Serum C3d levels in tropical pulmonary eosinophilia

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Background & objectives: Results of earlier studies to evaluate the possible role of complement system in tropical pulmonary eosinophilia (TPE) using classical methods like serum haemolyte component CH50, C3 and C4 levels were inconclusive. In this study we determined levels of serum C3d which is a catabolic fragment of C3, to find out any direct evidence of activation of the complement system in TPE.

Methods: The study population consisted of 3 groups. Group A consisted of 37 patients with well characterized TPE. In group B, 26 patients with pulmonary eosinophilia had similar respiratory and haematological features as in Group A but had associated worm infestation in stool. The control group consisted of 39 healthy volunteers. Serum C3d levels were determined by sandwich ELISA technique.

Results: The serum C3d levels in TPE patients were not significantly different from those of the patients of group B or the normal controls.

Interpretation & conclusions: Absence of significant change in serum C3d goes against the possibility of complement activation in TPE. Results of our study suggest that complement system is unlikely to play a pivotal role in pathogenesis of TPE.

Key words Complement - C3d - tropical pulmonary eosinophilia

Tropical pulmonary eosinophilia (TPE) is an interstitial lung disease that results from an exaggerated immune response to filarial parasites Wuchereria bancrofti and Brugia malayi. TPE has been reported from filarial endemic regions from all over the world but is especially common in India and South East Asia. The disease is characterized by wheezing, pulmonary infiltrates and marked peripheral blood eosinophilia. Patients with TPE demonstrate very high levels of IgE and filarial specific antibodies. Studies on possible role of complement system in TPE has so far used only the classical methods like serum haemolytic component of the complement (CH50), serum C3 and C4 levels. The results so far have been inconclusive. A rise in serum C3 level was reported by some workers while others observed a rise in CH50 only. Moreover, quantification of complement components using haemolytic or immunochemical methods does not provide dynamic information and static profiles of complement system can be misleading. Quantitation of the breakdown product of complement components was found to be the most suitable way of looking at the functioning of the complement system. In the present study, we determined serum levels of C3d, which is a catabolic fragment of C3, to further evaluate the possibility of the involvement of complement system in TPE.
Material & Methods

The present study formed part of a comprehensive research project on various aspects of TPE which was carried out at the Tuberculosis Research Centre, Chennai, during mid and late 1990s.

Study population: Sixty three consecutive patients with pulmonary eosinophilia (PE) attending the Tropical Eosinophilia Clinic of Tuberculosis Research Centre at Chennai during January 1996 to March 1997 were studied. The patients were from in and around Chennai city, a region endemic for *W. bancrofti* filariasis.

For the diagnosis of TPE, the following criteria were taken into account: (i) symptoms of paroxymal nocturnal cough with or without sputum; (ii) presence of bilateral audible ronchi; (iii) absolute blood eosinophilia count of 2000 cell/µl or above; (iv) absence of circulating microfilaria in night blood and absence of eggs, ova or cysts in stool; (v) successful clinical and haematological remission with diethyl carbamazine (DEC) therapy; (vi) increased bronchovascular and reticular marking in chest roentgenogram and (vii) residence in an area endemic for human filariasis.

Though the specific anti-filarial antibodies were not determined\(^1\)\(^-\)\(^3\), 37 of the 63 patients fulfilled all other necessary criteria as mentioned above and were diagnosed as TPE (Group A). The other 26 patients with PE also had similar respiratory and haematological features but had associated worm infestation in stool; these patients were grouped and analysed separately (Group B).

Control subjects: Thirty nine healthy volunteers matched for sex and age who had no respiratory or other symptoms, were selected from the same ethnic population for study; they included laboratory volunteers and patients’ attendants. They were non-smokers, the stools were negative for ova or cysts and radiography of chest were normal.

Written informed consent from the study subjects was obtained. The study protocol was approved by ethics committee.

**Estimation of C3d:** Serum C3d was determined by sandwich ELISA technique. The standard was prepared as described earlier\(^7\). The microtitre plates were read in a semi-automated photometer (ELISA reader-Molecular Devices, USA) at 420 nm wavelength. The values were converted to arbietary units using a logit analysis and were expressed as AU/ml. Student t-test was used for data analysis.

**Results**

Of the total 63 patients with pulmonary eosinophilia, 49 were males and 14 were females. They had a mean age of 29.05 ± 13.16 (range 10-78 yr). The duration of symptoms varied from 3 wk to 3 months.

Of the 37 patients with TPE, 29 were males and 8 were females. They had a mean age of 29.95 ± 13.71 (range 11-78 yr), and had an absolute eosinophil count (AEC) of 8365.41 ± 15572.874 (range 2000-96,720) per µl. Mean serum C3d level was 1.7158 ± 2.21 (range 0.03-11.78) AU/ml. Of the 26 patients of PE with intestinal worm infestation, 20 were males. They had a mean age of 27.77 ± 12.49 (range 10-60) yr. Fifteen of these patients had metazoa in stool (*Trichorhis trichura*, *Ancylostoma duodenale* 3 and *Ascaris lumbricoides* 10) and the remaining 11 had protozoa (*Giardia lamblia* 7 and *Entamoeba hystolytica* cyst 4). The mean AEC was 7392.31 ± 6234.61 (range 2000-24500) per µl while the mean serum C3d level was 1.01 ± 1.01 (range 0.05-3.85) AU/ml. Further analysis showed that the 15 patients with metazoal infestation had an AEC of 8026.67 ± 7313.93 per µl and mean C3d level of 1.27 ± 1.22 AU/ml while the 11 patients with protozoa in stool had a mean AEC of 6527.27 ± 4567.08 per µl and a C3d level of 0.67 ± 0.51 AU/ml.

