

Filarial infection in chest disease patients from *Wuchereria bancrofti*-endemic areas of Uttar Pradesh, India

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The study determined the extent of co-occurrence and the influence of filariasis in chest-symptomatic patients from filaria-endemic areas of Uttar Pradesh, India. Patients with chest and respiratory symptoms were categorized on the basis of clinical, radiological, sputum and haematological examinations as cases of tropical pulmonary eosinophilia (TPE) with asthma-type syndrome ($n = 36$), pleural effusion (PE; $n = 13$) and pulmonary tuberculosis (PT; $n = 36$). Twenty-six asymptomatic (Asym) microfilaraemic cases, but without chest symptoms, were included for comparison. Patients were examined for filarial infection by filarial skin test (FST), microfilaria in blood and pleural aspirates and for filaria-specific IgG₄ and IgM in serum. Microfilariae were found in 19, 46 and 6% of TPE, PE and PT patients, respectively. FST positivity was shown by 100, 69, 67, and 92%, filaria-specific IgG₄ by 92, 85, 89 and 96% and filaria-specific IgM by 94, 69, 86, and 77% of TPE, PE, PT and Asym microfilaraemic cases, respectively. Treatment of some of the chest-symptomatic microfilaraemic or amicrofilaraemic patients (5 to 6 patients in each category) with the antifilarial, diethylcarbamazine, provided considerable improvement in the chest condition. Alterations in the filaria-specific antibody responses further supported that the patients displaying chest symptoms had concurrent filarial infection. The study also indicates that filarial infection may contribute to the aggravation of chest symptoms in non-TPE chest symptomatic patients of filaria-endemic areas.

CHEST pains with nocturnal asthmiform paroxysms, breathlessness, chronic cough, fever with or without pleural effusion are some of the symptoms commonly presented by chest-disease patients, particularly those living in filaria-endemic areas in tropical countries. These symptoms are generally considered suggestive of pulmonary tuberculosis (PT). Some of the symptoms are also common in patients of tropical pulmonary eosinophilia (TPE). Although TPE is known to be associated with filariasis^{1,2} and can be treated favourably with diethylcarbamazine³ (DEC), whether non-TPE categories of chest symptomatic

patients also harbour filarial infection and whether filarial infection in such cases contribute to the chest symptoms has not been established. In the present communication, we report the results of a systematic screening of chest-disease patients living in filaria-endemic areas for the extent of co-occurrence of filariasis. We also report here the results of a pilot study to determine the improvement provided by DEC in these non-TPE patients.

Eighty-five chest/respiratory-symptomatic patients (age: 15–65 years) admitted in the Kasturba Chest Hospital, Lucknow University or reporting to the out-door patients department (OPD) of KG's Medical College, Lucknow, were included in the present study. These subjects were from bancroftian filaria-endemic areas Behraich, Gonda, Basti, Gorakhpur and rural areas of Lucknow. Following routine clinical and haematological examinations and chest X-ray, the patients were subjected to detailed laboratory tests. On the basis of clinical, radiological and laboratory tests (sputum and haematological examination), the patients were categorized as TPE with more or less bronchial asthma-type syndrome ($n = 36$), pleural effusion (PE; $n = 13$), and PT cases ($n = 36$). Twenty-six asymptomatic (Asym) microfilaraemic cases free from chest symptoms were also included for comparison. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki (1975) and prior permission was obtained from institutional ethics committee on human research.

For demonstration of microfilaria, 5 ml heparinized night blood was membrane-filtered (5 μ m porosity) following the method of Sircar *et al.*⁴. Pleural aspirates (50–100 ml) were also examined for microfilaria using this technique.

Total and differential leucocyte counts were made according to Dacie and Lewis⁵. Absolute eosinophil count (AEC) was calculated from per cent eosinophil count and total leucocyte count.

Smears of 3–4 sputum samples of the each patient were stained by Ziehl–Neelsen method and examined microscopically for acid-fast bacilli.

Chest radiograms of the patients were obtained from Department of Radiology, KG's Medical College, Lucknow. Sputum and radiological investigations were not done in Asym microfilaraemics.

