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Antigen-Specific Immune Complexes in Urine of Patients With Lymphatic Filariasis

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Lymphatic filarial subjects with disease manifestations exhibit significantly elevated levels of immune complexes (ICs) in their circulation. The objective of the study was to explore the possible excretion of filaria-specific soluble ICs in urine of subjects with lymphatic filariasis. Paired urine (overnight) and serum samples were analyzed for complement activating filarial antigen containing immune complexes by enzyme-linked immunosorbent assay (ELISA). Antigen-specific ICs were detected in urine samples of 34% of subjects with filarial disease manifestations while the frequency of occurrence was low in microfilaremic subjects. The titer of urine ICs is significantly high in subjects with chronic filariasis as compared to microfilaria (mf) carriers. The occurrence of filaria-specific ICs in urine and their passage through the filtering structures of the kidney is suggestive of the focal or diffuse damage in those subjects. Detection of ICs in urine may provide a noninvasive means of assessing the extent of renal damage in patients with lymphatic filariasis. J. Clin. Lab. Anal. 21:46–48, 2007.

Key words: lymphatic filariasis; renal damage; complement; immune complexes; ELISA

INTRODUCTION

Lymphatic filariasis caused by *Wuchereria bancrofti* is prevalent in the tropical world, affecting about 250 million people. The infection is characterized by long-term persistence of parasites in the infected host, providing continuous antigenic stimulus to the host's immune system. The presence of filarial antigens and antigen-specific antibodies in the circulation of microfilaria (mf) carriers as well as chronic filarial subjects with clinical manifestations is well documented (1–3). The antibodies generated against various soluble products of mf and/or adult filarial parasites form immune complexes (ICs) with specific antigens (4), facilitating opsonization for phagocytosis. However, under certain circumstances, the ICs may get accumulated in circulation and deposited in various tissues. The most vulnerable sites include the choroid plexus and glomerulus. Renal damage is apparent in a few, if not in all, subjects with chronic lymphatic filariasis (5–7). Kidney biopsies from filarial patients with glomerulonephritis revealed mesangial deposits of immunoglobulins and complement components (8–10), which strongly advocates involvement of ICs in renal damage seen in filarial subjects. However, involvement of filarial antigen-specific ICs in the renal damage is unknown. The objective of the present study was to explore the presence of antigen-specific, complement activating ICs in urine of subjects with lymphatic filariasis in an attempt to predict their possible role in renal damage.

MATERIALS AND METHODS

Night Blood Examination

Subjects living in two rural filaria-endemic pockets of Central India were screened for microfilaremia by night blood smear (20 μL) examination between 20:00 and 22:00 hours. A total of 20 mf carriers were selected for the study after taking oral consent.

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Clinical Examination

All the subjects were examined for filarial manifestations such as hydrocele, elephantiasis, lymphedema, lymphangitis, etc. A total of 41 subjects with elephantiasis who agreed to give urine and serum samples were included in the study.

Controls

A total of 20 healthy subjects in the 25–60-year-old age group from the same region without any history of filariasis volunteered as control group.

Collection of Urine Samples

The morning urine samples (middle stream) were collected from all the subjects participating in the study in 1 liter plastic bottles, transported to the laboratory within 1 hr of collection, and preserved at –20°C until use.

A total of 81 urine specimens belonging to filarial and healthy subjects were screened for filaria-specific ICs by anti-C3 enzyme-linked immunosorbent assay (ELISA) in this study. Paired serum and urine samples were collected from five subjects in each group; serum was separated immediately from cells and stored frozen at –20°C with sodium azide (0.01%) as preservative.

Isolation of Filarial Immunoglobulin G (IgG) From Clinical Filarial Subjects

Isolation of immunoglobulin G (FSIgG), from pooled serum of patients with elephantiasis having rich amount of filarial IgG as tested by counter immunoelectrophoresis was carried out by salt precipitation and ion-exchange chromatography, as described elsewhere (4).

Conjugation of FSIgG with penicillinase (Sp.acty. 2,100/mg protein; Sigma, St. Louis, MO, USA) was done by the method of Avrameas (11). Starch-iodine-penicillin substrate for penicillinase consisted of 150 mg of soluble starch in 27.5 mL of sodium phosphate buffer 0.2 M, pH 7.0 containing 10.64 mg of penicillin-V, and 65 μL of 0.08 M iodine in 3.2 M KI solution. The substrate was prepared fresh before use every time.

