A survey of bancroftian filariasis for microfilariae & circulating antigenaemia in two villages of Madhya Pradesh

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Background & objectives: The estimation of filariasis prevalence in Panna district of Madhya Pradesh was so far relied upon clinical and night blood smear examination. However, night blood smear examination fails to detect the infection in individuals having low parasitaemia and cryptic filarial infection. The present study was undertaken to re-evaluate the prevalence of filariasis in two villages of Panna district by Og4C3 ELISA.

Methods: The study was carried out during 2002-2003 in two villages (namely Pista and Taroni) of Panna district, Madhya Pradesh. Clinical examination was performed according to WHO criteria to classify filarial disease. Night blood smears collected during 20:00 to 00:00 h were examined to detect microfilariae (Mf). For estimation of circulating filarial antigen (CFA) by Og4C3 ELISA, 2 ml blood was collected from each individual by venepuncture.

Results: With Og4C3 ELISA, 38 per cent serum samples of village Pista (n=332) and 47.7 per cent from village Taroni (n=88) were found positive for CFA. The overall disease rate was (243/420) 57.9 per cent by CFA while it was (182/420) 43.3 per cent by night blood smear examination. A total of 14.5 per cent individuals were having cryptic filarial infection detected by CFA.

Interpretation & conclusion: The study emphasizes the use of CFA estimation being a sensitive and specific diagnostic tool for the evaluation of the true prevalence of the disease. The high CFA prevalence in the study area necessitates early intervention measures to check its transmission.

Key words Circulating filarial antigen - filariasis - Madhya Pradesh - microfilariaemia

Lymphatic filariasis (LF) caused by the filarial nematode Wuchereria bancrofti affects more than 115 million people worldwide1. In India, 22 States/Union Territories are known to be endemic for LF and 553 million people are at risk of infection with 27 million parasite carrier and 21 million with symptomatic filariasis2. LF is endemic in fifteen districts of Madhya Pradesh3. In 1991, a survey was carried out by our Centre in Panna district of Madhya Pradesh based on clinical and night blood smear examination3,4.
The diagnosis of filarial infection by clinical examination and parasitological methods was the mainstay in detecting filarial infection up to early nineties. These methods though correctly assess the clinical cases and microfilaraemic subjects with high microfilariae (Mf) count, but fail to identify low Mf count and cryptic filarial infection in asymptomatic microfilaraemic individuals. In recent years, with the introduction of Og4C3 ELISA, the prevalence of filarial disease was redefined in many parts of the globe based on antigen detection. Therefore, we undertook this study to re-evaluate the prevalence of filariasis in two villages of Panna district using Og4C3 ELISA.

Material & Methods

Study area: The study was carried out in two villages namely Pista (total population 2228, census 2001) and Taroni (total population 1330, census 2001) of Panna district, Madhya Pradesh, India. These villages are situated adjacent to Uttar Pradesh, a State known to be endemic for filariasis. These villages are also known to be endemic for lymphatic filariasis.

Sample collection: A door-to-door survey was carried out from June 2002 to December 2003 in the selected villages to include individuals (adults and children >1 yr) in the study. Informed consent was obtained from study individuals (parents in case of minor). History suggestive of filariasis and diethyl carbamazine citrate (DEC) consumption was recorded.

Mf detection: Mf was detected by making two thick blood smears of 20 µl each on a clean glass slide from 20:00 to 00:00 h. The smears were air dried, dehaemoglobinised and stained with Wright’s stain to detect Mf. Study subjects were also examined by a clinician for LF as per the WHO guidelines.

Antigenemia detection: About 2 ml blood was collected from all the individuals (n=420; 318 males and 102 females) enrolled in the study. Sera were separated in the field and brought to the laboratory and stored at -20°C until tested. The Trop Bio ELISA kit (Tropical Biotechnology Pvt. Ltd. Townsville, Australia) was used for detecting and quantifying W. bancrofti antigen according to the manufacturer’s recommendations. The results were expressed as arbitrary antigen units per ml using Onchocerca gibsoni antigen provided as standard in the kit (cut-off =100 units/ml).

Statistical analyses: χ² for trend was used to find the relation of age with Mf and CFA prevalence while McNemar χ² test was used to compare the efficacy of the two methods.

The study was approved by Ethics Committee of the Centre.

Results

A total of 332 and 88 individuals were examined from Pista and Taroni, respectively. The prevalence of Mf and CFA was 19.6 and 38 per cent in Pista, while it was 19.3 and 47.7 per cent in Taroni (Table I). The youngest subject exhibiting a positive response for antigen and Mf was 6 yr old. In Pista, the percentage of Mf positive and CFA positive individuals increased steadily with age reaching a peak in 20-29 yr age group. After a slight decrease in the age group of 30-39 yr, the prevalence of Mf and CFA increased up to 60 yr. Beyond 60 yr there was a slight fall in CFA prevalence, whereas the Mf prevalence was increased. In Taroni, the percentage of Mf positive and CFA positive also increased with age. By and large, an increasing trend was observed in both the villages between Mf and CFA with age and the results were found to be significant (Table I).

