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Diagnostic potential of purified bovine filarial antigens

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Abstract

Lymphatic filariasis is a vector borne parasitic diseases causing an important public health problem, affecting a significant proportion of individuals in terms of disease affliction, economy and sociological status in India. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) launched in 1997 has covered over 570 million people in 48 countries with a motto of elimination of Lymphatic Filariasis as a public health problem by 2020. To monitor the elimination programme antigen detection tests using Og4C3 and immunochromatographic diagnostic test (ICT), developed in the recent past to detect the presence of infection are widely used. However, these tests may not be useful for assessment of transmission during post-MDA surveillance and hence there is a need for marker(s) that detect the exposure to filarial infection. In order to develop such marker(s), heterologous antigens from the filarial parasite *Setaria digitata* were explored by reacting with sera of individuals exposed to infection (antigen negative, antibody positive) of bancroftian filariasis. The results showed that one protein (~37 kDa) reacted with convalescent sera sample (antigen negative, antibody positive individuals) were isolated and characterized for possible application to be used in a diagnostic test for assessing the transmission of filarial infection during post-MDA surveillance.

Keywords: *Setaria digitata*, characterization, cross reacting antigen, isolation

Introduction

Lymphatic Filariasis (LF), caused by *Wuchereria bancrofti*, *Brugia timori*, and *Brugia malayi*, is a major vector-borne parasitic disease, commonly known as elephantiasis. It is transmitted by the mosquito vector *Culex quinquefasciatus*. Globally, 120 million people across 72 countries are affected by this disease, with over 51 million individuals infected as of 2018, of which 43 million suffer from irreversible chronic manifestations (WHO, 2024) ^[1]. LF causes significant physical and socio-economic problems for affected individuals (Durrheim *et al.*, 2004 ^[2]; Gbakima *et al.*, 2005 ^[3]; Sabesan *et al.*, 2006 ^[4]; Kreuels *et al.*, 2008 ^[5]; Streit and Lafontant, 2008^[6]). In India, approximately 48.11 million people were infected with LF, primarily residing in 18 endemic states and Union Territories as of 2011 (Upadhyayula *et al.*, 2012) ^[7].

The Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched in 2000, aims to eliminate the disease through the Mass Drug Administration (MDA) program in co-endemic regions and to alleviate suffering by managing the disease's impact (WHO, 2017) ^[8]. In 2018, over 886 million people in 49 countries were at risk of LF (Collyer *et al.*, 2020) ^[9]. The WHO reported a global decline of 74% in LF cases by 2018 due to active GPELF strategies. However, as stated in the GPELF progress report of 2019, India failed in 3 Implementation Units (IUs) during the Pre-transmission Assessment Survey (TAS), and 13 IUs failed in TAS while assessing the impact of MDA (Cromwell *et al.*, 2020) ^[10].

Accurate and early detection tools, impact assessment, and post-MDA surveillance are critical for the success of LF elimination efforts. Recently, highly sensitive and specific immunodiagnostic kits have been developed, which have proven effective for monitoring the MDA program. Advanced techniques are also available for LF detection, such as *W. bancrofti*-specific DNA probes (Hoti *et al.*, 2002) ^[11], molecular xenomonitoring protocols using mosquito vectors (Albuquerque *et al.*, 2020) ^[12], and polymerase chain reaction (PCR) assays using night blood samples from infected patients (McCarthy, 2000) ^[13].

However, night blood sampling remains problematic in endemic countries, including India, due to its high cost, labor-intensive nature, and the public's lack of compliance.

Two of the immunological test kits such as the ICT card test (Weil *et al.*, 1997; Kamkumo *et al.*, 2015) [14, 15] and Og4c3 ELISA kit (Hoti *et al.*, 2002 [11]; Wattal *et al.*, 2007 [16]; Mary *et al.* 2016 [17]) are available currently commercially for the diagnosis of filarial infection that can detect the presence of circulating filarial antigens. These tests detect active infection, but during the post-MDA situation, when the transmission is expected to be very low tests that can detect the exposure are warranted. The commercially available antigen detection assays for bancroftian filariasis, namely the antigen test kit based on monoclonal antibody Og4C3 (Trop-Ag *W. bancrofti*, JCU Tropical Biotechnology, Pvt. Ltd, Townsville, Queensland, Australia) and the ICT card test (ICT Diagnostics, Balgowlah, New South Wales, Australia) CTK-Biotech-ICT, Encode Filariasis IgG/IgM Combo Rapid Test, PanLF Rapid-ICT, are very costly and therefore cannot be used for routine work including epidemiological surveys and control programs in India. Obtaining adequate amount of *W. bancrofti* antigens from human filarial infection is difficult.