Screening of Samples for Complement-Bound Antigen-Specific ICs

Anti-C3 ELISA, as described elsewhere (4), was employed to detect antigen-specific ICs in sera and/or neat urine samples. In brief, the wells in polyvinyl chloride (PVC) microtiter plates (Dynatech, Singapore) were coated with 100 μL of optimally diluted anti-C3 (1 μg/mL) in 0.06 M carbonate buffer, pH 9.6 by incubation at 37°C for 3 hr. Blocking was done with 1% bovine serum albumin in coating buffer. Following washing with phosphate buffered saline containing Tween 20 (PBS/T), the wells were incubated with serial dilutions of neat urine samples and/or 1:100 diluted sera samples (100 μL). The final incubation was with FSIgG-penicillinase conjugate (1:1,000) for 3 hr at 37°C or overnight at 4°C. The substrate used was freshly made starch-iodine-substrate. Complete decolorization of the substrate as visualized by naked eye was taken as positive reaction.

The data was analyzed using Costat Software.

RESULTS AND DISCUSSION

Infection with lymphatic filarial parasites not only affects the lymphatic system but also extra lymphatic tissue (12). ICs are formed more frequently and in greater amounts in chronic infections like lymphatic filariasis (4) and play an important role in modulating host immune responses. Formation of ICs involving antigens released from the living or dying parasites and specific antibodies of the host is a regular phenomenon that helps in neutralization and ultimate elimination of parasite antigens from the circulation. However, under certain circumstances, probably due to overloading of host's reticuloendothelial system or blockade of Fc and/or complement receptors on cells of reticuloendothelial system, ICs may get localized in various tissues and may inflict damage to the surrounding tissue by eliciting type-III hypersensitivity reactions.

Presence of C3-bound filarial-specific ICs in urine specimens of 14 out of 41 subjects with clinical manifestations, four out of 20 microfilaremic subjects, is evident from this study. (Table 1). The highest titers of urine ICs recorded in microfilaremic and clinical filariasis groups were 32 (Table 2) and 256 (Table 3), respectively. The load of filarial-specific ICs is found more in chronic filarial subjects as compared to that of microfilaria carriers in both serum as well as urine specimens. However, the serum titers of soluble ICs appeared to bear no relationship with their excretion pattern in urine samples, suggesting variation in the degree of damage mediated by antigen-specific ICs in different filarial patients. Also, there was no relation of

TABLE 1. Detection of filarial antigen-specific ICs in urine specimens by anti-C3 ELISA

Sera	Number of examination	Number positive for IC	Percentage positivity
Clinical filariasis	41	14	34
Microfilaremia	20	4	20
Controls	20	0	0

TABLE 2. Analysis of paired urine and serum samples of microfilaremic subjects for filarial immune complexes by anti-C3 ELISA

Microfilaremic group	IC titer[a] in urine	IC titer[a] in serum	mf count[b] per 20 microlitres of blood[a]
Subject 1	2	600	12
Subject 2	4	300	03
Subject 3	8	300	10
Subject 4	32	1,200	9

[a]Reciprocal of the highest dilution of serum/urine positive for ICs.
[b]As detected by night wet blood smear examination of peripheral and venous blood.

TABLE 3. Analysis of paired urine and serum samples of clinical filarial subjects for filarial immune complexes by anti-C3 ELISA

Clinical filariasis group	IC titer in urine	IC titer in serum
Subject 1	16	4,800
Subject 2	16	19,200
Subject 3	32	4,800
Subject 4	128	19,200
Subject 5	256	9,600

urine IC titers with mf load in blood or duration and/or severity of clinical manifestations.

The assay employed for detection for immune complexes in this study was free from all limitations associated with many IC detection assays. The assay detects only complement-activating filaria-specific ICs. The occurrence of ICs containing the third component of complement (C3) was measured previously (13) in the urine of 98 patients with a variety of renal diseases. Urinary C3 was detected in cases of membranous glomerulonephritis, mesangiocapillary glomerulonephritis, rapidly progressive glomerulonephritis, and renal amyloidosis. Concentrations of C3 were low or undetectable in cases of nonimmunological renal diseases. The authors did find a good correlation between urinary C3 concentrations and the deposition of C3 in glomerular capillary walls. Urinary C3 excretion appears to be an accurate indicator of continuing activity of disease. The findings of the present study coupled with observations of other workers on renal biopsies are indicative of IC-mediated renal damage in lymphatic filariasis. The occurrence of filaria-specific ICs in urine samples is being reported for the first time and the passage of ICs through filtering structures of the kidney is suggestive of the extent of kidney damage inflicted by tissue-localized filaria-specific ICs. The occurrence and level of antigen-specific ICs may serve as noninvasive markers in assessing the extent of kidney damage in filarial subjects. Thus, this study provides new insight in understanding the pathogenesis of this age-old disease.

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