

Usefulness of Sd30 in the diagnosis of arthritis of filarial origin

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Human lymphatic filariasis is characterized by the common clinical manifestations like acute adenolymphangitis and chronic lymphoedema. But a number of other occult manifestations like asthmatic bronchitis, pulmonary eosinophilia, recurrent URI, pneumonia, pain in abdomen and mono-arthritis of filarial origin in filarial endemic areas remain undiagnosed because of lack of suitable diagnostic tool¹. The present study has identified Sd30 (Sd—*Setaria digitata*, 30 - MW-30 kDa), an allergenic fraction purified from *Setaria digitata* (a cattle filarial parasite) that can detect the antifilarial (*Wuchereria bancrofti*) antibodies (IgM and IgG4) produced during the acute/chronic manifestation of extra lymphatic pathology like arthritis caused by *W. bancrofti* infection in individuals residing in filaria endemic regions.

The study was conducted at a Rheumatology Clinic, situated at Bhubaneswar, the capital city of Odisha, India. Total 80 arthritis patients from Khurda district, a *W. bancrofti* endemic region² and 20 arthritis patients from filarial non-endemic region attending the clinic for treatment were enrolled in this study. These non-filaria endemic arthritis subjects were considered as a control group. Informed consent was obtained from the patients. The Institutional Ethical Committee has approved the study. All the patients had evidence of single joint inflammation (monoarticular arthritis) and redness over the infected joint. Filarial symptom like lymphangitis-axillary/lymphadenopathy-inguinal was also observed in a number of patients. Patients were treated with standard dose of diethylcarbamazine (DEC), antibiotics and anti-inflammatory drugs as per the necessity of the patients.

The presence of circulating filarial antigen, filarial specific antibodies and pro-inflammatory cytokines were examined in blood and synovial fluid. Different haematological parameters like haemoglobin level, total WBC count (TWBC), eosinophils percentage, absolute eosinophils count (AEC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were examined. The Sd-30 antigen (MW-30 kDa) purified in our laboratory³ from *Setaria digitata* was used to determine the filarial

specific antibodies (IgG, IgM and IgG4) levels by ELISA⁴. Presence of circulating filarial antigen was detected both in serum and in synovial fluid using Og4C3 ELISA kit (Trop Bio, Australia)⁵. The antigen unit 100 was used as a cut-off value for identification of antigenemia as per the manufacturer's instruction. The pro-inflammatory cytokines such as interferon (IFN)- γ , interleukin (IL)-2 and interleukin (IL)-6 levels were measured by ELISA kit both in serum and synovial fluid. Antibody unit and O.D. value of each individual was plotted in the graph. Statistical analyses of the data were performed by using Graph Pad Prism (Version 5.01). Data were expressed as mean \pm S.D. (Standard deviation). Significance level was determined by Student's *t*-test.

The patients were categorized into two groups depending on the presence of circulating filarial antigen (CFA) in serum or in synovial fluid. The individuals without detectable circulating filarial antigen in serum or synovial fluid were designated as CFA⁻ (CFA negative) group and with circulating filarial antigen either in serum or in synovial fluid were designated as CFA⁺ (CFA positive) group. The filaria non-endemic arthritis patients were CFA⁻. Initially, the patients were treated with DEC (6 mg/kg body weight) for six days. All the CFA⁺ arthritis patients and some CFA⁻ patients were found to respond positively and the symptoms (pain, swelling of the joint, fever and filarial symptoms) were subsided after five days of treatment and subsequently the treatment was continued up to 12 days. Complete recovery was observed after 10 days of treatment. But majority of the CFA⁻ patients (either from filaria endemic region or from filaria non-endemic region) did not respond to DEC till Day 5 (Table 1). Therefore, these patients were treated with doxycycline (Standard dose) and NSAID (non-steroid anti-inflammatory drug) from Day 6 onward and were followed-up.

The levels of anti-filarial antibodies (IgG, IgM and IgG4) to filarial antigen (Sd30) in both serum and synovial fluid are shown in Fig. 1. The representative dot plot depicts the antibody level of each individual. The

Table 1. Clinical and laboratory findings of the patients

Group (Area)	No. of patients	Age range in yr (Mean \pm SD)	Mean \pm SD of hemoglobin value (g/l)	Mean \pm SD of total WBC count ($10^9/l$)*	Mean \pm SD of eosinophil %	Mean \pm SD of AEC ($10^9/l$)*	Onset of disease with fever (days)	ESR mm/h (Mean \pm SD)	Filarial symptoms
CFA ⁻ group (Endemic area)	40	25–80 (49.45 \pm 9.9)	11.6 \pm 1.4	9.1 \pm 1.4	9.6 \pm 2.3	0.5 \pm 0.3	Nil	24–62 (41.6 \pm 12.6)	Nil
CFA ⁺ group (Endemic area)	40	20–51 (41.07 \pm 7.9)	11.41 \pm 1.85	9.20 \pm 1.08	18.25 \pm 3.36	0.91 \pm 0.2	2–4	48–88 (66.7 \pm 13.7)	Lymphangitis, Lymphadenopathy
CFA ⁻ control group (Non-endemic area)	20	36–56 (43.2 \pm 7.02)	11.1 \pm 1.7	8.7 \pm 0.7	7.3 \pm 2.6	0.4 \pm 0.4	3–4	40–86 (66.3 \pm 17.8)	Nil