Infective larval antigen was prepared from infective larvae (L₃) of *Brugia malayi*⁶. Briefly, L₃ were isolated from infected *Aedes aegypti* mosquitoes fed on microfilaraemic *Mastomys coucha* as described earlier⁷. Protein content of the soluble antigen preparation was estimated following the method of Lowry *et al.*⁸ and adjusted to 40 μ g/ml. The antigen was used in filarial skin test. The soluble somatic antigen from adult worms of *B. malayi* recovered from *B. malayi*-infected *Meriones unguiculatus* was prepared by the method of Tandon *et al.*⁹. Protein was estimated as mentioned earlier. The antigen was used for the detection of IgG₄ and IgM.

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Skin test was carried out as described by Chandra *et al.*⁶. Briefly, 0.05 ml of antigen (2 µg antigen protein) was injected intradermally into the volar surface of the arm and the area was demarcated with a ballpoint pen. After 15–20 min, the wheal area was again marked and the ratio of 0 min to 15–20 min wheal area (reaction ratio; RR) was determined. An RR of more than 2.0 was considered as positive.

Filaria-specific IgM and IgG₄ were determined in the sera of patients by ELISA^{10,11}. ELISA strips (Nunc, Denmark) were coated with adult worm antigen at 1 µg (IgM) or 10 µg (IgG₄)/ml in carbonate buffer (0.06 M; pH 9.6). Human sera (primary antibody) were used at 1 : 200 (IgM) and 1 : 25 (IgG₄) dilutions; rabbit antihuman IgM peroxidase and mouse antihuman IgG₄ (Sigma Co., St. Louis) were used at 1 : 2500 and 1 : 5000 dilutions, respectively. For the detection of IgG₄ goat antimouse IgG peroxidase (Sigma) was used at 1 : 2000. The colour reaction was measured at 492 nm in an ELISA reader (Multiscan). ELISA values higher than the mean + 2 S.D. of normal chest disease-free subjects (10) from filaria non-endemic area (Anantnag, Kashmir), but infested with *Ascaris/Trichuris/Enterobius vermicularis* were considered to be positive for filariasis (IgM: 0.092 ± 0.026; IgG₄: 0.055 ± 0.012).

Chest symptomatic cases with or without microfilaria in the circulation (6 TPE, 5 PE, 5 PT and 6 Asym cases) were treated with DEC citrate at a dose of 6 mg/kg, p.o. for 21 days. After day 30 of treatment, these patients were re-examined and all the parameters were repeated.

Data were analysed by one-way ANOVA followed by Newman Keuls test. TLC and AEC data were analysed by the non-parametric Mann–Whitney test. Differences between initial and post-treatment were analysed by paired *t*-test. Differences were considered significant if $P < 0.05$.

In TPE group, 13/36 cases (36%) showed positive chest radiograms. PE and PT groups showed atypical abnormalities of PE and PT, respectively. Twenty-five of 36 (69.4%) PT patients were positive, while all TPE and PE

cases were negative for AFB in sputum. In TPE group, the total leucocyte counts (TLC) in chest radiograph-positive cases were marginally higher (median: 11500/cmm; range: 8600–13500/cmm) than in chest radiograph-negative patients (median: 9400/cmm; range: 7800–12800/cmm), though the difference was not statistically significant. The AEC was, however, almost identical among radiograph-positive (median: 2743/cmm; range: 1400–6844/cmm) and negative patients (median: 2314/cmm; range: 564–15300/cmm). One of the patients who had microfilaria in blood and pleural aspirate did not show eosinophilia (data not shown). AEC of patients of PE (median: 116/cmm; range: 0–1892/cmm), PT (median: 184/cmm; range: 0–784/cmm) and Asym microfilaraemic (median: 440/cmm; range: 214–665/cmm) groups were within normal range, though it was significantly lower ($P < 0.01$) than the counts in TPE group.

