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Research Article

SCREENING AND EVALUATION OF ANTIGENIC B-CELL EPITOPES FOR THE SERODIAGNOSIS OF LYMPHATIC FILARIASIS

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ABSTRACT

Lymphatic Filariasis (LF) is a parasitic disease transmitted to humans via mosquitoes and a major public health problem in tropical countries which requires transmission assessment tools for elimination. The objective of this study is to predict B-cell epitopes from 3D structures of antigens using *In silico* tools and also evaluate their diagnostic potential using clinical sera samples. The epitopes were predicted on the basis of Amino Acid Pair/ Kernel method by using ABCpred, BCpred and Bepipred servers. The amino acid sequences of *Wuchereria bancrofti* Abundant Larval Transcript -2 (*Wb*-ALT-2) and Cuticlin (*Wb*-CUT) were submitted to the servers and the epitopes obtained. Further, protein homology models of *Wb*-ALT-2 and *Wb*-CUT were developed using the SWISS-MODEL database and the epitopes were analysed for its position in 3D models. Finally, seven epitopes from *Wb*-ALT-2 and *Wb*-CUT were selected and their diagnostic potential in clinical samples by indirect Enzyme Linked Immuno Sorbent Assay (ELISA) was evaluated. Peptides P2 and P3 have potential in detecting the exposure to LF infection. Similarly, peptide P6 of *Wb*-CUT showed good specificity-sensitivity in IgG4 format and also detected a large number of EN samples as positives. The results indicate that peptide P1 and P2 of *Wb*-ALT-2 antigen and P6 of *Wb*-CUT have potential application in the diagnosis of LF.

KEYWORDS: Lymphatic Filariasis, *Wuchereria bancrofti*, Abundant Larval Transcript-2, Cuticlin

INTRODUCTION

Lymphatic filariasis (LF), a mosquito borne neglected tropical disease caused by parasitic nematodes. These nematodes are found in the individual's lymph vessels and develop with the nutrients in the lymph fluid[1]. They develop and damage a lymph system that induces body deformities which cause a continuing disability during the progression of the disease over a long period[2]. Although this disease is not life-threatening, it can induce severe socio-economic burden to the infected individuals present in economically backward countries[3]. The global government's support in the elimination of filariasis using methods introduced by the World Health Organization (WHO) such as Mass Drug Administration (MDA), Vector control and Transmission Assessment surveys (TAS)[4]. Effective implementation of treatment measures has reduced the parasite burden throughout the globe and it is still necessary to take care of a few hot spot regions that have active transmission[5]. To assess the transmission effective antibody monitoring tools were required. Antigens from the infective stage of lymphatic filarial parasites expressed at the time of host invasion have been identified as potential diagnostic candidates[6]. In addition, recombinant antigens were also used to simultaneously diagnose these parasitic infections; however the cost of manufacturing the recombinant antigens was high [7]. Therefore to overcome these problem peptides from these antigens can be used as an alternative to whole antigens. Compared to biologically derived molecules, synthetic peptides representing B-cell epitopes are ideal for use in diagnostic assays. Synthetic peptides have the advantage that can be produced indefinitely, stored and transported. Many diagnostic peptide based systems are under development and some are commercially available[8].

The present study is a novel attempt to aid in developing ELISA based diagnostic assay for the detection of Lymphatic Filariasis (LF) using peptide epitopes from Abundant Larval Transcript-2 (*Wb*-ALT-2) and Cuticlin (*Wb*-CUT) of *W. bancrofti*. These peptides were used as a biomarker for the assessment of infection (reactivity to detect) and transmission.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Human Ethics Committee (NITM/ICMR/BGV/17-18/EC/218/03) of ICMR-National Institute of Traditional Medicine (NITM), Belagavi. The blood samples from LF infected individuals residing in the endemic regions were collected, after obtaining informed written consent, as per the ethical guidelines.

