DETERMINANTS OF BRONCHIAL HYPERRESPONSIVENESS IN SUBJECTS WITH RHINITIS

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Subjects with rhinitis but without asthma may have coexisting bronchial hyperresponsiveness, although the reasons for this are uncertain. To evaluate the factors that determine BHR in rhinitis we examined 410 patients with symptomatic rhinitis with forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) ≥ 80% of the predicted value. In all subjects a skin prick test (SPT) was performed, a determination of total serum IgE and an eosinophils count in the blood. Of the 410 subjects we found that 161 (39.3%) exhibited a methacholine PD_{20} of 800 mg or less (Group A), whereas 249 (60.7%) had a methacholine PD_{20} more of 800 mg (Group B). Despite the matched mean values for FEV_1 and FVC, compared with Group B, Group A had a lower predicted forced expiratory flow between 25% and 75% ($FEF_{25\%-75\%}$) (86.7 ± 12.0 *vs.* 93.7±7.3, $P < 0.0001$). A great portion of the subjects of the Group A in respect to subjects of the Group B were exposed to passive smoke (37.8% *vs.* 22.0%, $P = 0.0008$), reported having mothers with asthma (34.1% *vs.* 6.0%, $P < 0.0001$), presented a positive skin prick test (93.7% *vs.* 67.0%, $P < 0.0001$), had higher levels of total serum IgE (geometric mean of Log_{10} 2.46 ± 0.27 kU/L *vs.* 2.06 ± 0.38 kU/L, $P < 0.0001$) and higher blood eosinophil counts (geometric mean of Log_{10} 2.67 ± 0.07 x 10^{-3} mL *vs.* 2.57 ± 0.09 x 10^{-3} mL, $P < 0.0001$), and reported increased nasal obstruction (2.0 (95%CI 1.8 to 2.2) *vs.* 0.6 (95%CI 0.5 to 0.7), $P < 0.0001$). Logistic regression demonstrates that nasal obstruction (OR 2.19, 95%CI 1.72 to 2.80) and the presence of positive SPT (OR 6.15, 95%CI 2.42 to 15.61) were the most available predictors to discriminate between subjects with BHR and subjects without BHR. In addition, BHR was positively related to blood eosinophil counts (OR= 2.80, 95%CI 1.54 to 5.07), $FEF_{25\%-75\%}$ values (OR= 2.72, 95%CI 1.23 to 5.99) and familiarity (mother) for asthma (OR = 2.45, 95%CI 1.10 to 5.46). Whereas passive smoke and total serum IgE were not positively related to BHR. Increased nasal obstruction and the presence of positive SPT were the most available predictors to discriminate between subjects with and without BHR. Finally, BHR was positively related to blood eosinophil counts, $FEF_{25\%-75\%}$ values and to familiarity (mother) for asthma.

Bronchial hyperresponsiveness (BHR) is a hallmark feature of asthma. However, BHR is also present in 19 to 62% of subjects without respiratory symptoms in the general population (1). The mechanisms underlying BHR in non-asthmatic subjects are still unclear. BHR, in subjects with rhinitis, can be regarded as a surrogate marker of inflammation and has also been linked to long term deleterious consequences, such as bronchial remodelling (2-3). Epidemiological, pathophysiological and clinical studies strongly suggested a relationship between allergic rhinitis and

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allergic asthma (4-5). Allergic rhinitis is correlated with and probably constitutes a risk factor for the occurrence of asthma (5). Some investigators have suggested that BHR in the asthmatic range may help to identify patients with rhinitis who are at risk for asthma (6-7).

Allergy is a process mediated by immunoglobulin E (IgE). Mast cells in the bronchial wall are activated by IgE and, as a consequence, release mediators that may cause BHR directly (histamine, prostaglandins, and leukotrienes) or indirectly (interleukin-4 (IL-4) and tumor necrosis factor α (TNF-α). IL-4 stimulates B cells to produce IgE, which maintains mast cell activation. Further, IL-4 and TNF-α enable eosinophils to migrate from the vessels into the bronchial mucosa by upregulation of vascular adhesion molecules (8-9). Eosinophils, once activated, release mediators, which damage the epithelium and cause BHR due to increased permeability (8, 10-11). However, increased total serum IgE levels and peripheral blood eosinophil counts are neither closely related or exclusively present in atopic individuals. Total serum IgE levels are also increased in nonatopic smokers, and peripheral blood eosinophils are also elevated in parasitic infections and in certain neoplasms (12). Thus, skin test responses, total serum IgE levels, and the number of peripheral blood eosinophils are independent features of a common underlying mechanism. The objective of our study was, therefore, to identify any putative factors that determined BHR in patients with allergic and nonallergic rhinitis.