The cattle filarial parasite *Setaria digitata*, which resembles the human species in having microfilarial periodicity and chemotherapeutic response to known anti-filarial agents has been widely used as a model in various studies (Tandon *et al.*, 1988) [18] including the immunological studies on diagnosis of *W. bancrofti* infection. Immunological studies on human bancroftian filariasis revealed that the sera of infected patients from high endemic region were showed higher specific antibody response than the sera collected from low endemic infected individuals (Jaoko *et al.* 2006) [19]. *Setaria digitata*, a filarial nematode dwelling in the peritoneal cavity of cattle has immunological determinants of filarial infection. The worm is quite similar with the human filarial parasite, *W. bancrofti* by morphology (Madathiparambil *et al.*, 2011) [20], histology (Decruse and Kaleysaraj, 1990) [21] and genome sequence (Senanayake *et al.*, 2020) [22]. Hence it has been

used as a model system in present study. The present study was carried out to identify the cross reacting protein of bovine filarial worm, *S. digitata* that could be used as a diagnostic marker to detect human filariasis at the early stage of infection by evaluating against the known human filarial sera sample.

Materials and Methods

Sample collection

Adult *S. digitata* parasites were collected from the peritoneal cavity of cattle slaughtered at the Greater Chennai Corporation abattoir and transferred to 1X Phosphate Buffered Saline (PBS, pH 7.2). The worms were transported to the laboratory, washed three times with sterile PBS, and stored at -20°C until use.

Preparation and purification of somatic antigens

Adult *S. digitata* worms were homogenized in sterile 1X PBS (pH 7.2) containing 0.1% of TritonX-100. The homogenate was dialysed against sterile 1XPBS (pH 7.2) at 4°C for overnight. The soluble material was extracted by centrifugation at 10,000 rpm for 20 min and the supernatant was stored at -20°C.

Ion exchange chromatography using DEAE-Sepharose as an anion exchanger was performed for further purification. The DEAE-Sepharose column was equilibrated with 20 mM Tris-HCl buffer, pH 8.0. A 2.5 mL sample of *S. digitata* adult antigen extract was applied to the column at a flow rate of 0.5 mL/min. The proteins in the crude antigen were either eluted as a single fraction using 50 mM Tris-sodium chloride or fractionated with increasing concentrations of sodium chloride (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM). The column was washed with the same buffer at a flow rate of 1 mL/min, and 2 mL fractions were collected (Fig 1). Absorbance of the column fractions was measured at 280 nm, and fractions corresponding to the eluted peak were analyzed by SDS-PAGE. The protein concentration of various purified protein fractions was estimated using the Bicinchoninic Acid (BCA) method (Smith *et al.*, 1985) [23].

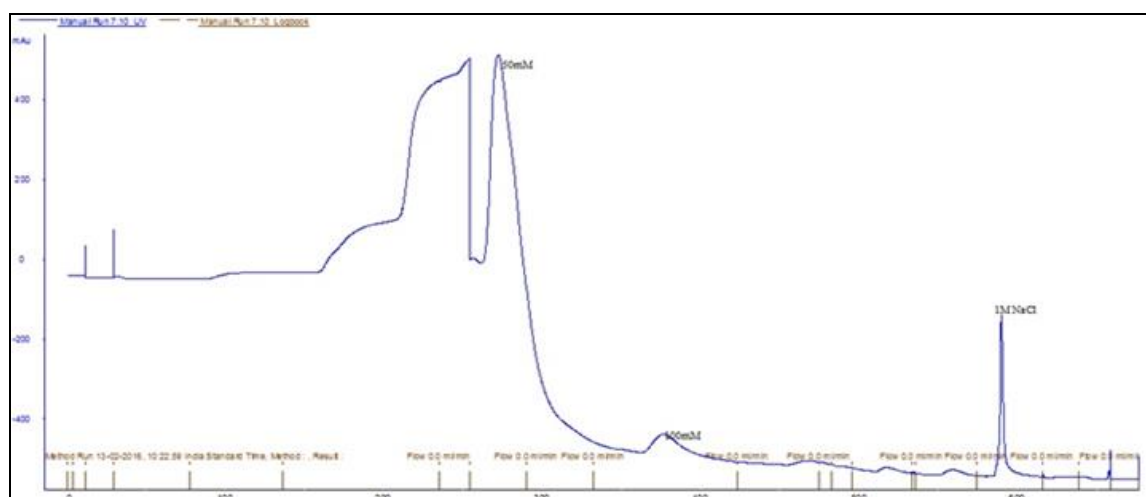


Fig 1: Chromatogram output of the purification using wash buffer containing 50mM Tris, 50mM salt concentration and 5 ml column volume

