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A study on human leukocyte antigen class I molecules in paediatric bronchial asthma

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Childhood asthma, often associated with atopy, is more common in boys and may persist throughout life in 50% of cases. This case-control study was carried out to examine if any association of paediatric bronchial asthma with human leukocyte antigen (HLA) class I antigens. Thirty-six children with bronchial asthma diagnosed on basis of Global Initiative for Asthma (GINA) criteria and an equal number of healthy controls without history of bronchial asthma were studied. Low resolution HLA- ABC typing was performed by sequence specific primers (SSP) and the frequency of HLA–ABC antigens in the two groups was compared. Total serum immunoglobulin E (IgE) estimation was done as a marker of atopy by ELISA. The study included 24 boys and 12 girls aged 13 months to 11 yrs, of which 16 (44%) had positive family history. Serum IgE levels were elevated in 20 (55%) of the cases and 33% of controls with peak values of 4877 and 627 IU/ml, respectively. No statistically significant correlation was observed between childhood asthma and HLA class I antigens, however, a statistically significant correlation was observed between serum IgE levels and asthma, which was elevated in cases, as compared to normal population. Serum IgE levels did not show a linear trend, in that a direct correlation with the severity of disease was not observed.

Keywords: Human leukocyte antigen class I, Paediatric bronchial asthma, Serum immunoglobulin E

Bronchial asthma may be regarded a complex, multi-factorial disease of the bronchi and bronchioles in which both the genetic micro-heterogeneity and environmental factors are involved. Childhood asthma is usually of extrinsic type characterized by airway hyperreactivity, episodes of reversible bronchoconstriction, mucus hypersecretion and eosinophilia[1]. Exposure to allergens may lead to development of symptoms of acute asthma. Immunoglobulin E (IgE) comprises a very small fraction of the immunoglobulins in the serum and its levels are high in individuals with atopy. Atopic asthma is commonest type of asthma and is a classic example of type 1 associated hypersensitivity reaction which usually commences in childhood. The function of human leukocyte antigen (HLA) molecules is antigen presentation; class I molecules present endogenous antigens and class II molecules present predominantly exogenous antigens. Atopic asthma is due to environmental antigens, so class I molecules are unlikely to have a role in its pathogenesis.

Although many genes have been implicated in the pathogenesis of asthma, in the current study, we have examined the association of HLA class I antigens with childhood asthma[2]. Most of the studies on HLA association with asthma have examined the association with HLA class II molecules and only a handful of studies have been made on HLA class I association[3-5].

Subjects and Methods

Subjects

The study was carried out at Command Hospital (SC), Pune. Thirty-six consecutive children (24 boys and 12 girls) aged between 13 moths to 11 yrs with bronchial asthma diagnosed on the basis of Global Initiative for Asthma (GINA) criteria who were under treatment in the outpatient department (OPD) or in the paediatric ward were included in this study[6]. All cases had been under regular follow-up in the OPD – many of them for over 2-5 yrs with regular clinical and laboratory evaluation. Although GINA categorizes children as those below 16 yrs age, we restricted the age to 14 yrs because only individuals <14 yrs were recognized as children for purpose of hospital admission.

The inclusion criteria included: (i) Age < 14 yrs, (ii) Definite clinical diagnosis of bronchial asthma by paediatrician supported by pulmonary function tests

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Abbreviations: CI, confidence interval; GINA, Global initiative for asthma; HLA, human leukocyte antigen; IgE, immunoglobulin E; OR, odds ratio; SSP, sequence specific primers.

in compliant children, (iii) Recurring episodes of non-productive cough, breathlessness and wheezing, having family history in some cases with reversibility of airway obstruction when treated with bronchodilators alone or with corticosteroids, and (iv) Cough variant asthma where asthma causes chronic cough which can be the only manifestation. The cases were classified according to the GINA criteria into intermittent, mild, moderate and severe persistent types of asthma[5]. Exclusion criteria included history of pulmonary tuberculosis or other restrictive lung diseases. The control group comprised of 36 (25 boys and 11 girls aged between 2-12 yrs) unrelated children with no history of early onset asthma. They were matched for age and gender with respect to the subjects.

Clearance had been accorded by the Institutional Ethical committee for carrying out the study. Informed consent was obtained from parents of all cases and also from control population.

Methods

Whole blood samples were collected in paediatric EDTA and sterile vacutainers for DNA extraction and serum IgE estimation respectively. DNA was extracted by column based method using kits from Qiagen (Germany) as described previously[7].

Low resolution HLA class I typing for ABC antigens was done by Olerup SSP^{TM} kits (Austria) for all cases and controls as per the instructions in product insert. HLA – A*80 which was identified in three patients was reconfirmed on fresh samples because of its rarity in Indian population. Total serum IgE estimation was done in duplicate by enzyme linked immunosorbent assay (ELISA) kits from (Lilac, India) which contained high affinity and specificity antibodies with different and distinct epitope recognition. The kit also consisted of six human serum references which were used as calibrators from 0-400 IU/ml. The normal values of serum IgE till age of 3 yrs was 0-46 IU s/ml and for those between 4-12 yrs – 0-280 IU/ml. With a cut-off of 280 IU/ml, the serum IgE levels in patient population was analyzed for their correlation with class I antigens.

Statistical analysis

Statistical analysis of accrued data was done using Epi-info-6 software. The frequencies of HLA class I antigens in patients and controls were compared. Odds ratio (OR) was calculated as the cross product in a 2 × 2 table and 95% confidence intervals (CI) were estimated. OR and P values were calculated for all antigens.

Results

The patients' age ranged from 13 months to 11 yrs with 16 (44.4%) children less than 5 yrs age. The gender distribution was skewed in favour of boys with a ratio of 2:1. Fifty-seven percent of our cases had a positive family history. The distribution of cases as per the severity of disease was: severe n = 6 (16.6%), moderate n = 22 (61.2%) and mild n = 8 (22.2%).