Table 2. Sd30 antigen positivity in respect to IgM level in different groups of arthritis patients from filaria endemic region

Cases	Sd30 (+)ve No. (%)	Sd30 (–)ve No. (%)
CFA ⁺ (n = 40)	38 (95%) (Responding to DEC therapy)	2 (5%) (Responding to DEC therapy)
CFA ⁻ (n = 40)	8 (20%) (Responding to DEC therapy)	32 (80%) (Not responding to DEC therapy)

cut-off value of each group has been indicated by horizontal bar. The IgG4 and IgM antibody levels to Sd30 were found to be above the cut-off level in 38 out of 40 CFA⁺ arthritis patients (Fig. 1a) which was significantly high ($p < 0.05$) as compared to control group of patients (Fig. 1c). On the other hand, though eight cases of CFA⁻ arthritis patients had Sd30 specific anti-filaria antibody level above the cut-off point, yet majority (32 out of 40) of them were having the antifilarial antibody level below the cut-off level (Fig. 1b). Notably, no remarkable differ-

ence was observed in IgG responses among different groups (Figs. 2a and b). As would appear from Table 2, the Sd30 positivity to IgM is significantly higher in CFA⁺ arthritis patients who were responding to DEC therapy as compared to CFA⁻ arthritis patients (patients were not responding to DEC therapy). This indicates that Sd30 can be used as a diagnostic marker to distinguish arthritis of filarial origin from arthritis of non filarial origin.

The pro-inflammatory cytokine IL-2, IL-6 and IFN- γ levels were measured in serum and synovial fluid in the study patients and are shown in Figs. 2 (a) and (b). No significant difference was observed in the cytokine level among the study subjects.

The etiology of arthritis is not fully understood. It was established that lymphatic dwelling filarial parasite produces some extra lymphatic pathology in endemic population^{1–2, 6}. In the present study, clinical and immunological evaluation of arthritis patients from a filarial endemic region had shown a strong evidence of association between filarial infections and arthritis which can be diagnosed. Several studies have demonstrated that the

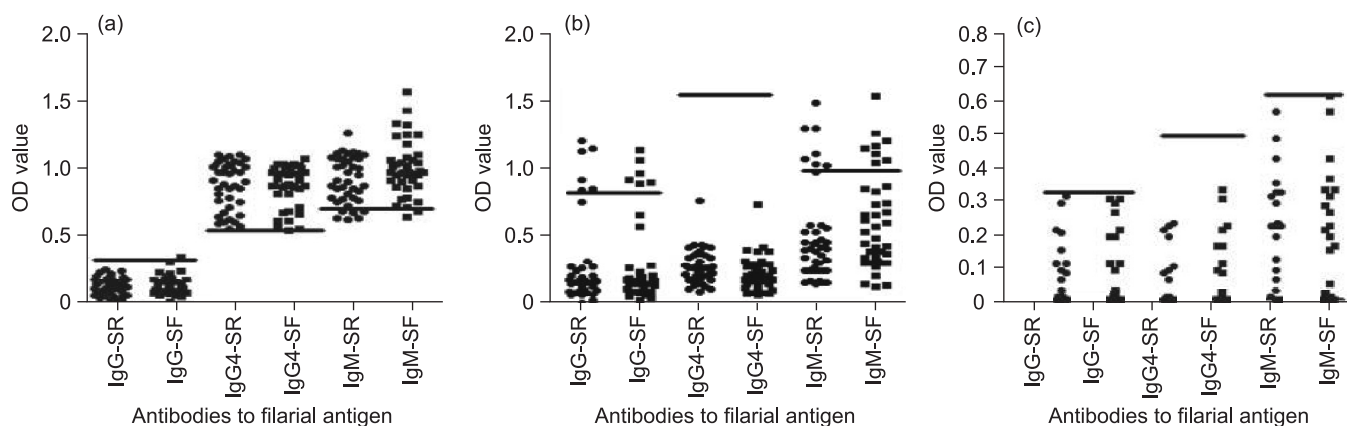


Fig. 1: Antibody level in serum [SR] and synovial fluid [SF] to filarial antigen (Sd30) in different groups—(a) CFA⁺ group from filaria endemic region; (b) CFA⁻ group from filaria endemic region; and (c) CFA⁻ group from filaria non-endemic region of arthritis patients. Each dot represents the value of single patient and horizontal bar represents the cut-off value (value of filaria endemic individuals, without arthritis). The CFA⁺ arthritis patients' cut-off with CFA⁻ pool sera, CFA⁻ arthritis patient's cut-off with CFA⁺ pool sera and filaria non-endemic arthritis patient's cut-off with CFA⁻ pool sera.