MATERIALS AND METHODS

We examined 410 volunteers with rhinitis, non smokers, over 18 years old, with and without positive skin prick test (SPT). All patients had a forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) of 80% or more than predicted. We grouped rhinitic subjects into those with BHR to methacholine (MCh) (Group A; provocative dose causing a 20% decrease in FEV_1 (PD_{20}), between 100 and 800 μg, equivalent to a provocative concentration causing a 20% decrease in FEV_1 (PC_{20}) < 1.5 mg/mL) and those without BHR (Group B; PD_{20} > 800 μg; equivalent to a PC_{20} > 1.6 mg/mL). Categories were decided according to American Thoracic Society guidelines (13).

In all subjects we excluded the presence of asthma or asthma-like symptoms. Furthermore, no respiratory tract infection was reported within 3 months before clinical and functional evaluation.

Skin Prick Test

Skin Prick Tests (Alk Abellò, Milan, Italy) for common aeroallergens of West Sicily (trees (*Olea* and *Cupressus*), weeds (Grass, Mugwort and *Parietaria judaica*), house-dust mites (*Dermatophagoides pteronyssimus* and *Dermatophagoides farinae*), *Aspergillus*, dog and cat dander) were performed on the volar aspect of the forearm after antihistamines had been withheld for at least 5 days. Glicerine and histamine (10 mg/mL) were used as negative and positive controls, respectively. A positive response was defined as any wheal with a diameter 3 mm greater than the negative control 15 minutes after application (14).

Spirometry

Lung function measurements

FEV_1 was measured with a Gould 2400 automated system, taking into account the highest of 3 successive measurements, provided that the difference between measurements was within 100 mL. MCh challenge was performed according to the Chai's method (13,15-16). Increasing concentrations were administered with a MEFAR nebulizer (Markos, Monza, Italy). After taking baseline measurements of FEV_1, subjects inhaled five puffs of saline solution. The diluent value was considered as the control, and if the FEV_1 variations were within 10% of baseline, the subjects inhaled increasing concentrations of MCh, ranging from 16 to 5120 μg/mL, at 5-minute intervals, until a 20% decrease from the baseline FEV_1 was recorded. The PD_{20} was determined by computer-assisted logarithmic interpolation of the log dose-response curve. If the patients did not react, a censored value of 5120 μg was given (16).

Laboratory analyses

Total serum IgE

Measurement of total serum IgE was carried out according to the fluoro enzyme immunoassay method (Pharmacia Diagnostic, Uppsala, Sweden). The reported values represent the mean of duplicate tests and were expressed in kU/L. The normal range of total serum IgE is 2-100 kU/L.

Eosinophil blood counts

A venous blood sample was collected, and absolute peripheral blood eosinophil counts were determined with Technicon-H1 blood cell counter (Bayer Leverkusen, Germany). The normal range is 0.10-0.40 x 10^{-3} cells μL (14,16).

Clinical score

The symptoms of nasal obstruction, sneezing and rhinorrhea were rated on an arbitrary four-point rating scale, from 0 to 3. Nasal obstruction was scored as follows: 0 = not present; 1 = slightly difficult breathing through the nose; 2 = moderately difficult breathing through the nose; 3 = very difficult or impossible breathing through the nose. Any other recorded symptom was scored as follows: 0 = none; 1 = mild (occasional present); 2 = moderate (rather frequent); 3 severe (persistent) (17-19).

Statistical analys

Data of total serum IgE and blood eosinophil counts were not normally distributed, so Log_{10}-transformed data were used in all calculations. Data are presented as geometric mean of Log_{10} values ± SD. Chi-square test and Mann-Whitney U test were used to compare the characteristics of subjects with and without BHR. Logistic regression analyses were performed to determine the relationship between BHR and skin test responses, total serum IgE levels, peripheral blood eosinophilia, familiarity for asthma, passive smoke, $FEF_{25\%-75\%}$ and nasal obstruction. Each variable was dichotomized, i.e. the presence or absence of skin test positivity, levels of total serum IgE >100 kU/L or < 100 kU/L, blood eosinophil counts > 0.40 x 10^{-3} or < 0.40 x 10^{-3} μL, presence or absence of familiarity for asthma, and finally presence or absence of nasal obstruction. All analyses were repeated with serum total IgE levels, peripheral blood eosinophil counts and nasal obstruction as continuous variables. A *P* value < 0.05 was considered significant.

