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Filarial selenium glutathione peroxidase: a probable immunodiagnostic marker for lymphatic filariasis

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ABSTRACT

Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is widely prevalent in tropical and subtropical countries. Night blood film examination is most commonly used for diagnosis of filariasis but is cumbersome and labour intensive. In order to develop an indirect ELISA-based immunodiagnostic test, the importance of antifilarial IgG subclasses was evaluated in bancroftian filariasis patients. Blood samples from healthy individuals and different categories of LF patients were used to estimate the diagnostic potential of selenium glutathione peroxidase antigen purified from the bovine filarial parasite *Setaria cervi*. This antigen reacted with both IgG_1 and IgG_4; however, the IgG1 response was greater in microfilaraemic patients and the IgG_4 response was higher in chronic filarial patients. The diagnostic sensitivity of IgG_1 and IgG_4 was 97% and 96% whereas specificity was determined to be 95% and 98% respectively. Our observations suggest that SeGSHPx could be an alternative diagnostic marker for the detection of bancroftian filariasis in an endemic area.

1. Introduction

Lymphatic filariasis (LF) is a complex human infection with a wide spectrum of clinical manifestations. Worldwide, more than 1.2 billion people are at risk of filariasis, and 120 million are already known to be infected[1]. It has been estimated recently that there are more than 27 million microfilaraemic carriers, around 20.8 million cases of symptomatic filariasis and about 429 million individuals at risk of filarial infections in India.[2] The World Health Assembly has inaugurated the Global Program to Eliminate Lymphatic Filariasis, and India is one of its signatories.[3] Since 2006, a two-drug regimen using diethylcarbamazine (DEC) and albendazole has been adopted in India as a strategy to eliminate and eradicate LF; however, obtaining adequate supplies of both drugs is a major challenge for India.[4]

The decisions that affect the outcome of the mass drug administration (MDA) programme depend upon monitoring its effects, and defining target populations and endpoints for termination of MDA. Consequently there is a need for a reliable and accurate filarial diagnostic test.[5] Until recently, diagnosis of filarial infection has depended upon night blood film examination to detect circulating microfilariae in the blood of infected individuals. This test has major drawbacks, as it is neither convenient nor reliable.

Active filarial infections are characterized by elevated levels of antifilarial IgG_4, which decline to normal levels after treatment.[6] Within endemic populations different IgG subclasses have been used for filarial diagnosis. An elevated level of filarial specific IgG_4 in urine has also been used as a diagnostic tool for screening among young children

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infected with filariasis.[7] Currently, the immunochromatographic card test is used for filariasis detection, although it is facing challenges over its sensitivity, production and storage, as well as cost-effectiveness for field trials.[8]

Setaria cervi, a bovine filarial parasite, has a resemblance to *Wuchereria bancrofti* in its nocturnal periodicity and antigenic pattern and various biochemical,[9] MALDI mass sequencing[10] and immunoprophylactic studies[11] have unequivocally established its closeness to *W. bancrofti*. Previously we reported that different antigens of *S. cervi* cross-react with sera of human patients infected with filaria.[12,13] We observed that selenium glutathione peroxidase (SeGSHPx) was present in different life stages of *S. cervi*, including its excretory-secretory products.[14] Since the antigen is present in all stages of the worm and is cross-reactive to *W. bancrofti*, SeGSHPx appears to be a very good candidate for use as a diagnostic antigen for detection of bancroftian filariasis in an endemic area (S. Rathaur, unpublished data). In this study, we report on SeGSHPx reactivity with sera from humans infected with filaria and from healthy controls in an indirect ELISA. The efficacy of this test for the detection of an antifilarial IgG subclass specific for the filarial parasite was also evaluated.

2. Materials and methods

2.1. Study site and population

The study was carried out in Narottampur, a suburban area of Varanasi district during July 2006 and October 2006. Inhabitants of this area vary in their socioeconomic status, from very low to lower-middle class. Participants were selected without any bias for age, sex and caste, until nearly 20% of the total population of this area had been investigated. For each individual age, sex, duration within the community, and any previous treatment with DEC were recorded. Oral informed consent was obtained from all the participants. None of them had received any antifilarial drugs in any form during the previous 5 years.

2.2. Parasitological examination and collection of different categories of sera

Finger-prick samples of night blood were taken from all those participants who had been examined clinically for LF. Thick smears of night blood were prepared from finger-prick samples and stained with Leishman's stain for identification of microfilariae to species. Microfilariae were identified based on morphological features and size. Peripheral blood samples were collected using sterile needles and syringes after cleaning the volar surface of the arm with cotton wool moistened with methylated spirit, between 21:00 and 01:00 h. A 100-μl blood sample was drawn into a heparinized capillary tube and immediately transferred to 900 ml of 3% acetic acid. The microfilariae in the specimen were quantified later in laboratory, using the counting-chamber technique[15] and microfilaraemia was expressed as mf/ml. On the basis of examination of night blood films and clinical examination, cases were divided into three groups: (i) microfilaraemic, (ii) asymptomatic microfilaraemic and (iii) symptomatic amicrofilaraemic. The average (mean ± SD) ages in these groups were 33 ± 4 years, 35 ± 3 years and 38 ± 4 years respectively. The ratio of males to females in all groups was approximately 1:1. Twenty-five healthy individuals without any evidence of past filarial infection from the endemic area were selected as endemic normal. The average age of the endemic normal group was 32 ± 4 years.

Sera from 15 normal individuals from a non-filariasis endemic area were used as controls to establish the cut-off for the indirect ELISA (see below). For cross-reactivity studies, well-defined ascariasis and hookworm sera obtained from patients living outside the endemic area were used. These sera were generously provided by Prof. F.M. Tripathi, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

2.3. Parasite collection and SeGSHPx purification from adult female Setaria cervi

Setaria cervi female worms were collected from a local abattoir and somatic extract was prepared as described previously.[14] SeGSHPx was purified to homogeneity using a combination of glutathione agarose affinity and Sephadex G-200 column chromatography.[14]

2.4. Indirect ELISA for filarial specific IgG subclass antibodies against SeGSHPx antigen

ELISA plates were coated with 2 μg/ml of purified SeGSHPx and incubated overnight at 4 °C. Antigen-coated plates were incubated with sera at 1:100 dilution from different categories of participants: (i) normal, (ii) endemic normal, (iii) symptomatic amicrofilaremic, (iv) asymptomatic microfilaremic, (v) ascariasis and (vi) hookworm sera. Plates were washed and anti-human IgG_1-biotin or IgG_4-biotin (Sigma-Aldrich, Bangalore, Karnataka, India) at 1:1000 dilution was added. After completion of incubation, ELISA plates were washed three times with washing buffer. Finally colour was developed using streptavidin peroxidase (Sigma) (1:500) and orthophenylenediamine (Sigma) chromogen at 37 °C and optical density (OD) was taken at 595 nm in a BioRad ELISA reader (BioRad Laboratories, Hercules, CA, USA). The mean + 5 SD of the negative control was used as the cut-off value for further analysis of data.

2.5. Diagnostic evaluation

Sensitivity and specificity of assay were calculated as described by Galan and Gambino.[16] Individuals who had microfilariae in night blood films are referred to as true positives. Individuals who did not have microfilariae in night blood films are referred to as true negatives. False negative refers to those individuals who had microfilariae in night blood films but who failed to be identified as positive for microfilarial infection by ELISA. False positive refers to those individuals who were negative for microfilariae in night blood films but positive by ELISA test.