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PREVALENCE OF NITRATE AND NITRITE IN URINE OF *SCHISTOSOMA HAEMATOBIUM* INFECTED SUBJECTS IN ADIM, CROSS RIVER STATE

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ABSTRACT

Urinary nitrite and nitrate concentrations were estimated in forty subjects infected with *Schistosoma haematobium* from Adim Village, Biase Local Government Area in Cross River State and compared with age-matched forty uninfected normal subjects from Calabar Municipality using colorimetric methods. The mean urinary nitrate concentration of schistosoma-infected subjects was found to be 20.46 ± 6.88 $\mu gNO_3/ml$, while that of normal subjects was 24.40± 8.05 $\mu gNO_3/ml$ ($p<0.05$). On the other hand, the mean urinary nitrite concentration of schistosoma-infected subjects was found to be 0.84 ± 0.58 $\mu gNO_2/ml$, and that for the normal subjects contained no detectable nitrite in their urine ($p<0.05$). It is suggestive that the activity of nitrate reductase bacteria inhabiting the bladder of schistosoma-infected subjects might be responsible for the nitrite content in the urine of these subjects.

KEYWORDS: nitrate, nitrite, urine, *Schistosoma haematobium*

INTRODUCTION

Schistosomiasis is an helminthic infection, which is endemic in Nigeria and has been found prevalent in the middle belt and south eastern regions of Nigeria (Mandony, 1997). *Schistosoma haematobium* infection causes severe damage to the bladder and other underlying tissues of the urinary tract (Ezigbo, 1990). The infection is characterized by haematuria, dysuria, granulomas with fibrosis and calcification forms in the bladder mucosa and may be present as papillomas capable of simulating neoplasia (Grist et al, 1993). Schistosomiasis infection occurs through drinking, bathing, washing and agricultural use of water infected with eggs of *Schistosoma haematobium* (Cheesbrough, 1992).

Nitrates and nitrites are common inorganic pollutant of water. Exposure to these substances may be from septic tanks, barnyards, sewage treatment plants and heavily fertilized crops (Lansing, 1993). Nitrates and nitrites are widely distributed in consumable food items especially traditionally preserved fish and meat; where these compounds are deliberately employed as preservatives (Walker, 1990). Exogenous exposure to these compounds includes inhalation of atmospheric nitrogen oxides and tobacco smoke. Nitrate on its own is not toxic but its reduction to nitrite in vivo has brought it under increasing scrutiny. Bacteria inhabiting the stomach and bladder posses nitrate reducing abilities. Microbial reduction *in vivo* is determined by microbial count which is high in the mouth and stomach and also in infections of the bladder, urinary tract and vagina (Walker, 1990).

The most important toxic property of nitrite is its ability to combine with certain amino groups to yield mutagenic and carcinogenic N-nitroso compounds (Bartsch and Montessano, 1984). Epidemiological studies have indicated correlation of gastric cancer with nitrate ingestion (Carrosa et al, 2001). In *Schistosoma haematobium* infection, secondary bacterial colonization of bladder is one of the complications of the disease. These microorganisms convert nitrate present in urine to nitrite. The nitrite formed reacts with amines to produce toxic and carcinogenic N-nitroso compounds (Leach, 1988).

The aim of this study therefore is to experimentally estimate nitrate and nitrite levels in urine of *Schistosoma haematobium* infected subjects in Cross River State and in particular those in the following local government areas –Biase, Ugep, Ogoja and Bekwarra respectively which have not been previously reported and compare it with normal (uninfected) subjects.

SUBJECTS AND METHODS

Test subjects were forty primary school pupils below the age of thirteen from Adim village, Biase Local Government Area in Cross River State, with obvious haematuria due to schistosomiasis. The subjects were made to exercise (run a distance of 50m) before urine samples were collected. Primary school pupils of same age bracket from Calabar Municipality were used as control subjects.

Five millimeter of urine were collected into sterile universal specimen containers with 1ml of 5N NaOH serving as preservative against microbial reduction of nitrate to nitrite. Samples were stored at refrigerator temperature of 4°C prior to analysis for nitrate and nitrite.