RESULTS

A total of 410 subjects with complete information on rhinitis symptoms, BHR, SPT, serum total IgE levels, eosinophil blood counts, family history of asthma, active and/or passive smoke, living (urban or rural), and lung function measurements (FEV_1, FVC, $FEF_{25\%-75\%}$) have been included. No subjects reported asthma or asthma-like symptoms at the clinical visit. Characteristics of the examined subjects are displayed in Table I. All subjects reported the onset of the symptoms of rhinitis over at least two years (mean of years 7.6 ± 4.1).

Lifestyle

Two hundred ninety five subjects (72.0%) lived in urban areas and 115 (28.0%) in rural areas. With regards to smoking, all subjects declared not to be active smokers, whereas 116 subjects were exposed to passive smoking.

SPT

On the basis of the SPT, 92 subjects resulted negative to twelve allergens tested and 318 subjects resulted positive to one or more of them. Of 318 subjects with SPTs positive, 157 subjects were positive to only pollen allergens tested, 58 subject were positive only to perennial allergens tested and finally 103 subjects were positive to pollen and perennial allergens.

Total serum IgE

Three hundred seven subjects (75.1%) have total serum IgE higher of 100 kU/L (> 2 Log_{10}) and 103 subjects (24.9%) have serum total IgE ≤ 100 UI/mL (≤ 2 Log_{10}). The geometric mean of Log_{10} total serum IgE was 2.25 ± 0.39 kU/L (~180 kU/L).

Blood eosinophil counts

172 subjects (42.0%) had blood eosinophil higher of 0.45 x 10^{-3} μL and 238 (58.0%) had eosinophil count ≤ 0.45 x 10^{-3} μL. The geometric mean of Log_{10} blood eosinophil counts was 2.61 x 10^{-3} μL ± 0.20 (~ 0.41 x 10^{-3} μL)

BHR

BHR evaluated as MCh PD_{20} resulted in the asthmatic range (≤ 800 μg/mL of MCh) in 161 subjects (39.3%), whereas in 249 subjects (60.7%) was > 800 μg/mL of MCh. The mean of dose provocation of MCh was 1634 ± 1081 μg/mL.

Symptoms of rhinitis

For the diagnosis of rhinitis we considered the presence of at least two of the following symptoms re-occurring more than once a day per month for at least two years: nasal obstruction, sneezing and rhinorrhea. Two hundred and fifty eight subjects (62.9%) reported two symptoms and 152 subjects (37.1%) three symptoms. In particular, the nasal obstruction was present in 220 subjects (53.7%) and was absent in 190 subjects (46.3%). Sneezing was reported by 364 subjects (88.8%) whereas this was not reported by 46 subjects (11.2%). Finally, rhinorrhea was reported by 386 subjects (87.1%) and was absent in 24 subjects (5.9%).

Table I. *Characteristics of Study subjects.*

Subjects (No)	410
Age (mean years (CI95%))	29.3 (28.3-30.2)
Gender (Female/Male)	243 / 167
Onset of the symptoms of rhinitis (mean of years ± SD)	7.6 ± 4.1
Living (Urban/Rural)	295 / 115
Familiarity for asthma (Yes/No)	145 / 265
- Father	40 / 370
- Mother	70 / 340
- Brother and/or Sister	79 / 331
Passive Smokes (Yes/No)	116 / 294
SPT (Positive/Negative)	318 / 92
Number of subjects with total serum IgE	
> 100 UI/mL / <100 UI/mL	307 / 103
Number of subjects with Blood Eosinophil count	
> $0.45x10^{-3}$µL / < $0.45x10^{-3}$µL	172 / 238
Number of subjects with BHR in asthmatic range	
(PD_{20} ≤ 800 mg/mL of MCh)	161 / 249
Nasal obstruction (Present/Absent)	220 / 190
Sneezing (Present/Absent)	364 / 46
Rhinorrhea (Present/Absent)	386 / 24