While comparing the efficacy of the two testing methods (Table II), it was observed that in asymptomatic individuals (n=296), Og4C3 ELISA could detect infections in 119 (40.2%) individuals, while night blood smear in 58 (19.6%) only. In symptomatic individuals (n=124), the prevalence was 39.5 and 19.4 per cent by Og4C3 ELISA and night blood smear, respectively. Infection rate detected by CFA assay was significantly (P<0.0001) higher compared to that by night blood smear examination.

Of the 420 individuals included in the study, 124 had clinical symptoms of filariasis (elephantiasis, hydrocele and acute lymphangitis). Among the 81 individuals presented with hydrocele, Mf was present in 21 (26%) and CFA in 40 (49.4%) cases. All the 20 individuals presented with elephantiasis were microfilaraemic and only 2 of them were found positive for CFA. Three (13%) and seven (30.4%) of
the 23 individuals presented with acute lymphangitis were positive for Mf and CFA, respectively.

It was observed that all the microfilaraemic individuals were CFA positive but all the CFA positive individuals were not microfilaraemic (Fig.). A total of 86 individuals were CFA positive but having no circulating Mf. From the 86 amicrofilaraemic antigen positive individuals, 61 were asymptomatic and amicrofilaraemic having cryptic infection detected by Og4C3 ELISA (Fig.).

**Discussion**

Filariasis is a major public health problem in India. With the continuous change in environmental factors, urbanization and availability of newer diagnostic tools, the estimation of 40 per cent global burden due to filariasis in India is bound to increase. With the widespread availability of the CFA assay, which reflects adult worm burden, it can now be demonstrated that a majority of the earlier studies underestimated the prevalence of filariasis in endemic communities.

This study reports on the prevalence of filariasis in two villages of Madhya Pradesh determined by CFA assay. As commonly observed, the prevalence of CFA was considerably higher than Mf prevalence in all the age groups. In the present study, in Pista the prevalence of filarial infection in the population was

**Table I.** Age group-wise prevalence of Wuchereria bancrofti microfilaraemia and circulating filarial antigenaemia

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Pista</th>
<th></th>
<th>Taroni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>Mf + (%)</td>
<td>CFA + (%)</td>
</tr>
<tr>
<td>1-9</td>
<td>39</td>
<td>4 (10.3)</td>
<td>11 (28.2)</td>
</tr>
<tr>
<td>10-19</td>
<td>90</td>
<td>10 (11.1)</td>
<td>21 (23.3)</td>
</tr>
<tr>
<td>20-29</td>
<td>63</td>
<td>16 (25.4)</td>
<td>30 (47.6)</td>
</tr>
<tr>
<td>30-39</td>
<td>57</td>
<td>11 (19.3)</td>
<td>22 (38.6)</td>
</tr>
<tr>
<td>40-49</td>
<td>31</td>
<td>8 (25.8)</td>
<td>16 (51.6)</td>
</tr>
<tr>
<td>50-59</td>
<td>23</td>
<td>6 (26.1)</td>
<td>13 (56.6)</td>
</tr>
<tr>
<td>60+</td>
<td>29</td>
<td>10 (34.5)</td>
<td>13 (44.8)</td>
</tr>
<tr>
<td>Total</td>
<td>332</td>
<td>65 (19.6)</td>
<td>126 (38.0)</td>
</tr>
</tbody>
</table>

Mf, microfilariae; CFA, circulating filarial antigen

$P < 0.001$ for CFA and Mf in Pista

$P < 0.05$ for CFA and Mf in Taroni

($\chi^2$ for trend)

**Table II.** Relationship between circulating filarial antigen (CFA) and microfilariae (Mf) detection with clinical status

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFA+ve/ Mf+ve</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>58</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
</tr>
</tbody>
</table>

$P < 0.0001$ for both symptomatic and asymptomatic (McNemar $\chi^2$ test)
approximately two times higher when determined by CFA positivity compared to Mf examination in all the age groups except 1-9 and 60+ yr age groups. In the context of filariasis elimination programme, use of antigen detection in the diagnosis of filariasis, particularly in young children is important as treatment at an earlier age may prevent subsequent development of clinical disease.

The average CFA prevalence was about 2 times higher than the Mf prevalence indicating that a majority of infection was antigen positive but Mf negative. In this case however, Mf prevalence was estimated by a relatively less sensitive 20 µl blood smear and the present CFA+/Mf- might include low-density Mf carriers.

The prevalence of microfilaraemia and antigenaemia seemed to increase with age in Pista and peak prevalence was seen in 30-39 yr age group. In Cook Islands the percentage of CFA positive subjects increased steadily with age reaching a peak in the 30-40 yr age group.

To conclude, about 40 per cent antigenaemia in the study population is a matter of concern and necessary control programme is needed to check the transmission of filariasis.

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References


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