Indian J Med Res 134, July 2011, pp 79-82

Acquired alpha 1-antitrypsin deficiency in tropical pulmonary eosinophilia

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Received August 20, 2009

***Background & objectives*: Observation of an increased frequency of an intermediate deficiency of serum alpha1-antitrypsin (α1-AT) in patients with Tropical Pulmonary Eosinophilia (TPE) was earlier reported. Though the possibility of existence of an acquired deficiency was suggested, without phenotyping a hereditary α1-AT deficiency in TPE could not totally be ruled out. In this study, we have done Pi (Protease inhibitor) phenotyping to investigate the possibility of association of any heterozygous (or homozygous) α1-AT deficiency in patients with TPE.**

***Methods*: Serum a1antitrypsin (α1-AT) was measured in 103 patients (Group A) with TPE, 99 patients with pulmonary eosinophilia who had associated intestinal worm infestation (Group B) and 43 healthy volunteers who served as controls. In 19 α1-AT deficient patients (9 of Group A and 10 of Group B), α1-AT level was measured before and after treatment. In 58 patients with TPE and in 5 controls, phenotyping was done.**

***Results*: Fifteen patients of Group A and 16 from Group B showed intermediate α1-AT deficiency (150 mg % or less. None of the control subjects had α1-AT deficiency (<200 mg%). After treatment with DEC and/or deworming, in 19 patients there was a significant ($P < 0.001$) rise in α1-AT levels. Results of phenotyping showed that all had M_1 or M_2 allele and none had S or Z variant (either homozygous or heterozygous) thus ruling out any underlying genetic cause for the observed α1-AT deficiency.**

***Interpretation & conclusions*: The observed α1-AT deficiency may be due to the chronic inflammation in TPE and associated oxidative stress. However, in such α1-AT deficient patients with TPE and those with worm infested pulmonary eosinophilia, faecal α1-AT concentration and faecal α1-AT clearance should be routinely estimated to rule out the possibility of any intestinal protein loss.**

Key words α1-antitrypsin - acquired deficiency - tropical pulmonary eosinophilia - phenotyping

Previous studies in tropical pulmonary eosinophilia (TPE) have mostly been on clinical, histopathologic, lung function and immunological aspects. However, studies which address the important issue of host factor in TPE are not available. Observation of an increased occurrence of alpha1-antitrypsin (α1-AT) deficiency in patients with TPE was earlier reported from Vellore[1]. The observed intermediate deficiency was suggested to be acquired in nature. The present study was undertaken to confirm an increased occurrence of

α1-AT deficiency in TPE and also to further look into the possibility of any underlying hereditary cause for such deficiency.

Material & Methods

Selection of patients: The present study is part of a comprehensive research project on various aspects of TPE carried out at the Tuberculosis Research Centre, Chennai, during mid and late 1990s. A total of 1157 patients attending the Tropical Eosinophilia Clinic at the Tuberculosis Research Centre, Chennai, with respiratory symptoms were screened for eosinophilia and 202 consecutive patients with elevated absolute blood eosinophil counts of 2000/µl or above were identified. All these patients who were residents of Chennai city were divided into two groups: Group A consisted of 103 patients with TPE who fulfilled the following criteria for diagnosis of TPE: (*i*) symptoms of recent onset of paroxysmal nocturnal cough with or without sputum, (*ii*) presence of bilateral audible rhonchi, (*iii*) absolute blood eosinophil count (AEC) of 2000/µl or above, (*iv*) absence of circulating microfilaria in blood and absence of egg, ova or cysts in stool, (*v*) successful clinical and haematological remission with diethylcarbamazine (DEC) therapy, (*vi*) increased bronchovascular and reticular markings in chest roentgenograms, and (*vii*) residence in an area endemic for *Wuchereria bancrofti* filariasis.

Group B consisted of 99 patients with pulmonary eosinophilia who had similar respiratory symptoms and other features of TPE as described above and associated intestinal parasites in stool. The majority of the patients required DEC therapy for successful remission usually following deworming. Presence of parasites did not automatically exclude them from being categorized as TPE. However, all patients with intestinal parasitic infestations were grouped separately.

The filarial specific IgG done for confirmation of diagnosis of TPE[2] showed very high serum titre in 58 patients and they were analysed separately.

Selection of control subjects: Forty three healthy volunteers who were free of intestinal parasites and matched for age and sex from the same population were selected as controls. They gave no history of smoking and had no significant respiratory symptoms or any personal or family history of allergy; their chest radiography was normal.

All selected patients were non-smokers and had no past history of jaundice or liver disease or any other serious illness. Chronic diseases such as diabetes mellitus was excluded in the study population and their sputum smears for acid fast bacilli (AFB) were negative (patients and controls). All female subjects (both control and the patients) were not taking oral contraceptive pills.

Informed consent was obtained from all the study subjects. The study protocol was approved by the ethics committee of TRC.

Laboratory studies: For routine tests, 4 ml of venous blood was collected and another 3 ml of blood was withdrawn in a separate syringe, the serum from which was stored at –80°C for performing further tests as and when the necessary reagents/kits became available. All experiments could be completed only at a later date well after initiation of the project. Serum concentration of α1-AT was measured by radial immunodiffusion method employing specific antiserum (Sigma Immunochemistry, USA) using α1-AT standards (Behringwerke AG, Marburg, Germany)[3]. Pretreatment α1-AT levels between 10-60 per cent of the mean serum value of normal were considered as intermediate deficiency and a level below 10 per cent as severe deficiency[1]. Anti-filarial IgG antibodies were estimated by enzyme immunoassay, using antigen from adult filarial worms and microfilaria[2]. α1-AT phenotyping was done by isoelectric focusing at *p*H between 4-5 using ampholytes[4].