Serum IgE levels were normal in only 43% of patients, with 11% having higher than 4000 IU/ml and peak value was 4877 IU/ml and mean value of 731 IU/ml. Corresponding values in control population were much lower. The antigen HLA – A*33 was noted in patients with raised serum IgE, but the prevalence of other antigens was similar in both cases and controls. During the study, we identified A*80 in three unrelated children which was a rare in Indians, as we did not encounter the antigen in over 2000 clinical typings performed in the lab.

There was no statistically significant correlation between presence of HLA class A, B and C antigens in cases and controls. 95% CI of odds ratio was calculated (Tables 1-3). 95% CI of all calculated odds ratio included 1 and P values for all antigens were more than 0.05, hence they were not statistically significant.

Table 1—Frequency of HLA-A antigens in cases and controls

Antigen	Cases (n = 35)	Controls (n = 36)	OR (95 %CI)
A*01	9	13	0.61 (0.22-1.70)
A*02	9	12	0.69 (0.25-1.93)
A*03	5	1	5.83 (0.65-52.74)
A*11	5	7	0.69 (0.20-2.42)
A*23	2	02	1.03 (0.14-7.75
A*24	14	13	1.18 (0.45-3.08)
A*26	1	1	1.03 (0.06-17.13)
A*29	3	2	1.59 (0.25-10.17)
A*30	0	2	---
A*31	3	1	3.28 (0.32-33.17)
A*32	0	4	----
A*33	10	9	1.20 (0.42-3.44)
A*34	1	0	---
A*36	3	0	-----
A*68	3	3	1.03 (0.19-5.49)
A*80	3	0	----

Table 2—Frequency of HLA-B antigens in cases and controls

Antigen	Cases (n = 35)	Controls (n = 36)	OR (95 %CI)
*07	4	4	1.03 (0.24-4.50)
*08	2	2	1.03 (0.14-7.75)
*13	3	5	0.58 (0.13-2.64)
*15	14	7	2.76 (0.95-8.03)
*18	2	1	2.12 (0.18-24.51)
*27	1	2	0.50 (0.04-5.78)
*35	12	11	1.19 (0.44-3.21)
*37	2	2	1.03 (0.14-7.75)
*38	0	3	---
*40	9	8	1.21 (0.41-3.61)
*44	8	4	2.37 (0.64-8.74)
*50	1	0	----
*51	3	8	0.33 (0.08-1.36)
*52	1	4	0.24 (0.02-2.2)
*53	1	1	1.03 (0.06-17.13)
*55	3	0	---
*56	2	0	------
*57	3	5	0.58 (0.13-2.64)
*58	4	6	0.67 (0.17-2.60)

Table 3—Frequency of HLA-C antigens in cases and controls

Antigen	Cases (n = 35)	Controls (n = 36)	OR (95 %CI)
*01	6	1	-
*02	1	5	0.18 (0.02-1.65)
*03	9	10	0.94 (0.33-2.69)
*04	11	12	0.92 (0.34-2.48)
*06	3	2	1.59 (0.25-10.17)
*07	20	18	1.33 (0.52-3.40)
*08	3	2	1.59 (0.25-10.17)
*12	1	6	0.15 (0.02-1.29)
*13	0	3	-
*14	6	3	2.28 (0.52-9.93)
*15	9	13	0.61 (0.22-1.70)
*16	3	3	1.03 (0.19-5.49)

Discussion

Asthma runs strongly in families and its heritability has been estimated at 60%[8]. Childhood asthma is more common in boys, whereas adult onset asthma is more common in women[2]. Our study also had a preponderance of boys and a positive family history was present in 57% of cases which was lower than that mentioned in another study[9]. A hereditary prevalence study in an Indian population revealed that 81 of the 100 asthmatics had a positive family history of allergic diathesis in contrast to 36.6% of controls, which was consistent with findings of Burke *et al.*[10]. that the risk of having asthma increased by 2-4 times in case of a child having one asthmatic parent.

Cutaneous allergy testing was not available in our centre, so we used raised serum total IgE as a marker of atopy and it was raised in two-third of the children with much higher peak values, as compared to controls. Serum IgE was elevated in all cases with severe asthma, but the severity did not correlate with serum IgE levels. The data base of www.allele.frequencies .com did not show any report of HLA A*80 from India and is more common in African population. The product insert confirmed that the kit was tested against cell lines with A*80 and hence capable of correctly identifying the antigen.

Earlier, no association was found between HLA class I antigens and childhood asthma in mite-sensitive Greek children[3]. Two other studies, one from Spain[11] and another from United Kingdom[3] also did not show any definitive HLA association in both atopic and non-atopic asthmatics. However, in a recent study from Croatia, the antigen HLA-B8 was found to be significantly increased among those with elevated serum IgE, asthmatics as well as in patients sensitized to *Dermatophagoides pteronyssinus*[12].

Strengths of this study included the fact that it was done on children all of whom had atopic asthma and the diagnosis was established by clinical and pulmonary function tests. Secondly, all patients included in this study had been under regular follow-up for 2–3 yrs. However, the study was limited by small number of cases. Borish *et al.* reported higher total IgE levels in males, children, smokers, non-white racial/ethnic groups and adults with childhood-onset disease in patients with severe or difficult-to-treat asthma[13]. We did not find such association, which could be due to small number of severe form of disease.

The difference in antigen association with paediatric asthma in different populations may be dependent on their ethnicity and environmental exposure. However, more studies are required with larger sample size to confirm or negate the association of HLA class I antigens in Indians, before we can label a particular antigen as being protective or causative for asthma.

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