Of the 39 normal controls, 33 were males and 6 females. They had a mean age of 31.44 ± 11.054 (range 16-56) yr and had an AEC of 625.64 ± 356.68 (range 50-1600) per cmm. Their mean serum C3d level was 1.01 ± 1.01 (range 0.00-29) AU/ml. ACE levels in groups A and B patients were significantly (*P<0.001*) higher than normal control (Table).

In Group B, the pre-treatment AEC and C3d values of the 15 patients with metazoal and those of 11 patients with protozoa were not significantly different.

In 14 patients of Group A and 13 of Group B, the pre- and post-treatment serum C3d levels were compared. Analysis of the data showed that 14 patients

| Table. Group-wise details of absolute eosinophil counts (AEC) and serum C3d level of the patients and their comparison with normal controls |
|------------------------|-----------------|------------------------|
| Group                  | AEC* (per µl)   | C3d level (AU/ml)       |
| Group A (TPE)          | 37              | 8365.41 ± 15572.87*     | 1.72 ± 2.21 |
| Group B (PE            | 26              | 7392.31 ± 6234.61*      | 1.01 ± 1.01 |
| with worms)            | Normal control  | 39                      | 625.64 ± 356.68 | 1.56 ± 4.60 |

* Values are mean ± SD *P<0.001 compared to controls
of the group A had pre-treatment C3d level of 1.01 ± 1.18 AU/ml while the post-treatment value of C3d was 1.29 ± 1.93 AU/ml; the difference was not significant. Similarly, the 13 patients of group B who had pre-treatment C3d level of 0.73 ± 0.63 AU/ml, showed a post-treatment C3d level of 0.99 ± 1.58 AU/ml, the difference was not significant.

Discussion

Elevation of serum IgE has been observed in TPE, and a IgE mediated type I hypersensitivity reaction was thought to play an important part in the pathogenesis of TPE. Raised serum immunoglobulin levels (IgA, IgG and IgM) were also observed and this was further confirmed in bronchoalveolar lavage (BAL). Elevated serum levels of specific antifilarial antibodies have also been reported. Using BAL, striking elevation of total IgE along with high levels of specific IgG, IgM and IgE were found in lower respiratory tract epithelium which suggested that these specific antibodies play an important part in the pathogenesis of TPE. Earlier Udwadia found an increased IgG fluorescence in lung biopsy specimen of patients with TPE and suggested possible presence of a Type III IgG mediated reaction in TPE. Marshall et al favoured the existence of a mixed type I / type II immune response in TPE. Zumla and James suggested that the clinicopathological features in TPE are due to interplay of type I and type III reaction to microfilarial antigen.

In situ formation of antigen-antibody complexes can trigger a Gell & Coombs type III hypersensitivity reaction mediated by the complement system. An earlier study observed an increase in circulating immune complexes in patients with TPE but serum C3 levels in their patients were within normal limits. Ray and Saha observed a significant increase in serum C3 level in patients of TPE while the serum C4 levels in their patients were normal. Sharma et al did not observe any significant rise in serum C3 levels in their patients with TPE whereas the serum haemolytic complement CH50 level was found to be significantly increased. Both reports suggested that the rise either in serum C3 or in CH50 levels was due to an acute phase response to ongoing inflammation rather than any actual involvement of the complement system in pathogenesis of TPE. Interestingly, a significant rise in serum C3 levels in pulmonary eosinophilia associated with worm infestation has also been reported and similar explanation for the rise in C3 level in these patients was provided.

In the present study, we have estimated the serum levels of C3d which is a catabolic fragment of C3 as an increase in C3d level would indicate activation of the complement system. We found the serum levels of C3d in our patients with TPE not to be significantly different from those of the patients of pulmonary eosinophilia associated with worm infestation or the normal controls.

A critical role for complement C3d and the B cell receptor (CD19/CD21) complex in the initiation of inflammatory arthritis has been reported. Immune complex production and deposition as well as complement activation have been regarded as the principal aetiology of erythema nodosum leprosum (ENL). In a recent review of the pathology of ENL, the findings of deposition of immune complexes and complement C3 and C3d together with Mycobacterium leprae in the skin lesions with significant elevation of serum levels of C3 and C3d have been critically analysed. An earlier study had shown a significant and specific correlation between elevated serum C3d levels and ENL.

In respiratory tract immune defence mechanism against pathogens, complement system has important inflammatory and antimicrobial function. Deposition of C3b clevage product, C3d, greatly enhances the uptake and presentation of microbial antigen by antigen specific B cells and so potentiates the development of strong antibody response. In conclusion, the results of our study suggest that complement system is unlikely to play a pivotal role in the pathogenesis of TPE. However, active participation of the complement system in the pathogenesis of TPE can be ruled out completely only by studying the local response at the site of the lesion.

References

5. Sharma SK, Pande JN, Khilnani GC, Verma K, Khanna M. Immunologic & pulmonary function abnormalities in tropical


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