In 19 patients (Group A 9, Group B 10) who had intermediate α1-AT deficiency, the effect of drug therapy was followed up. Blood samples were collected before and after treatment with DEC alone in 9 patients, deworming alone in 4 and 6 patients had deworming therapy followed by DEC. Student t-test was used for data analysis.

Results & Discussion

Pretreatment characteristics of the study population are outlined in the Table. None of the control subjects had α1-AT at below 200 mg%. Fifteen patients of Group A showed a serum α1-AT level of < 150mg% which was considered as intermediate deficiency. Of the 99 patients of pulmonary eosinophilia with associated worm infestation in stool (Group B), 45 patients had metazoal infestations. (*Ancylostoma duodenale* 8, *Ascaries lumbricoides* 20 and *Trichuris trichura* 8) while the other 54 had protozoa (*Giardia lamblia*, 25,

Table. Pre-treatment characteristics of the study population

Characteristics	Group A (n= 103)	Group B (n=99)	Controls (n=43)
Age (yr)	28 ± 12	24 ± 12	33.2 ± 8.7
Sex (M:F)	69:34	69:30	25:18
AEC levels (per μl)	6736 ± 10559	6304.55 ± 5395.53	300.00 ± 195.5
α1AT levels (mg %)	267 ± 117	259.76 ± 95.01	248.1 ± 44.7

Values are mean ± SD. AEC, absolute eosinphil count

Entamoeba histolytica cyst, 29) infestation in stool. Sixteen patients of Group B showed an intermediate α1-AT deficiency below <150 mg%.

In 19 patients (group A 9, group B 10) with α1-AT level <150 mg% pre- and post-treatment values of AEC and α1-AT were compared by paired t-test. Following therapy the pre-treatment AEC level of 4792.11 ± 3491.3 per μl fell significantly to 1428.95 ± 812.6 per μl (P<0.001) while the pretreatment α1-AT level of 117.8 ± 21.2 mg% rose significantly to 192.74 ± 57.9 mg% (P<0.001). Groupwise analysis before and after treatment also showed similar results.

In 58 patients with TPE, the serum titres of specific IgG filarial antibody was very high (P< 0.05) at 2665.149 ± 2369.397 mg/ml while it was 130.55 ± 99.48 mg/ml in five normal subjects. Among these 58 patients, 8 had intermediate deficiency with α1-AT <150 mg%.

α1-AT phenotyping done in the 58 patients with TPE and 5 normal subjects showed all of them having exclusively variants of normal M allele. None of the normals or the patients (including 8 with α1-AT deficiency) showed S or Z variant (either homozygous or heterozygous). Six of the α1-AT deficient patients showed M_1M_1 pattern while the other two showed M_2M_2 pattern.

α1-AT deficiency, a common genetic disorder, is now recognized as a classic example of a conformational disease of the liver and a disequilibrium in the lung proteases[5]. The previous report of an increased prevalence of α1-AT deficiency in TPE had not adequately investigated the genetic basis of the disorder. Hitherto, all Indian studies including the one from Vellore[1] have relied on low α1-AT levels as basis for determining heterozygous or homozygous status which cannot be done without phenotyping. In this study, we have again demonstrated the presence of an increased frequency of α1-AT deficiency in patients with TPE. Improvement in α1-AT levels following successful therapy suggests that the observed α1-AT deficiency was acquired in nature. This observation was further confirmed by the results of phenotyping and the absence of S or Z variant ruled out any hereditory cause for α1-AT deficiency (hetero- or homozygous) in TPE.

Increased oxidative stress in smoking and chronic obstructive pulmonary disease (COPD) with important consequences which include oxidative inactivation of antiprotease and reduced α1-AT level has been observed earlier[6,7]. Oxidative stress has a role in enhancing the inflammation that occurs in smokers and in COPD[8]. Our patients had no history of COPD and were non-smokers. However, oxidative stress has been found to occur in TPE. Increased oxidative stress in chronic and occult filariasis which improved significantly following DEC therapy has been reported[9]. Also, bronchoalveolar lavage studies with long term observation of patients with TPE following conventional treatment with DEC had demonstrated presence of chromic respiratory tract inflammation and persistent eosinophilic alveolits[2]. Thus it is possible that the observed α1-AT deficiency may be due to the chronic inflammaton in TPE and associated oxidative stress which needs further study.

While exploring the other possible causes of the acquired deficiency in TPE, it is worth noting that the present study as well as the Vellore study[1] demonstrated an increased frequency of α1-AT deficiency in patients of pulmonary eosinophilia associated with intestinal worm infestation also. A non-filarial TPE-like syndrome caused by other helminths with elevated levels of antifilarial antibodies has been described[10]. Besides, occult helminthiasis in patients with TPE can never be totally excluded and in fact, it has been reported that in 20 per cent of patients with TPE, examination of stool may reveal other helminths[11]. It should also be noted that protein-losing enteropathy has been documented in parasitic infection of the gut including hook worm, round worm and giardia infestations[12-14]. Thus the possibility of intestinal protein loss in patients with TPE and also in patients with worm infested pulmonary eosinophilia being a possible

cause of the observed α1-AT deficiency cannot be ignored and therefore estimation of faecal α1-AT concentration and faecal α1-AT clearance which has been found to be easy and reliable methods[12-14] should be routinely done to exclude such a possibility.

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