BRIEF COMMUNICATION

MICROSCOPIC HAEMATURIA AS AN OCCULT FILARIAL INFECTION IN BHUBANESHWAR AN ENDEMIC AREA FOR BANCROFTIAN FILARIASIS

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RUNNING TITLE : Microscopic haematuria- an occult filarial infection

ABSTRACT

Sera samples of 7 microscopic haematuria cases collected before and after treatment with Diethylcarbamazine citrate, (DEC) , 9 microfilaraemic cases and 19 endemic normal individuals were analysed for filarial antigen and IgG antibody levels. Filarial antigen was detected in 5 of the 7 microscopic haematuria cases, of which 3 turned negative for antigen after treatment with DEC. While none of the 7 haematuria cases were positive for filarial IgG antibodies, before the DEC treatment, all of them turned positive after DEC treatment. The sensitivity and specificity values(to detect mf +ve cases) were 89% and 90% respectively for the detection of filarial antigen and 78% and 95% respectively for the detection of filarial IgG antibodies.

KEY WORDS

Filariasis, *Wuchereria bancrofti*, *Brugia malayi*, microscopic haematuria.

INTRODUCTION

Among wide spectrum of clinical manifestations in humans infected with *Wuchereria bancrofti* or *Brugia malayi* occult infections represent an important category. These include different clinical conditions prevalent in filarial endemic areas such as tropical pulmonary eosinophilia (TPE), monoarthritis, endomyocardial fibrosis etc. (1,2,3) resulting from the pathology mediated by filarial parasites or host's immune response against infection. The symptoms being atypical and with no detectable microfilariae (mf) in peripheral circulation, it is difficult to precisely diagnose and manage such cases. There are studies reporting the presence of filarial antibodies and / or antigen in different occult infections confirming filarial aetiology (4,5,6). Haematuria

(usually microscopic) is one of the clinical entities known to occur in microfilaraemic cases (3). The present study is conducted to determine the association of haematuria bancroftian filariasis by screening microscopic haematuria cases before and after treatment with Diethylcarbamazine citrate (DEC) for filarial antibody and antigen.

MATERIALS AND METHODS

Sera samples of microfilaraemic cases and 7 confirmed microscopic haematuria patients, attending the pathology clinic of Regional Medical Research Centre (ICMR) Bhubaneswar, a coastal endemic region for bancroftian filariasis in Orissa were kindly gifted by Dr. G. P. Chhotray. Blood samples from the above mentioned 7 patients were collected before and after 30 days treatment with DEC (6 mg/kg body weight/ day for 12 days).

Blood samples were also collected from endemic normals (asymptomatic and amicrofilaraemic individuals) and microfilaraemic cases in and around Bhubaneswar. Presence of microfilariae was confirmed by night blood smear examination. Sera

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were separated and stored with 0.1% sodium azide as preservative at -20°C until used.

Brugia malayi microfilarial excretory-secretory (Bm mf ES) antigen was obtained by *in-vitro* culture of microfilariae (mf) in RPMI 1640 medium (15,000 mf/ml) at 37°C for 48 hrs as described earlier (7). The supernatant was collected, concentrated by ultramembrane filtration, dialyzed against 0.1M sodium phosphate buffer (SPB), pH 7.2 and the antigen (Bm mf ES) was stored with protease inhibitors at -20°C until use (7).

Ig Fraction of filarial serum immunoglobulins (FSIg) were separated from pooled clinical sera by 33% ammonium sulphate precipitation and the IgG fraction (FSIgG) was isolated from it by DEAE cellulose (Whatman DE 52) ion-exchange chromatography (8).

Presence of filarial IgG antibodies in sera samples were detected by dipstick Indirect penicillinase ELISA (7) using Bm mf ES antigen coated cellulose acetate membrane (CAM) sticks (100pg/5 μl /stick), serially diluted sera, optimally diluted antihuman IgG penicillinase conjugate (1:1000) and starch-iodine-penicillin 'v' substrate. The sera showing complete decolorization of blue color of substrate at least 3 minutes earlier than the referral pooled negative control (endemic normal sera) sample are considered positive.