Prediction of Epitopes

The amino acid sequences of the antigens *Wb*-ALT-2 (ADC54122.1) and *Wb*-CUT (AAD28743.2) of *W. bancrofti* were submitted to the server viz. ABCpred and BCpred, the predicted epitopes were obtained. The proteins were subjected to homology modeling using I-TASSER (Iterative Threading ASSEmbly Refinement) server[9] and the peptides were mapped in the protein structure using Biovia Discovery Studio 4.1 visualizer (BIOVIA Scientific Innovation). The peptides which were found to be present in the outer layer of the antigens were selected and custom synthesized from GL-BioCHEM and Peptron, China[10].

Study population

The serum samples from *W. bancrofti* infected individuals were collected from an area endemic for bancroftian filariasis in and around Karnataka, India. The microfilarial status of the individuals was assessed by visualization under a light microscope and ICT card test. 1 ml of venous blood were collected between 9.00 PM and 2.00 PM after informed consent from 10 microfilaremic patients (MF), 10 with chronic lymphatic disease (CP), and 10 microfilariae negative endemic normals (EN) healthy individuals residing in the endemic areas. Non-endemic normal (NEN) sera were obtained from individuals residing in the deep forest of Karnataka with no filarial exposure (n=10).

Antibody Detection

Synthesized epitopes were coated at 20 µg/well concentration onto 96 well-plates in coating buffer ($NaHCO_3/Na_2CO_3$, 0.067 M, pH 9.6) in 100 µL of volume and incubated overnight at 4°C. Antigen blank (coating buffer alone without antigen) and antibody blank (antigen without antibody) were also maintained. The plates were washed 4 times with PBS containing 0.1% Tween-20 (PBST). The unbound or non-specific sites were blocked with 100 µL per well of 3% bovine serum albumin (BSA) at 37°C for 2 h. The plates were washed 4 times with PBST. Then 100 µL of sera samples at 1:100 dilutions were added to each well in duplicate. The plate was incubated at 37°C for 2 h. Thereafter, the wells were washed 4 times with PBST and 100 µL of anti-human IgG1 and IgG4 labeled with horseradish peroxidase (HRP) was added and incubated at 37°C for 1 h. The plates were washed 4 times with PBST. 100 µL of the OPD (o-Phenylenediamine dihydrochloride) substrate substrate at a concentration of 1 mg/mL in substrate buffer ($NaHCO_3$, Na_2CO_3 and $MgCl_2.6H_2O$, pH 8.6) was added to the wells and colour developed. The reaction was arrested after 20 min by the addition of 100 µL of 1M H_2So_4 solution per well. The absorbance was measured at 492 nm in a microplate reader (Thermo Scientific, USA)[11].

Statistical Analysis

Statistical analysis was done using GraphPad Prism Software version 5.0 (GraphPad Software, San Diego, CA). The comparison of more than 2 means was done using 1-way analysis of variance (ANOVA) and $P \leq 0.05$ was considered as statistically significant[12].

RESULTS

Homology Modeling and Epitope Prediction

Structural mapping was used to analyze the epitopes. First, I-TASSER server was used to predict the homology models of *Wb*-ALT-2 and *Wb*-CUT proteins. The model with high coverage, identity and TM score (global fold similarity) were selected for structural peptide mapping. The amino acid sequences for the antigens *Wb*-ALT-2 and *Wb*-CUT were retrieved from National Center for Biotechnology Information (NCBI) and submitted to ABCpred, Bepipred and BCpred (B-cell epitope prediction servers). The predicted epitopes sequences were represented in a 3D structure consisting of alpha helices and beta strands, mapping and analysis of the spatial arrangement of the epitopes (Fig. 1). Out of 40 epitopes obtained from the above-said servers, seven were selected (Table 1) based on their position in the outer layer of the protein structure (Table 1).