Table 1 summarizes the microfilaria positivity, FST positivity/reactivity and levels of filaria specific circulating antibodies in different groups of chest-symptomatic patients and Asym microfilaraemics. Microfilaria could be detected in 7/36 (19.44%) of TPE group, 6/13 of PE (4 with microfilaria in blood and PEs, and 1 each with microfilaria in blood or PE, total 46.15%) and 2/36 (5.56%) of PT patients. In these cases, microfilaraemia ranged from 3 to 1053/5 ml of blood. FST was positive in 100, 69.23, 66.67 and 92.30% of the cases of TPE, PE, PT and Asym microfilaraemic groups, respectively. Among TPE cases, 94.44% were IgM-positive and 91.67% were IgG₄-positive. In PT patients, the responses were 88.99 and 86.11%, respectively. While the per cent positivity for IgG₄ in PE (84.62%) was almost identical to that in PT patients, that of IgM (69.23%) was lower than that in PT cases. However, significantly higher ($P < 0.01$) levels of specific IgM were shown by TPE patients compared to PE. There was no significant difference in the levels of IgM between PE and PT patients, while IgG₄ levels were comparable among all the groups. In Asym microfilaraemics, 76.92% were positive for IgM antibodies and 96.15% cases were positive for IgG₄. These cases showed

Table 1. Microfilarial count, filaria skin test positivity and filaria-specific IgG₄ and IgM levels in different categories of chest-symptomatic patients from filaria-endemic areas (values are mean ± SD)

Group	No.		FST		IgG ₄		IgM	
	Microfilaria +ve	No. +ve	No. +ve	A ₄₉₂	No. +ve	A ₄₉₂	No. +ve	
TPE (<i>n</i> = 36)	7 [†] (19.44)*	36 (100)	0.71 ± 0.31	33 (91.67)	0.38 ± 0.21	34 (94.44)		
PE (<i>n</i> = 13)	6 (46.15)	9 (69.23)	0.64 ± 0.24	11 (84.62)	0.28 ± 0.15	9 (69.23)		
PT (<i>n</i> = 36)	2 (5.56)	24 (66.67)	0.63 ± 0.23	32 (88.89)	0.31 ± 0.17	31 (86.11)		
Asym (<i>n</i> = 26)	26 (100)	24 (92.30)	0.65 ± 0.46	25 (96.15)	0.22 ± 0.14	205 (76.92)		

TPE, tropical pulmonary eosinophilia; PE, pleural effusion; PT, pulmonary tuberculosis; Asym, asymptomatic microfilaraemics.

n, Number of cases; *, Per cent.

[†]Includes 4 cases with microfilaria in blood and PEs, and 1 each with microfilaria in blood or PE.

A₄₉₂, Absorbance at 492 nm.

O.D. cut-off values for seropositivity: IgG₄: 0.055 ± 0.012; IgM: 0.092 ± 0.026.

significantly lower response of specific IgM compared to TPE ($P < 0.01$) and PT ($P < 0.05$) cases.

Table 2 shows alterations in various parameters following DEC therapy in various categories of chest-symptomatic patients and Asym microfilaraemic subjects. Chest symptoms treated with DEC showed favourable response with considerable amelioration of the symptoms. In TPE cases, absolute eosinophil count significantly ($P < 0.01$) dropped after treatment. However, there was no change in AEC in other groups. Though skin test response in TPE cases was not affected by treatment, PE and PT patients showed significant ($P < 0.05$) decrease in the response. Both IgG₄ ($P < 0.01$) and IgM ($P < 0.05$) responses in TPE cases showed increase following DEC treatment. In contrast, alteration in IgG₄ level after therapy in the remaining two groups (PE and PT) was not statistically significant. IgM level in PE patients significantly increased ($P < 0.01$) after treatment. As expected, skin test reaction was negative ($P < 0.05$) in microfilaria carrier cases (Asym) after treatment. On the contrary, DEC treatment increased IgG₄ and IgM ($P < 0.01$) response.