Characterization and immunoblotting of purified somatic antigen of *S. digitata*

The protein profile of purified somatic antigen of *S. digitata* was analyzed by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) using 12% (w/v) gel under reducing condition as described by Laemmli (1970) [24]. Each well was loaded with 40µl of purified antigen. Electrophoresis

was performed at 100V current in a Tris-glycine buffer system. The gel was stained with Coomassie brilliant blue R-250 (Sigma-Aldrich, USA).

The reactivity of filarial antigens with known positive sera was analysed by immunoblotting technique as described by Towbin *et al* (1979) [25]. In brief, the setarial antigen was resolved by SDS-PAGE. The antigen components were

electrophoretically transferred onto PVDF membrane with 0.6 amps current for 90 min at 4°C. After being blocked the PVDF membrane with 5% skimmed milk powder in 1X PBS, pH 7.2 for 1 hours at 37°C. The membrane was washed three times with PBST for 10 min and incubated further with a (1:50) dilution of convalescent sera of filaria exposed individual obtained from clinically suspected cases from JIPMER, Pondicherry for 1 hours at 37°C. The membrane was washed and incubated with a 1:8000 dilution of Recombinant Protein A\G Horse radish peroxidase-conjugate as secondary antibody and revealed with Diaminobenzidine (DAB) and 0.005% Hydrogen peroxide solution.

Results

Total protein from *Setaria digitata* was extracted using tissue homogenizer (Braun Biotech Homogenizer, Germany) followed by a freeze-thaw cycle. The protein sample was then run on a 12% SDS-PAGE gel, which revealed multiple bands of varying sizes. Four weak bands were observed in the range of 25 to 40 kDa, with one prominent band approximately at 37 kDa. Additionally, multiple sharp protein bands appeared in the range of 45 to 65 kDa (Figure 2).

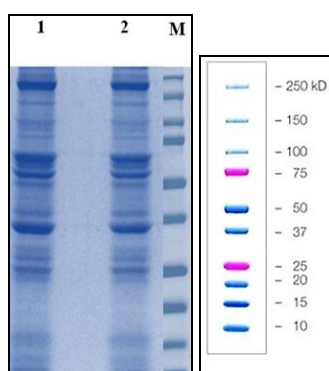


Fig 2: 12 per cent SDS-PAGE analysis showing protein profile of total crude antigen of *Setaria digitata*. Lane 1 and 2→ Total crude antigen of *Setaria digitata*, M→ Pre-stained protein marker

Western blotting using convalescent serum was done but the sera showed nonspecific binding (data not shown). The total protein concentration of crude lysate of *Setaria digitata* was quantified by using BCA method. The average total protein yield was approximately 2.2mg per worm (4.7mg/ml), (Figure 3).

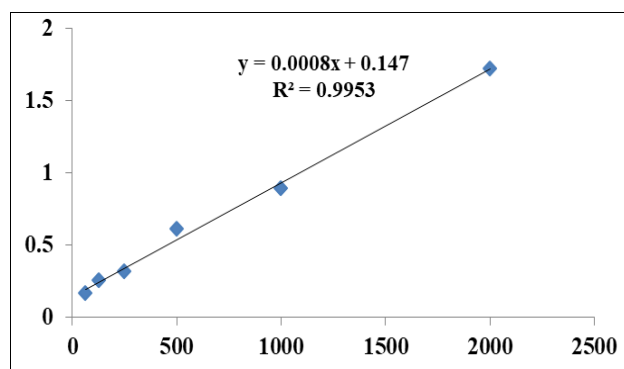


Fig 3: Estimation of protein concentration of purified crude protein fractions of *Setaria digitata* using BCA method

The 12% SDS-PAGE analysis of the purified somatic antigen of *S. digitata* at a salt concentration of 50 mM in fractions 6 and 7 revealed a prominent protein band in the range of 23-40 kDa, along with a single band in the 75-95 kDa range.

Additionally, four weaker protein bands were observed between 40-70 kDa (Figure 4a).