Subjects with and without BHR

In Table II we reported the characteristics of subjects with BHR (Group A) and without BHR (Group B). All subjects have a FEV_1 and a FVC of 80% or more than predicted, whereas significant differences between Group A and Group B were found in predicted $FEF_{25\%-75\%}$ (86.7 ± 12.0 *vs.* 93.7 ± 7.3, P < 0.0001), in the proportion of subjects with positive SPT (93.7% *vs.* 67.0%, P < 0.0001), in the geometric mean of Log_{10} of total serum IgE (2.46 ± 0.27 kU/L *vs.* 2.06 ± 0.38 kU/L, P < 0.0001) and in the geometric mean of Log_{10} of blood eosinophil counts (2.67 ± 0.07 x 10^{-3} µL *vs.* 2.57 ± 0.09 x 10^{-3} µL, P < 0.0001). Finally, it has been found significant differences between the Group A and Group B in the proportion of subjects who were exposed to passive smoking (37.8% *vs.* 22.0%, P = 0.0008%), in the proportion of familiarity (mother) for asthma (34.1% *vs.* 6.0%, P < 0.0001) and in the mean of nasal obstruction (2.0 (1.8-2.2) *vs.* 0.6 (0.5-0.7), P < 0.0001).

Regarding the results of SPT (Table III), we found no differences between the two groups for median of number of positive allergens per patient (2 (IQ range

Table II. *Characteristic of subjects with and without BHR.*

	Group A *With BHR (n=161)*	Group B *Without BHR (n = 249)*	*P*
Males/females	71/90	96/153	0.3
Age, yr, mean ± SD	28.3 ± 8.8	29.9 ± 10.2	0.1
Duration of rhinitis, yr, mean ± SD	7.9 ± 3.6	7.3 ± 4.3	0.2
FEV_1 % predictive value	99.1 ± 4.1	98.5 ± 5.0	0.1
FVC % predictive value	95.1 ± 4.8	96.0 ± 3.9	0.06
FEF_{25-75} % predictive value	86.7 ± 12.0	93.7 ± 7.3	< 0.0001
Living in city, n (%)	117 (72.6)	178 (71.4)	0.8
Passive smoke (%)	61 (37.8)	55 (22.0)	0.0008
Familiarity for asthma			
- Father (n, %)	21 (13.0)	19 (7.6)	0.1
- Mother (n, %)	55 (34.1)	15 (6.0)	< 0.0001
- Brothers and/or sister (n), (%)	38 (23.6)	41 (16.4)	0.09
Positive SPT, n (%)	151 (93.7)	167 (67.0)	< 0.0001
Log_{10} total serum IgE (kU/L) geometric mean ± SD	2.46 ± 0.27	2.06 ± 0.38	< 0.0001
Log_{10} Blood eosinophil count (x 10^{-3} µL) geometric mean ± SD	2.67 ± 0.07	2.57 ± 0.09	< 0.0001
Nasal obstruction, mean (95% CI)	2.0 (1.8 – 2.2)	0.6 (0.5 - 0.7)	< 0.0001
Sneezing, mean (95% CI)	1.6 (1.5 – 1.8)	1.5 (1.4 – 1.6)	0.1
Rhinorrhea, mean (95% CI)	1.9 (1.7 – 2.0)	1.8 (1.7 -1.9)	0.4

1-3) *vs.* 1 (IQ range 0-2), P = 0.8) and the total number of positive SPT (349 *vs.* 353, P = 0.1). Analyzing the single allergen tested, significant differences have been found between the Group A and the Group B to *Parietaria* (67.0% *vs.* 37.7%, P < 0.0001), to house dust mites (46.5% *vs.* 29.7%, P = 0.0005) and to cat dander (7.4% *vs.* 1.6%, P = 0.003). No other allergen was statistically different between the two groups.

To study risk factors for the development of BHR in subjects with rhinitis, associations were tested on a binary model. Of the seven background variables (nasal obstruction, SPT, blood eosinophil counts, $FEF_{25\%-75\%}$, familiarity for asthma (mother), passive smoke, and total serum IgE), nasal obstruction (OR 2.19, 95%CI 1.72-2.80) and the presence of positive