Chest diseases and filariasis are very common in tropical countries. The two diseases may co-exist in a patient. For devising effective treatment strategies, it is therefore necessary to study the prevalence of the filarial infection and its contribution to the symptoms in chest-symptomatic patients from filaria-endemic areas. In the present study four reliable tools, namely microfilaria counting in membrane-filtered night blood, filaria-specific IgG₄ and IgM levels in sera and FST were employed to detect filariasis in patients with chest symptoms. The superiority of membrane filtration over the conventional thick smear technique for microfilaria detection is well established^{12,13} and we have earlier established the sensitivity and specificity of FST in detecting all the stages of filarial infection^{4,14}. The larval antigen did not cross-react with intestinal helminths and only 3.4% of 500 subjects infected with *Dracunculus medinensis* (guinea worm) were positive in FST¹⁴. FST gave very high percentage positivity (around 80% of the endemic population), while the percentage of microfilarial positivity in blood examination was depend-

ent on the volume of blood sample and the method of examination. Compared with an improved examination method such as membrane filtration and use of large volume of blood sample, FST was found to be 2-fold more sensitive⁴.

It is known that the levels of IgG₄ increase due to constant stimulation of the antigen. Since filariasis is a chronic disease, filaria-specific circulating IgG₄ is increased in filarial patients, the highest levels being found in Asym microfilaraemics, followed by amicrofilaraemics with or without symptoms^{15,16}. In the latter group, elevated IgG₄ could be due to adult worms, since circulating antigens are detected in these subjects¹⁷. Therefore, IgG₄ is considered an indicator of active infection^{18,19}. On the other hand, IgM was found involved in clearing microfilaria from circulation²⁰. IgM response was found highest in endemic normals and minimal in microfilaraemic subjects²¹. However, DEC therapy enhanced IgM response in microfilaraemics²².

In the present study, all TPE cases were positive for FST, as expected, and showed high levels of filaria-specific IgM and IgG₄, though only about 19% showed microfilaria in blood. The entity of TPE is well-recognized. Although the exact etiology of TPE is not known, high filaria-specific IgM, IgE and IgG₄ levels and low microfilaraemia or amicrofilaraemia are characteristics of TPE^{23,24} and reflect a hyperimmune response. In the present study 6 such cases treated with DEC showed a remarkable drop in eosinophil count and significant alterations in IgG₄ and IgM responses. These alterations further supported the fact that the TPE syndrome was due to filarial infection. Recently, Cooray and Ismail²⁵ have reported eosinophil count-dependent response to DEC in TPE cases. They have shown that patients from filaria-endemic areas who had eosinophil count greater than 3600/cmm, responded to DEC treatment with 92.5% fall in the count 3 months after administration of DEC. More than 45% of PE cases showed microfilaria in blood and pleural aspirates and 70–85% of the cases also showed FST reactivity and specific IgG₄ and IgM. This high incidence of microfilaraemia in PE cases shows the com-

Table 2. Total leucocyte count, absolute eosinophil count, filaria skin test reaction ratio (FST-RR) and filaria-specific IgG₄ and IgM levels in different categories of chest-symptomatic patients from filaria-endemic areas following treatment with diethylcarbamazine citrate (values are mean \pm SD)

Group	Treatment status	TLC/cmm blood	AEC/cmm blood	FST-RR	IgG ₄ (A ₄₉₂)	IgM (A ₄₉₂)
TPE* (n = 6)	Before DEC	11133.3 \pm 1163.9	4840.7 \pm 1836.6	3.31 \pm 1.33	0.79 \pm 0.30	0.29 \pm 0.12
	After DEC	9900.0 \pm 1218.2	1077.0** \pm 587.3	3.92 \pm 2.74	1.43** \pm 0.52	0.44 [®] \pm 0.15
PE (n = 5)	Before DEC	7140.0 \pm 1753.0	574.6 \pm 742.6	3.04 \pm 1.17	0.67 \pm 0.25	0.17 \pm 0.10
	After DEC	7860.0 \pm 1136.7	252.8 \pm 283.79	1.54 [®] \pm 0.49	0.81 \pm 0.08	0.28** \pm 0.08
PT (n = 5)	Before DEC	9220.0 \pm 769.4	157.2 \pm 267.7	4.23 \pm 1.77	0.46 \pm 0.20	0.25 \pm 0.08
	After DEC	8640.0 \pm 622.9	103.2 \pm 95.0	2.19 [®] \pm 0.98	0.91 \pm 0.56	0.37 \pm 0.16
Asym (n = 6)	Before DEC	6950.52 \pm 624.8	520.18 \pm 0.23	4.19 \pm 1.45	1.13 \pm 0.30	0.56 \pm 0.10
	After DEC	7106.42 \pm 1105.5	432.16 \pm 0.33	1.92 [®] \pm 1.15	1.99** \pm 0.40	0.90** \pm 0.29