Reactivity of Peptides

The immuno-reactivity of the peptides was checked against microfilaria positive (mf+ve) and microfilaria negative (mf–ve) sera samples. The peptide P1 was found to react only with mf+ve samples but not with the mf-ve samples. The peptides were tested against 10mf+ve (MF), 10 chronic Pathology (CP), 10 Endemic Normals (EN) and 10 Non-Endemic Normal samples (NEN). The peptide P2 (Fig. 2B) reacted with MF, CP and EN samples but not with NEN samples in IgG1 and IgG4 antibody assay and the peptide P3 (Fig. 2C) reacted with MF, CP, and EN samples but not with NEN samples in IgG1 antibody assay. Hence, it can be used as a marker for the detection of Non-endemic normals in the overall population. The peptides P4, P5, P6, and P7 (Fig. 3A, 3B, 4A & 4B) has reacted with all the clinical categories of LF sera samples in IgG1 and IgG4 assays. Therefore, it cannot be used as a marker.

DISCUSSION

The reactivity of peptides was determined using sera samples of individuals residing in endemic areas as well as in non-endemic areas. Ten sera samples from each group namely MF, CP, EN and NEN were used for determining the reactivity of the peptides against IgG1 and IgG4 antibodies. IgG1 ELISA may be useful in detecting more recent infections, especially among children[13]. It has reported that the IgG1 levels decreased significantly in visceral Leishmaniasis patients six months after treatment[14]. However, using this assay in children living in endemic areas can provide accurate filarial transmission status[15]. In addition, elevated IgG4 levels were reported in filarial mf carriers[16], this assay can be used for the detection of filarial infection[17]. Therefore, IgG4 antibodies assay can be used to enhance specificity without loss of sensitivity to detect the filarial infection[18].

Among the peptides of ALT-2 antigen, P1 showed high reactivity (0.17-1.2) against MF sera samples in IgG1 assay while moderate reactivity in IgG4 assay. On the contrary, peptide P2 showed less reactivity in IgG1 assay and high reactivity in IgG4 assays. Peptide P3 showed low and negligible reactivity in IgG1 and IgG4 assays respectively (Fig. 2C). The peptides of cuticlin generally exhibited very low reactivity against both the antibodies (0.01- 0.10). It may be noted that peptide P2 of ALT-2 antigen (Fig. 2B) reacted with the majority of the endemic normal samples and its reactivity in these individuals was almost similar to that against MF positive individuals while it did not show negligible reactivity against a few sera samples of CPs indicating the possibility that these ENs are exposed to filarial infection.

In these tests, all the three peptides of ALT-2 antigen could differentiate between MF positives and NEN in IgG1 assays. While peptide P1 and P2 could differentiate between MF positives and NEN in IgG4 assays that peptide P3 could not do so (Fig. 2C). Interestingly, peptide P2 and P3 detected all the MF positives, CP and EN as positives in IgG1 assays unlike peptide P1 on the other hand peptide P2 tested a majority of the EN sera in IgG4 assay. Among the peptides of cuticlin P4 (Fig. 3A), P5 (Fig. 3B), P6 (Fig. 4A) and P7 (Fig. 4B) reacted almost equally against sera of all the groups, so that they were not suitable for antibody detection. Thus among all the peptides tested peptide P1 of ALT-2 antigen showed high specific reactivity against MF positive sera indicating its potential for diagnosing MF individuals and therefore was evaluated further for its sensitivity and specificity.

Table 1: Amino acid sequences of the synthesized peptides

Antigen	Peptide sequence	Code
Wb-ALT-2 (L3 stage)	SESDEEFDDGSNDETDDKEDEGNS	P1
	EVVETDGKKKECSSHEACYD	P2
	QREPQAWCRPNENQSWTD	P3
Wb-CUTICLIN 2 (MF stage)	PVDNGVEGEPEIECGPTS	P4
	DQQGCRNDEGGRQV	P5
	LDGGPSGQPVQF	P6
	KEPNSECQRPQCTEPQGFGAI	P7

Table 2: Sensitivity, specificity, efficiency, positive predictive and negative predictive values of P1, P2, P3, P4, P5, P6 and P7 based IgG1 and IgG4 antibody assays for detecting filarial specific exposure