Urine samples collected without preservatives were used for *Schistosoma haematobium* ova identification and count. Examination was carried out using filtration technique. 10ml of urine was aseptically transferred into a labeled conical tube and centrifuged for 5min. The supernatant was decanted and the sediment remixed by tapping the bottom of the tube. A drop of well-mixed urine was transferred to a slide and covered with a cover glass. The preparation was microscopically examined using the 10X and 40X objective (Cheesbrough, 1992).

Nitrite was estimated using the Montgomery and Dymock (1961) method. The principle of the reaction is that nitrite is diazotized with sulphanillic which forms a pink coloured complex with 1-(Napthylethylenediamine) dihydrochloride and is measured colorimetrically at an absorbance of 550nm.

Nitrate was estimated using the Follet and Ratcliff method (1963). The principle of the reaction is that nitrate is reduced to

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nitrite by a cadmium reduction column and the eluate analyzed for nitrite content. Nitrate concentration is deduced from a standard recovery experiment.

RESULTS

Table 1 shows the values of both nitrates and nitrites for the infected subjects compared to the non-infected subjects. The urinary nitrate concentration is significantly higher ($p < 0.05$) in normal pupils than in *Schistosoma haematobuim* infected subjects. The nitrite values obtained for the infected subjects urine samples was also significantly higher ($p < 0.05$) compared with the non-detectable nitrite values for the uninfected subjects.

TABLE 1: COMPARATIVE VALUES OF NITRATES AND NITRITE IN URINE OF NORMAL AND *SCHISTOSOMA HAEMATOBIUM* INFECTED SUBJECTS

Subjects	n	Concentration in µg/ml	
		Nitrate (NO_3)	Nitrite (NO_2)
Infected	40	20.46 ± 6.88	0.84 ± 0.58
Normal	40	24.40 ± 8.05	Not detected
Significance level		$p < 0.05$	$p < 0.05$

DISCUSSION

The result obtained from this study shows the presence of nitrate and nitrite in urine of subjects suffering from schistosomiasis in Adim village,Biase Local Government Area of Cross River State. The significant amount of nitrite detected in the urine samples of the *Schistosoma haematobium* infected subjects is consistent with the findings of some earlier researchers (Hicks et al, 1977; El-Merzabani and El-Hasser, 1979; Leach, 1988). The long term exposure of these pupils to nitrate and nitrite *in vivo* is a threat to their well-being as more severe and complicated infection may likely develop. Nitrate and nitrite have been found to be mutagenic and can react with amines *in vivo* to form carcinogenic N-nitroso compounds, this reaction has been found to occur in all animal species so far tested (International Agency for Research on Cancer, 1978, International Agency for Research on Cancer, 1985).

Nitrite is an abnormal constituent of urine and is present in urine in cases of urinary tract infection such as *Schistosoma haematobium* infection as shown from the result of the study of Hicks et al (1977). The nitrite found in urine is attributed to the activity of bacteria inhabiting the bladder and urinary tract (Leach, 1988). This relationship has been found to be consistent with the formation of nitrite in the bladder as a result of secondary bacteria infection subsequent to infection with schistosomiasis (Wrunnzel, 1998).

A greater percentage of cases of bladder carcinoma in East Africa and the Middle-east is attributed to chronic urinary tract disease caused by *Schistosoma haematobium* infection (Warren et al, 1995). Endogenous nitrosation of aliphatic, cyclic and heterocyclic secondary amines is the source of exposure to carcinogenic nitrosamines (Tricker and Pfundstein, 1992).

Nitrate alone has been implicated in the epidemiology of stomach cancer in Chile, Colombia and Japan (Harman, 1983). Nitrate and nitrite detected in saliva and urine in liver fluke infection has been found to be synonymous with cholangiocarcinoma prevalence in Northeast Thailand (Srianujata et al, 1984).

In conclusion, nitrite was not detected in the urine of the normal (uninfected) pupils, however, the mean urinary nitrate level of the uninfected pupils was higher than that of the *Schistosoma haematobium* infected pupils. The presence of nitrite in the urine of the infected subjects may be suggestive of bacterial reduction of nitrate, which may also be responsible for the lower mean urinary nitrate levels of the infected subjects when compared to the uninfected subjects.

Clinically, the gradual reduction of body nitrate to nitrite in the bladder of these pupils might predispose them to bladder carcinoma due to increased formation of carcinogenic N-nitroso compounds.

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