*Abbreviations as in Table 1; TLC, total leucocyte count; AEC, absolute eosinophil count; n, number of cases; A₄₉₂, Absorbance at 492 nm; RR, reaction ratio; ** $P < 0.01$; [®] $P < 0.05$.

plexity of this syndrome, though the extent to which microfilariae contribute in the pathogenesis of the chest disease is not known. However, chest symptoms in PE cases are, at least partly, due to filarial infection, since these patients responded well to DEC treatment in the present study. DEC therapy given to five patients provided considerable improvement in symptoms, decrease in skin reaction and further increase in IgM. There was a marginal increase in IgG₄ after therapy, but this was statistically insignificant. Arora and Gowrinath²⁶, who found excellent response of a PE case to DEC therapy, also suggested filarial etiology for PE. In PE cases, Annupindi *et al.*²⁷ found microfilaria in bronchial brush cytology and suggested that the presence of microfilaria was not incidental, but was responsible for the symptoms and lesions. These patients responded well to antifilarial treatment. It was interesting to note that IgM response of Asym microfilaraemics of the present study was significantly lower than PT and TPE cases, indicating neutralization of IgM with constant release of microfilaria antigen. Indeed, Asym microfilaraemics show IgM isotypes in the immune complexes²⁸. Bal and Das²¹ have shown elevated IgM response in endemic normals and minimum response in microfilaraemic subjects. Similarly, the moderate-to-high positivity of PT patients to FST and to filaria-specific IgG₄ and IgM clearly shows that PT is associated with filariasis and that, as in PE cases, it may partly be contributing to the chest symptoms. Five cases treated with DEC showed significant decrease in FST and this confirmed the association of filariasis in PT cases. Whether filarial infection in these cases is primary or secondary and whether any of these infections make the subjects susceptible to the other, remain to be studied. Notably, more than 65% of PT cases with no microfilaria in blood and with normal eosinophil counts were FST-positive and showed filaria-specific circulating antibodies. This can be explained since all these cases were from endemic areas where exposure to infected mosquitoes was inevitable. We have earlier shown that more than 70% of endemic normals are FST-positive⁴. FST positivity and circulating filaria-specific antibodies, but no microfilaraemia in these 'normals' has been suspected to be due to absence of mature parasites and/or presence of only one sex of the parasite at any given location in the lymphatics, with no chance to mate and produce microfilaria. Filarial antigen-specific IgG₄, that is increased as a result of chronic stimulation by the parasites, has been suggested to be a suitable diagnostic marker for filarial infection and IgG₄ levels correlate more with the active infection¹⁸ than microfilaraemia²⁹. IgG₄ and FST responses of Asym microfilaraemics in the present study were similar to those reported earlier^{4,11}. Interestingly, DEC caused increased IgM response in all the categories of subjects, except in PT patients who showed only a marginal, but insignificant increase; a larger number of PT subjects may clarify the intensity of IgM response.

The present study shows co-existence of filariasis in non-TPE chest symptomatic patients from filaria-endemic areas. The respiratory symptoms in PE and PT cases may also be attributed, at least partly, to filarial infection since treatment with DEC showed considerable alterations in specific immunological response and improvement in chest symptoms in these patients. Thus the findings emphasize the need for inclusion of filaria diagnostic test(s) in the routine diagnosis of chest symptomatics, especially when the patients come from filaria-endemic areas. This may help in designing effective treatment strategies.

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ACKNOWLEDGEMENTS. This work was supported by grant CST/SERC/Med (3)/93 from UPCST, Lucknow. We thank Mr R. P. Ghosh for technical assistance, Mr S. K. Mandal, Division of Biometry, CDRI, for statistical analysis and all the patients who willingly participated in the study.