Antigen	Antibody	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Efficiency (%)
P1	IgG1	100.00	96.77	90.91	100.00	97.56
P1	IgG4	100.00	100.00	100.00	100.00	100.00
P2	IgG1	100.00	44.12	20.83	100.00	51.28
P2	IgG4	100.00	68.18	41.67	100.00	74.07
P3	IgG1	50.00	90.91	76.92	75.00	75.47
P3	IgG4	100.00	73.17	47.62	100.00	78.43
P4	IgG1	55.56	76.92	52.63	78.95	70.18
P4	IgG4	52.63	85.71	66.67	76.92	74.07
P5	IgG1	71.43	65.22	38.46	88.24	66.67
P5	IgG4	62.50	83.33	62.50	83.33	76.92
P6	IgG1	58.82	100.00	100.00	81.08	85.11
P6	IgG4	90.91	76.92	52.63	96.77	80.00
P7	IgG1	52.63	85.71	66.67	76.92	74.07
P7	IgG4	71.43	66.67	40.00	88.24	67.80

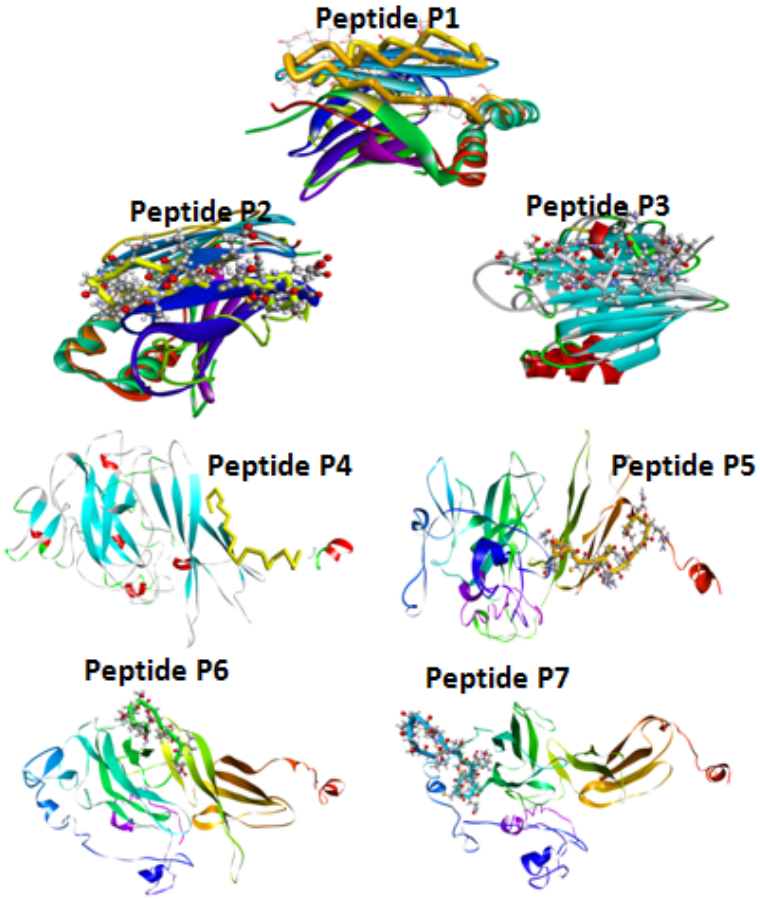

Figure 1: Structural epitope mapping of peptides P1, P2, P3 in *Wb*-ALT-2 and P4, P5, P6, P7 in *Wb*-CUT protein