Inhibition stick ELISA for detection of circulating filarial antigen in samples was carried out as described earlier (9) with few modifications. The CAM sticks were coated with 5 μl of optimally diluted FSIgG (20 μg /ml) and then blocked with 3% gelatin. The sticks were incubated with 0.5 ml of serially diluted test sera at 37°C for 2 hrs. followed by incubation with optimally diluted Bm mf ES Ag-penicillinase conjugate (1:1000 in PBS/T) at 37°C for 2 hrs. After a final wash with PBS/T, the sticks were incubated with 0.5 ml of starch-iodine-penicillin 'V' substrate. The sera showing persistence of blue colour about 3 min. after the decolorization in negative control serum are considered to be positive.

Statistical analysis was done by Mann Whitney 'U' Test.

RESULTS AND DISCUSSION

Of the 9 samples of microfilaraemic cases, 7 were positive for filarial antibody and 8 were positive for

filarial antigen. Only 1 and 2 of the 19 endemic normal samples of the same region were positive for filarial antibodies and antigen respectively. These results are comparable with the sensitivity and specificity values of filarial antibody assay and antigen assay, reported earlier from our laboratory (7,10).

In the present study none of the 7 microscopic haematuria cases showed microfilariae in peripheral circulation when tested by night blood smear examination using 20 μl samples. While as many as 5 samples of these cases collected before initiation of DEC were positive for circulating filarial antigen, only two samples showed filarial IgG antibodies (Table). In earlier studies from our laboratory (9) we had shown that filarial antigen assay is more sensitive than antibody assay for detection of occult infection like TPE. Dreyer *et al* (11) studied renal abnormalities in 20 microfilaraemic patients and reported that while half of them were positive for haematuria / proteinuria before DEC treatment, all of them showed their abnormalities after treatment with DEC.

Three of the haematuria cases turned negative for filarial antigen one month after treatment of DEC. All the 7 samples collected after DEC treatment were positive for antibody and there was significant increase in the GMT of antibody ($p < 0.03$) reflecting the elevated host's immune response against the released parasite components followed by treatment.

No haematuria was observed in any of these cases one month after DEC treatment. Filarial patients with clinical manifestations including those with haematuria are reported to respond well to treatment with DEC (3). However for the fact that all of them continued to be positive for filarial antibody and two cases positive for filarial antigen one month after DEC treatment suggests the need for their long term follow up if necessary with repeated DEC treatment. Earlier reports from our laboratory and other workers showed the necessity of such long term follow up in the management of clinical filariasis (10,12).

From the present study it can be envisaged that in filarial endemic areas the microscopic haematuria patients should be tested for filarial infection so that the positive cases can be effectively managed by treatment with antifilarial drug DEC.

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Table : Results of screening of sera of Microscopic Haematuria cases for filarial antibody and antigen before and after treatment with DEC.

Microscopic haematuria case No.	Before DEC Treatment		After DEC Treatment	
	Filarial antibody positivity* (Titre)	Filarial antigen positivity* (Titre)	Filarial antibody positivity* (Titre)	Filarial antigen positivity* (Titre)
1	- (150)	+ (600)	+ (600)	- (150)
2	- (150)	- (75)	+ (600)	- (75)
3	- (75)	+ (300)	+ (300)	- (75)
4	+ (1200)	+ (1200)	+ (2400)	+ (600)
5	- (75)	- (75)	+ (300)	- (75)
6	+ (600)	+ (600)	+ (1200)	+ (300)
7	- (75)	+ (300)	+ (300)	- (75)
Total no. positive	2	5	7	2
GMT	182.8 ^a	299.9 ^b	599.9 ^c	135.85 ^d

* Serum samples with filarial IgG antibody/antigen titre of $\geq 1:300$ are considered as positive. GMT = Geometrical mean titre of filarial IgG antibody or filarial antigen.

(a vs c $p < 0.02$; b vs d $p < 0.1$ by Mann Whitney U Test)

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