Received 25 September 2000; revised accepted 13 March 2001

Three-dimensional structure of *Mycobacterium tuberculosis* chaperonin-10 reveals a partially stable conformation of its mobile loop

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The 60 kDa and 10 kDa chaperonins form a unique multimeric complex that mediates several intracellular protein-folding reactions. The 10 kDa chaperonins interact with the 60 kDa chaperonins through a 17-residue long mobile loop which is believed to be highly flexible in the uncomplexed chaperonin-10 but adopts a well ordered conformation upon complex formation with chaperonin-60. We have now solved the three-dimensional structure of *Mycobacterium tuberculosis* chaperonin-10 and report here a partially stable conformation for its mobile loop. Evolutionary arguments and supporting experimental observations suggest additional conformational rearrangements for chaperonin-10s when associating with chaperonin-60.

THE 60 kDa and 10 kDa heat shock proteins are homologues of the well-characterized GroEL and GroES chaperonins of *Escherichia coli* and are known to be involved in various intracellular chaperone-mediated protein-folding processes^{1,2}. The larger chaperonin, GroEL, forms a tetradecameric assembly, with two heptameric rings arranged back to back^{3,4}, while the smaller 10 kDa chaperonin and its homologues from other species form a loose heptameric assembly^{5–7}. The site of functional importance is a large cavity in the tetradecameric 60 kDa chaperonins, where the nascent proteins are proposed to fold. This large cavity is closed upon capping by the heptameric

10 kDa chaperonins. The 10 kDa chaperonins interact with the 60 kDa chaperonins primarily through a 17-residue long mobile loop⁸ and regulate the release and binding of polypeptides from chaperonin-60. The mobile loop of chaperonin-10 is believed to be highly flexible in the uncomplexed chaperonin-10, but adopts a well-defined conformation in the chaperonin-60: chaperonin-10 complex⁴. The complex formation between the two chaperonins is accompanied by a large loss of conformational entropy. The entropic costs of loop immobilization, estimated to be around 8 kcal mol⁻¹, must be compensated by strong enthalpic contributions of interactions⁹.

We have now solved the crystal structure of *Mycobacterium tuberculosis* chaperonin-10 (*Mt*-cpn10) encoded by the Rv3418c ORF of *M. tuberculosis* genome¹⁰, at 3.5 Å resolution. The structure reveals that the mobile loop, can adopt a well-defined, partially stable conformation, which is essentially the same in all the seven subunits. Evolutionary arguments and supporting experimental observations suggest that chaperonin-10s undergo other conformational rearrangements apart from those observed in the mobile loop when associating with chaperonin-60.

Large amounts of *Mt*-cpn10 were purified as described earlier¹¹ and crystallizations set up in the presence of metal ions. The best crystals suitable for diffraction were obtained using hanging drops with a protein concentration of 14 mg ml⁻¹ in the presence of 10 mM CaCl₂ and with a well solution containing 26% PEG400, 210 mM Li₂SO₄ in 100 mM acetate buffer at pH 4.0. Crystals were obtained in the orthorhombic space group, C222₁, with one molecule per asymmetric unit. The structure was solved by molecular replacement using the main chain coordinates of *M. leprae* chaperonin-10 heptamer (1LEP) by Amore package available in the CCP4 suite¹². The crystal parameters and overall refinement statistics are shown in Table 1. The molecular replacement model contained all the non-Gly residues as alanines. A small stretch of residues, identified as part of the mobile loop in *M. leprae* chaperonin-10 coordinates was removed from the molecular replacement model. Coordinates were refined using either REFMAC¹³ or CNS¹⁴ programs. Five per cent randomly chosen reflections were set aside for *R*-free calculations and the same set was maintained while using either of the

Table 1. Crystal parameter, data reduction and refinement statistics

<i>a</i>	77.5 Å
<i>b</i>	162.5 Å
<i>c</i>	125.6 Å
Space group	C222 ₁
Overall <i>R</i> _{merge}	0.078
Overall completeness of data	96.4%
Overall redundancy in measurement of data	7.7
Final <i>R</i> -factor	0.202
Final <i>R</i> -free	0.265
Overall <i>G</i> -factor	-0.14

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