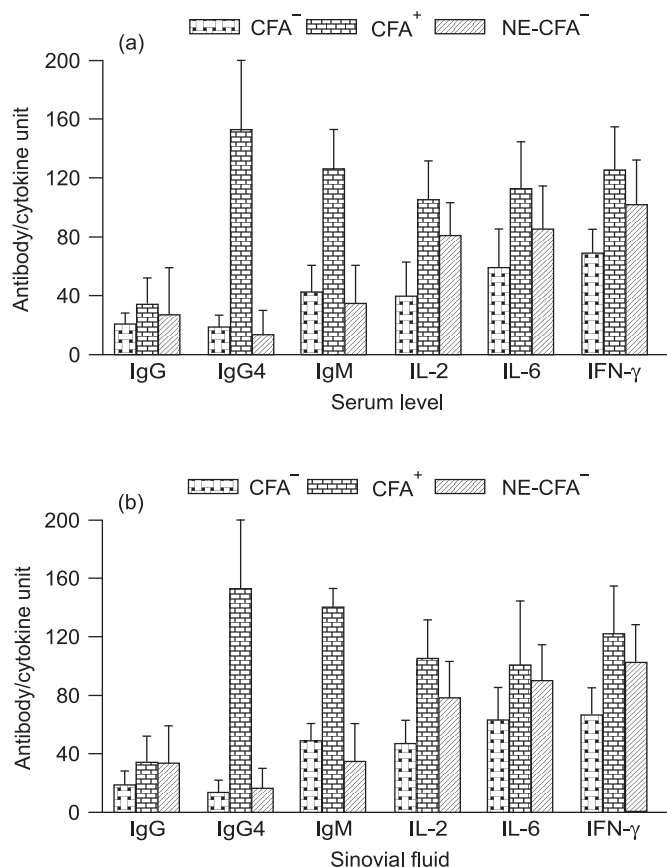


Fig. 2: Antibody and cytokine level in serum (a); and synovial fluid (b) to filarial antigen (Sd30) in different groups of arthritis patients. Data were expressed as antibody/cytokine unit and represent the mean and SD value.

presence of filaria specific IgG4, IgM and circulating filarial antigen is the marker of active filarial infection^{7–11}. Sarker *et al*¹² have established that lymphatic filariasis manifested acute arthritis which has been treated with oral diethylcarbamazine citrate (DEC) (150 mg/day) for three weeks, showed complete resolution of arthritis. The findings of the present study also support the above observations but the antigen Sd30 has an ability to diagnose the occult filarial infection in arthritis patients. The increased level of eosinophilia, circulating filarial antigen in arthritis patients is associated with elevated level of IgG4 and IgM antibody responses to Sd30 antigen. Most importantly, the CFA⁺ arthritis patients in our study have responded well to the DEC treatment and have cleared the symptoms like lymphangitis-axillary/lymphadenopathy-inguinal/hydrocele, fever, joint pain and swelling satisfactorily. A number of CFA⁻ patients from filaria endemic region have also exhibited higher levels of antifilarial antibodies (IgG4 and IgM) to Sd30 and responded to DEC as well. No side effect or relapse was encountered. It was assumed that during benign course of lymphatic filari-

asis, development of arthritis was due to the immunological reaction against some occult agents in the joints¹¹. Some literatures also reveal that filarial pathogen can induce arthritis, which can be distinguished from other cause of arthritis based on clinical criteria associated laboratory findings and prompt response to diethylcarbamazine (DEC) treatment¹². Studies have also reported that IL-6, IL-2 and IFN-γ play a central role in the pathogenesis of arthritis¹³. However, during the present study no significant difference was observed in the level of inflammatory cytokines (IL-6, IL-2 and IFN-γ) in different groups of patients indicating the induction of pro-inflammatory responses in all the patients.

The finding of this study provides the evidence that Sd30 can diagnose immunologically the filarial attributed arthritis from arthritis of non-filarial origin. This observation will help the physicians to institute appropriate treatment to filarial attributed arthritis patients in a cost-effective manner. However, the main limitation of the study is the number of samples. But this preliminary observation can be validated in other filarial endemic areas with larger number of samples.

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