ABSTRACT

Lymphatic filariasis (LF) is a major cause of acute and chronic morbidity affecting humans in tropical and subtropical areas of Asia, Africa, the Western Pacific, and some parts of the Americas. India accounts for a significant number of people suffering from parasitic infection. As part of the WHO sponsored Filarial Genome Project, many novel genes of *W. bancrofti* and *B. malayi* important for their diagnostic, prophylactic, chemotherapeutic potential and other genes involved in immune evasion and parasite survival mechanisms have been identified from various stages of the parasite life cycle. *Wuchereria bancrofti* protein WbSXP-1 was identified and established as a potential candidate for the diagnosis of lymphatic Filariasis at CBT, Anna University (Rao et al. 2000, Basker et al. 2004). WbSXP-1 is a surface antigen found abundantly in the hypodermis of the parasite. Recombinant WbSXP-1 was expressed in osmotically inducible *E. coli* GJ1158 and purified with immobilized metal affinity chromatography. In our center, cultivation and expression of GJ1158 in bioreactor level and purification of rWbSXP-1 his-tag fusion protein was optimized in technical scale using FPLC. Criteria for protein quality and immunoreactivity of purified rWbSXP-1 were established for diagnostic applications (Janardhan et al 2007).

In order to improve the diagnostic efficiency of antigen, WbSXP-1 was cloned in baculovirus expression system and expressed in Sf21
(Spodoptera frugiperda) insect cells which is used for expression of eukaryotic proteins for post-translational modifications, improved processing and targeting. The SXP-1 protein contains signal peptide at amino-terminal region with possible cleavage site. The SXP gene was cloned with signal sequence to Baculovirus expression system. The extracellular protein expressed as a glycosylated form with the maximum expression of 8.25µg/ml/10^6, which is comparable to soluble SXP-1 expressed in E.coli BL21 (4.2 µg/ml) and GJ1158 (12.4 µg/ml) in bioreactor (S. Janardhan et al, 2007). Protein (rBAC-WbSXP) purified from baculovirus expression system showed 100 % sensitivity and specificity with filarial clinical samples. SXP-1 protein expressed in eukaryotic baculovirus expression system found to be post-translationally modified processed and showed significantly (p < 0.0001) higher reactivity towards MF positive samples (1.122±0.254) compared to SXP-1 expressed in E.coli (0.911±0.205).

WHO has highlighted the importance of developing diagnostic assays, as a surveillance and measure to eliminate ‘Lymphatic Filariasis’. Presently, problems in the immuno-diagnosis are the specificity to detect brugian and bancroftian parasites, stability of diagnostic lines, cost of assays, time and manpower associated with use of ELISA kit and PCR etc.,. These affect their application for disease assessment in endemic area. WbSXP-1 is highly immunogenic and is present in all the stages of parasite life cycle with 84 % homology to BmSXP. The possible application of monoclonal antibodies developed against this protein for the detection of circulating filarial antigen in W. bancrofti and B.malayi clinical samples. Monoclonal
antibodies (MAb) were developed against SXP antigen to achieve the objective of development of antigen based diagnostic kit. Two monoclonal antibodies namely 1F6H3 (IgG2a) and 2E12E3 (IgM) with better sensitivity were selected for validating capture ELISA. Sandwich ELISA was developed with SXP monoclonals as capture antibody and rabbit anti-SXP-1 polyclonal as detection antibody and validated against recombinant as well as native microfilaria antigen. The efficiency of sandwich ELISA was analyzed with MF positive samples and used NEN as control. The evaluated results of sandwich assay showed 100% sensitivity in the data obtained from experimental test groups. Results showed that the MF groups carried significantly higher antigen units (p < 0.0001) compared to endemic normal, chronic pathology and non endemic normal groups. The MF group had the 100% antigen positive reactivity (0.769 ± 0.14) while EN and CP groups showed the 16.66 % (0.259 ± 0.084) and 0 % (0.271 ± 0.038) respectively.