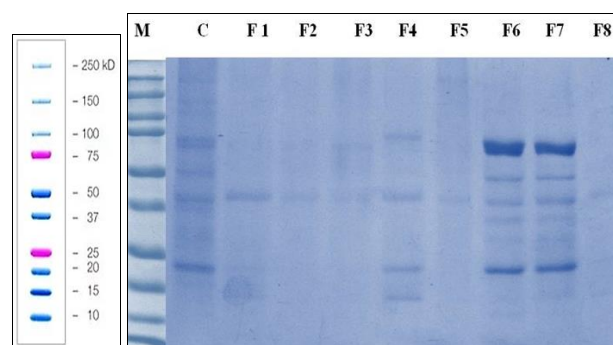


Fig 4a: 12 percent SDS-PAGE analysis of purified protein fractions of *Setaria digitata* somatic antigen at 50mM salt concentration

M→Marker, C→Crude antigen of *Setaria digitata*, F1-F8→Purified somatic antigen fractions of *S. digitata*

In contrast, the purified fractions at a 100 mM salt concentration showed two faint peptide bands in the 50-70 kDa range in the first four fractions (Figure 4b).

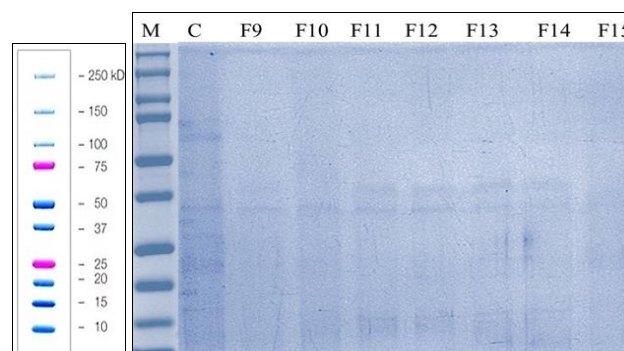


Fig 4b: 12 percent SDS-PAGE analysis of purified protein fractions of *Setaria digitata* somatic antigen at 50mM and 100 mM salt concentration

Immunoblot analysis of the purified somatic antigen from adult *S. digitata* (approximately 37 kDa) showed reactivity with convalescent sera samples collected from patients infected with filarial parasites (Figure 5a).

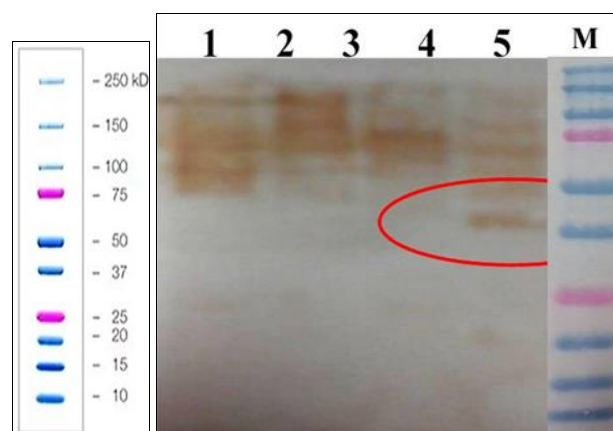


Fig 5a: Immunoblot analysis using positive anti filarial serum (from clinically infected patient) showing immunoreactivity at 37 kDa. Lane 1 to 4→Reactivity of purified somatic antigen of *S. digitata* with clinically suspected antifilarial sera; Lane 5→ Positive antifilarial sera (Lane 1-5: 300, 200, 100, and 50 mM salt concentrations, respectively); M→ Prestained protein marker

However, the sera collected from filaria negative individual showed no immunoreactivity against purified somatic antigen of *Setaria digitata* (Figure 5b).

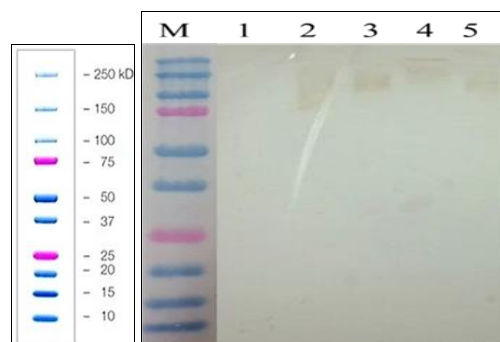


Fig 5b: Immunoblot analysis of purified somatic antigen of *Setaria digitata* showing no immunoreactivity against negative anti-filarial serum (from naive individual).

Lane 1 to 5→Reactivity of purified somatic antigen of *S. digitata* with negative antifilarial sera (Lane 1-5: 300, 200, 100, and 50 mM salt concentrations, respectively); M→Prestained protein marker.