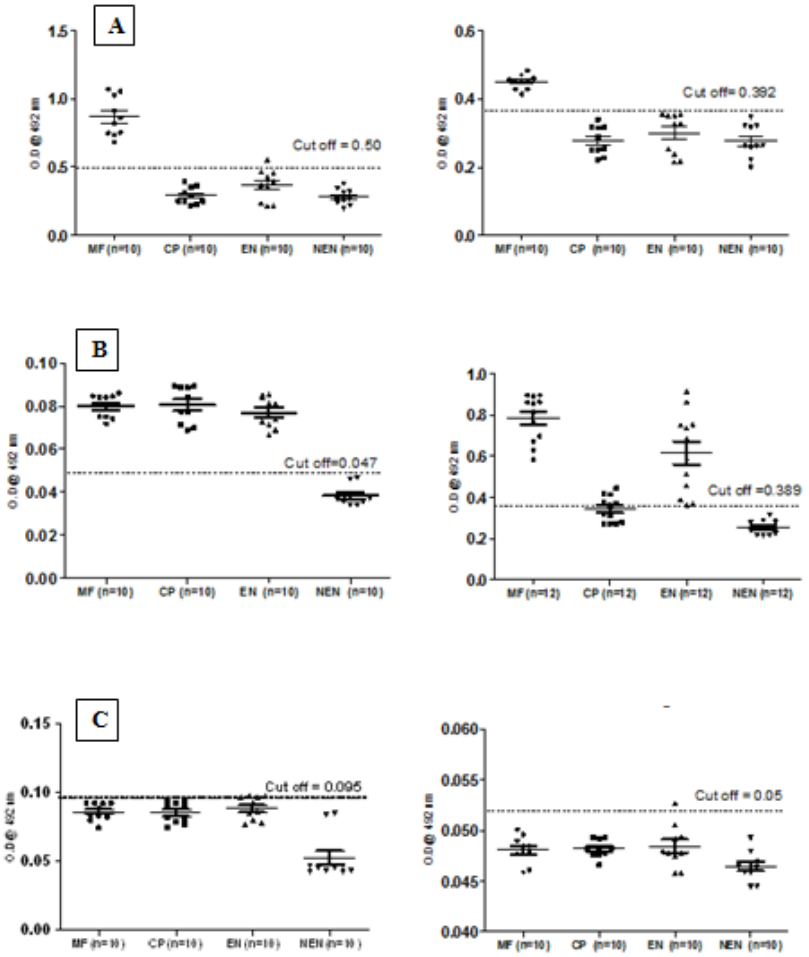

Figure 2: Reactivity of IgG1 and IgG4 antibodies in different clinical category sera against the peptides (A) P1, (B) P2 and (C) P3