Table III. *Positive Skin Prick Test according to BHR.*

	Group A **With BHR**	*Group B* **Without BHR**	**P**
Grass	61 (37.8)	75 (30.1)	0.1
Mugwort	22 (13.6)	23 (9.2)	0.1
Paritaria judaica	108 (67.0)	94 (37.7)	< 0.0001
Olea Europea	50 (31.0)	61 (24.5)	0.2
Cupressus	20 (12.4)	21 (8.4)	0.2
House-dust mites	75 (46.5)	74 (29.7)	0.0005
Dander (cat)	12 (7.4)	4 (1.6)	0.003
Dander (dog)	1 (0.6)	1 (0.4)	0.1
Aspergillus	0	0	NA
Median No. of positive SPT result per patient (interquartile range)	2 (1-3)	1 (0-2)	0.8
Total No. of positive SPT results	349	353	0.1

Table IV. *Risk factors. Odds ratio (OR) and 95% confidence interval (CI) for the presence of BHR in a population of rhinitic adults.*

	OR (95% CI)	*P* value
Nasal obstruction (0-3)	2.19 (1.72 to 2.80)	< 0.0001
Positive SPT	6.15 (2.42 to 15.61)	0.0001
Blood Eosinophil counts (cells x 10^{-3} μL)	2.80 (1.54 to 5.07)	0.0007
$FEF_{25\%-75\%}$	2.72 (1.23 to 5.99)	0.01
Familiarity for asthma (mother)	2.45 (1.10 to 5.46)	0.02
Passive smoke	1.12 (0.60 to 2.08)	0.7
Total serum total IgE (kU/L)	1.54 (0.72 to 3.29)	0.2

SPT (OR 6.15, 95%CI 2.42-15.61) were the most available predictors to discriminate between subjects with BHR and subjects without BHR. In addition, BHR was positively related to blood eosinophil counts (OR= 2.80, 95%CI 1.54-5.07), $FEF_{25-75\%}$ values (OR= 2.72, 95%CI 1.23-5.99) and familiarity (mother) for asthma (OR = 2.45, 95%CI 1.10-5.46). Whereas passive smoke and total serum IgE were not positively related to BHR (Table IV).

DISCUSSION

This study shows that, in subjects with rhinitis, the occurrence of BHR is related to nasal obstruction, atopy, sensitization to *Parietaria judaica* and house dust mites, blood eosinophil counts, maternal asthma and passive smoke. These parameters are all associated with asthma but have never been evaluated together in rhinitic subjects with or without BHR.

The pathogenesis of nasal obstruction is complex and consists of 3 main events: inflammatory oedema, vascular congestion and mucus hypersecretion (20). The nose is usually considered a protective barrier against inhalants, contributing to the reduction of allergen load to airways. It is possible that the upper airway inflammatory reaction *per se* influences the development of bronchial inflammation and hyperresponsiveness, for example by release of inflammatory mediators (16). However, the size of allergen, the intensity and duration of exposure may influence both nasal symptoms than the development of BHR. Many subjects with rhinitis have a unique physiological features that differentiate them from asthmatic and healthy subjects, including an increased bronchial sensitivity to methacholine or histamine (21-22). Patients with seasonal allergic rhinitis experience the development of seasonal BHR that is not associated with clinical bronchospasm (16, 23). In the ERCHS epidemiologic study BHR was increased in patients with rhinitis in comparison with non-rhinitis subjects, but to a lesser extent than in asthmatic patients. The analysis of the data of ERCHS showed that non-specific bronchial hyperresponsiveness was significantly more frequent in subjects with perennial rhinitis than in patients with seasonal rhinitis (24).

Our study showed a higher risk of BHR in subjects with blood eosinophilia. Eosinophils play an important role in the inflammatory process of the airways of asthmatics and rhinitics (25-26). Whether increased numbers of peripheral blood eosinophil reflect an inflammatory process in the airways of rhinitic subjects that is responsible for BHR is unknown. However, an increased bronchial hyperresponsiveness during the pollen season (27) suggests that inflammatory changes occurring in the mucosa increase its sensitivity to neuropeptides. In the present study, a high blood eosinophil count is a discriminating factor for having BHR associated with rhinitis.

With regard to familial asthma, only maternal asthma was discriminant for the presence of BHR in rhinitic subjects. Finally, passive smoke was also discriminant for the presence of BHR in rhinitic subjects. In conclusion, this study gives new insights into the relationships between rhinitis and BHR, showing the tight association, in rhinitic patients, of BHR and the presence of atopy, blood eosinophilia, passive smoke and maternal asthma. In atopic rhinitic subjects, BHR is strongly associated with sensitization

to *Parietaria judaica* and house dust mites allergens. Our results suggest that, in rhinitic subjects, these parameters should be taken into account for the prevention of the development of asthma.

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