Discussion

Recent advances in various fields related to lymphatic filariasis (LF) have led to the development of new approaches aimed at reducing the severity and impact of the disease (Ottesen *et al.*, 1997) [26]. The introduction of novel diagnostic tools for detecting *Wuchereria bancrofti* infection in humans has opened new possibilities for interrupting transmission and, consequently, eliminating filariasis (WHO, 1994) [27]; Crainey *et al.*, 2016 [28]; Sunish *et al.*, 2015 [29]. This goal was reaffirmed in 1997 when the World Health Organization (WHO) set the target of eliminating filariasis worldwide by 2020 (Ottesen, 2000) [30]. The global program for LF elimination has now reached a stage where diagnostic paradigms are shifting. Microscopic examination of the microfilarial stage of the parasite is no longer feasible in the post-MDA (Mass Drug Administration) scenario, necessitating the use of alternative tools to detect filarial-specific antibodies, which indicate exposure to infection. Currently, there are no commercially available point-of-care kits for the early diagnosis of filariasis. Therefore, there is a need to develop rapid diagnostic serological tools to detect specific antigens in the early stages of infection. One of the major challenges in this area is the lack of sufficient quantities of filarial parasites and their antigens.

This study aims to utilize heterogeneous antigens from the cattle parasite "*Setaria digitata*" as a diagnostic marker for human filariasis, to help prevent further disease transmission and enable early therapeutic intervention. Antigens were identified that reacted with the sera of individuals exposed to bancroftian filariasis. A 10% SDS-PAGE analysis of somatic antigens from *Setaria cervi*, purified using a preparative SDS-PAGE gel elution technique, revealed a prominent protein band at 37 kDa under reducing conditions, as visualized by silver staining (Priyadarshi *et al.*, 2017) [31]. Sephacryl S-200 column-purified somatic antigen fractions of adult *S. cervi* revealed a prominent protein band in the 35-40 kDa range, while DEAE-cellulose-purified antigens displayed two major and one minor protein bands in the 65-75 kDa range in 10% SDS-PAGE analysis (Kaushal *et al.*, 2009) [32]. Earlier studies on this parasite identified two adult worm antigens, 52 kDa and 130 kDa, which were used to detect *Wuchereria bancrofti*

microfilariae in endemic areas (Wickremanayake *et al.*, 2001) [33]. John and Raj (1998) [34] found that the heat-inactivated, detergent-soluble protein of *S. digitata*, with a molecular weight of 29 kDa, suppressed the host immune response and reported that this 29 kDa protein had diagnostic value in patients infected with bancroftian filariasis. Affinity-purified, *E. coli*-expressed recombinant protein of third-stage larvae of *Brugia malayi* (*rBm*-SL3) revealed a protein band at 37 kDa in 10% SDS-PAGE analysis. The *rBm*-SL3 protein showed a specific antibody response only with sera from individuals in endemic areas in immunoblot analysis (Dabir *et al.*, 2006) [35]. Surface antigens from *S. digitata* isolated by EDTA extraction and purified by affinity chromatography using *W. bancrofti* infected sera demonstrated approximately six antigenic bands (ranging from 10-89 kDa) in immunoblotting (Theodore and Kaliraj, 1990) [36]. Two monoclonal antibodies, namely 13B4 and 15D6, have been developed against antigenic epitopes that are common to both *S. cervi* and *B. malayi*. These antibodies have proven useful for detecting circulating antigens in filarial-infected individuals (Kaushal *et al.*, 1994) [37], but not for measuring exposure. Known positive human anti-filarial sera (IgG antibody) collected from endemic regions reacted with adult somatic antigens from *S. digitata* and *B. malayi*, but not with their microfilarial antigens (Satapathy and Sahoo, 2019) [38]. In contrast, the secretory glycoprotein (200 kDa) of *S. digitata* dissociated into 15 polypeptide bands in the 11-66 kDa range under reducing conditions in SDS-PAGE analysis, with all proteins reacting with the mouse monoclonal antibody MK6BE3 in immunoblotting (Madathiparambil *et al.*, 2009) [39].

Conclusion

This present study demonstrates that cross-reactivity of antigens derived from *S. digitata* with human sera from *W. bancrofti* infected individuals occurs, highlighting the presence of a potential sero-reactive protein at ~37 kDa. Further purification and proteomic analysis of this antigen may provide valuable insights into how human infection with filarial parasites affects the sensitivity of diagnostic tests for *W. bancrofti*. The promising nature of this antigen suggests its potential utility in measuring individual exposure to filarial infection and in assessing transmission dynamics during post-MDA surveillance, making it an important candidate for improving diagnostic and surveillance methods for filarial diseases.

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Conflict of Interest

Not available

Financial Support

Not available

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