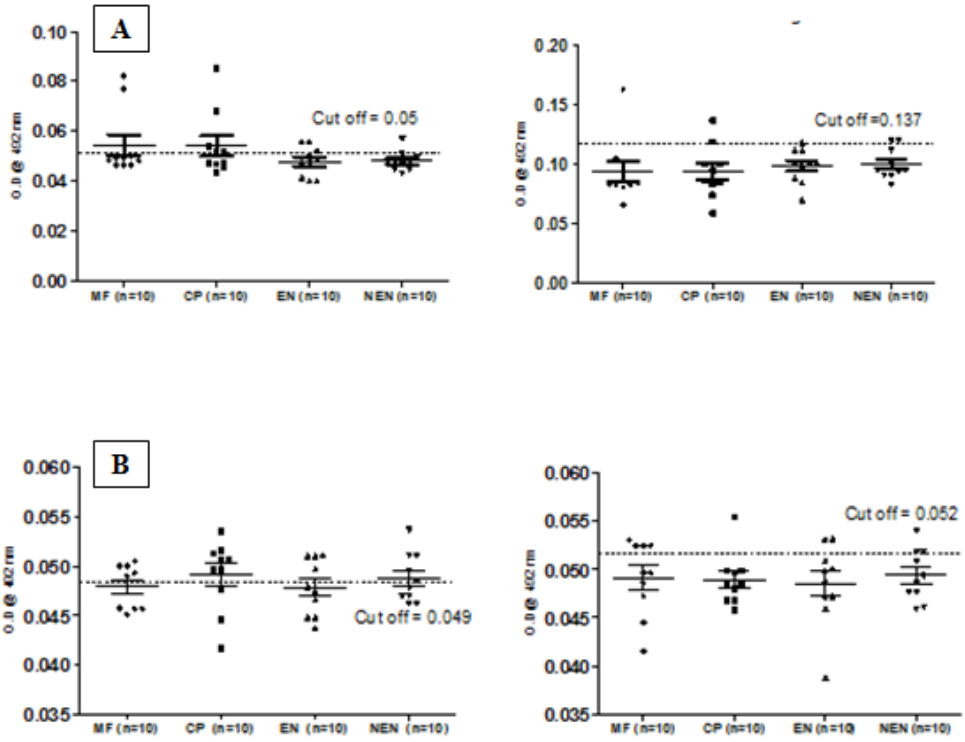

Figure 3: Reactivity of IgG1 and IgG4 antibodies in different clinical category sera against the peptides (A) P4 and (B) P5

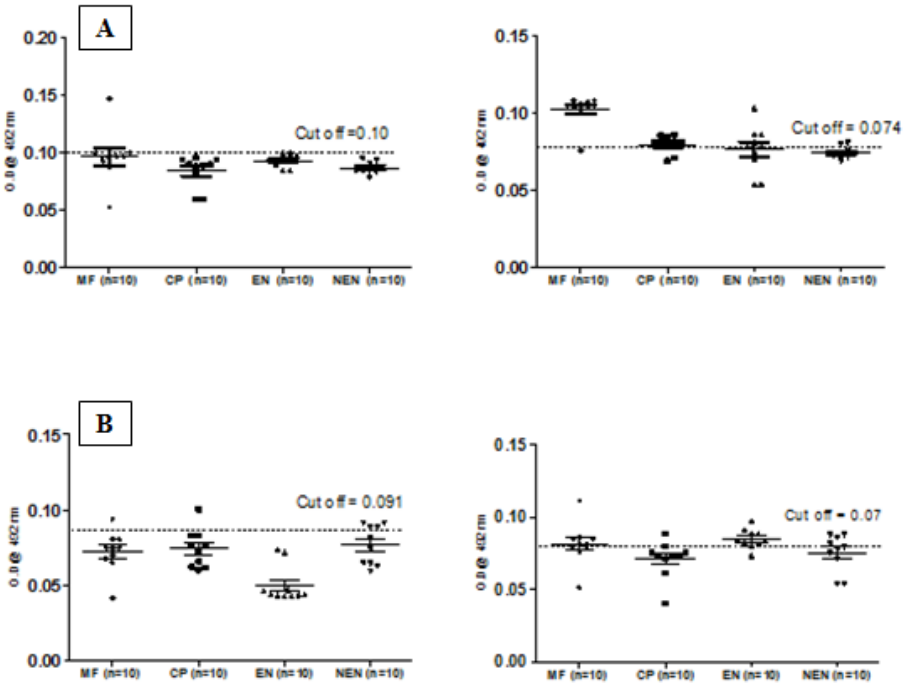

Figure 4: Reactivity of IgG1 and IgG4 antibodies in different clinical category sera against the peptides (A) P6 and (B) P7

It is interesting to note that peptide P2 of ALT-2 showed significant reactivity against EN sera in IgG4 assay while it showed weak reactivity against CP (lymphoedema) sera. This indicates that this peptide is recognizing the individuals who are exposed to LF infection. It may also be noted that these individuals are CFA negative as tested using *Alere*™ *Filariasis* antigen detection kit[19]. Therefore, the overall results indicate that the peptide P1 based IgG1 ELISA can be used for detecting the MF cases while peptide P2 can be used for detecting the exposure to LF infection. Peptide P6, although could differentiate between infected and uninfected and also detected some of the EN as filarial specific antibody positives, its low reactivity makes it unsuitable as a diagnostic marker. In view of this, the peptide P1 based IgG1 ELISA may be useful for detecting early infections/recent exposure to filarial infection.

CONCLUSION

The peptide P1 showed high reactivity with both IgG1 and IgG4 antibodies in the sera of all the mf+ve cases and some endemic normal individuals, indicating that it can detect the active infection as well as exposure to it. Thus, the peptide has good potential in detecting active LF infection and assessing its transmission in the endemic communities.

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