1	618.1±42.2
11-15	8	3	560.0±304.0	599.5±33.9
16-20	3	2	290.1±140.3	397.2±73.7
21-25	2	1	180.4±73.7	96.5±20.1
>26	1	1	113.3±46.2	42.1±21.9
	26	14	314.9±227.5	299.0±274.1

Table 2: The intensity of *S. haematobium* infection with serum TNF concentration

Age groups in years	Light infection (< 50 ova/10ml) of urine			Heavy infection (< 50 ova/10ml) of urine		
	No	Egg burden	TNF concentration (pg/ml).	No	Egg burden	TNF concentration (pg/ml).
1-5	2	15.5±9.5	41.5±10.5	1	65.5±40.8	189±130.1
6-10	7	46.2±4.0	150.0±50.1	9	1189.9±295.1	1110±501.1
11-15	6	34.1±8.5	130.0±70.1	4	1163.9±140.1	990±255.1
16-20	3	30.1±10.1	100.1±20.1	3	763.9±295.1	480±301.1
21-25	2	25.5±5.5	76.2±46.2	1	167.5±84.1	284±140.2
>26	1	20.5±10.1	65.0±25.1	1	63.7±10.7	161±95.6
	21	28.7±10.9	93.8±40.9	19	569.1±538.0	535.7±415.5

Table 3: The serum TNF concentration of the participants with urinary tract pathology

Urinary tract pathology	Classification	Infection (No)		Total Infection No (%)	Mean TNF concentration (pg/ml)
		Male	Female	Both Sexes	
Abnormal wall thickness	1	14	8	22	190.6±15.6
	2	4	2	6	290.6±40.1
	Total	18	10	28 (70)	240.6±65.2
Irregularity of bladder wall	1	10	6	16	241.2±101.1
	2	4	2	6	339.8±72.1
	Total	14	8	22 (55)	290.5±98.1
Echogenic particles	—	20	10	30 (75)	215.7±41.4
Calcification	1	8	8	16	230.2±18.1
	2	3	5	8	460.2±35.1
	Total	11	13	24 (60)	345.2±15.6
Pseudopolyp	2	3	2	5 (12.5)	560.9±32.4
Masses	2	1	3	4 (10)	630.6±15.6
Residual volume	—	7	5	12 (30)	185.0±50.1
Hydroureter	3	2	3	5 (7.5)	480.3±71.1

TNF concentration of the participants with both heavy infection (r =0.97) and light infection (r = 0.98).

The serum TNF concentration of the participants with urinary tract pathology is presented in Table 3. Eight types of urinary tract pathology, namely, abnormal wall thickness (70%), irregular bladder wall (55%), echogenic particles (75%), calcification (60%), pseudopolyp (12.5%), masses (10%), residual volume (30) and hydroureter (7.5%) were observed among the *S. haematobium* infected participants. These participants had a relatively high serum TNF concentration ranging from individuals with abnormal wall thickness >5mm (190.6±15.6 pg/ml) to individuals with masses

(630.6±15.6 pg/ml). Urinary tract pathology occurred in all participants who had heavy infections. The TNF of the participants with class 1 abnormal wall thickness (190.6± 15.6 pg/ml) were significantly lower than that of those with class 2 (290.6 ± 40.1 pg/ml) at (χ^2 = 20.8, p < 0.05). The difference between the TNF level of the volunteers with class 1 irregular bladder wall (241.2 ± 72.1) was significantly lower than that of those with class 2 irregular bladder wall (339.9 ± 72.1 pg/ml) at (χ^2 = 16.8, p < 0.05). The participants with category 2 calcification had a mean TNF level of 460.2 ± 35.1 pg/ml while those with class 1 calcification had a lower TNF concentration (230.2 ± 18.1 pg/ml). This difference was statistically significant (χ^2 = 26.6, p < 0.05).

DISCUSSION

We reported significantly elevated levels of serum TNF in association with the intensity of *S. haematobium* infection. Our findings support the documentation of King *et al*, that *S. haematobium* infection induces the production of TNF [21] and so implicates this cytokine in the disease pathogenicity. The relevance of TNF in the disease process is further supported by the enhancement of eosinophil toxicity by TNF elicited by *Schistosoma* larvae [22]. Moreover, eosinophils have been shown to express major histocompatibility class II and CD4 molecules and act as antigen presenting cells *in vitro* [23 - 25]. The net effect is that increased eosinophil production functions in parasite clearance. Although the relationship between eosinophil and TNF was not documented in the present study, this may be consistent with our observation of increased TNF level with intensity of infection.

The present investigation showed a significantly higher serum TNF level in the <16 years age group than >16 years age group. Our finding supports the report of Mwatha *et al.*, in which high a morbidity of schistosome infection was seen in children while a low morbidity was observed in adults [17]. Children with high morbidity of infection produced higher level of TNF than adults. This finding is compatible with the hypothesis that severe morbidity is associated with an over-exuberant Th1 response which may be modulated in disease-free individuals by a Th2 response [17].

We reported the association of a relatively high serum TNF level with urinary tract pathology. This observation corroborates the finding of King *et al.,* and so implicates TNF as an important factor in bladder pathology associated with *S. haematobium* infection [21]. The urinary tract pathology observed in this investigation, such as pseudopolyps, calcification and urinary masses has been implicated as a probable part of the stages of disease culminating in bladder cancer [1, 26, 27], and elevated levels of TNF have been observed in schistosomiasis patients with carcinoma of the urinary bladder [13]. It has been documented that TNF response to *S. haematobium* infection is likely to develop into an exaggerated granulomatous response to ova trapped in the bladder wall associated with urinary tract pathology [24]. Pathological changes in schistosome infestations are caused by the position of the eggs in various tissues where granulomas or pseudo tubercles form around them [28]. Granuloma formation may be the result of delayed type hypersensitivity reactions mediated through a T cell mediated immune response to soluble egg antigen [10, 12, 20].

In conclusion, the bladder and kidney pathology as well as the intensity of infection revealed in this investigation correlated with the levels of TNF among *S. haematobium* infected participants in Ihieve-Ogben, Nigeria. Also, we observed an association between high level productions of TNF and both heavy infection and urinary tract pathology.

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