Active smoking effect in allergic rhinitis

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ABSTRACT

Background: Tobacco smoke has been described as causing increased prevalence of rhinitis symptoms and decreased atopy. Furthermore, these nasal symptoms and quality of life in smokers with Allergic Rhinitis (AR) were not significantly different to non-smokers. As a result of this duality, a comparison study between the quality of life and inflammatory markers of atopy among active smokers and non-smokers having AR was put forward.

Material and methods: Cross-sectional study in adult smokers and non-smokers, with a clinical diagnosis of AR and positive Skin Prick Test (SPT). Smoking status was confirmed by salivary cotinine measurements. Functional respiratory evaluation was performed, and quality of life between groups was compared using Mini-RQLQ questionnaire. Immunological markers in serum and nasal washes (IgE, IL-4, IL 5, IL 13, IL 17, IL 33) were evaluated, while samples from a third group of passive smokers was incorporated for serological comparison exclusively. The statistical analysis included Student T test, x2, Mann Whitney U (Anova 2-way), and Kruskal Wallis for 3 groups analysis. Values of P < 0.05 were considered significant.

Results: Twenty-two patients per group with similar demographics and allergen sensitivity were studied. Regarding inflammatory markers, a reduction of IL 33 in the serum of smokers (P < 0.001) was the only statistically significant different parameter revealed, showing a remarkable trend in nasal lavage. Salivary cotinine levels were absolutely different (P < 0.0001), but pulmonary function evaluations were not statistically significant after multiple adjusting. There were no significant differences in quality of life parameters.

Conclusions: In our study of AR, active smokers do not demonstrate impaired nasal related quality of life or impact on atopic inflammatory parameters, compared to non-smokers. Reduced levels of IL33 could explain a lack of symptoms alerting smokers of the harmful consequences of smoking.

Keywords: Allergic rhinitis, Tobacco, Inflammation, Quality of life

BACKGROUND

Allergic Rhinitis (AR) is a chronic disease of the upper airways, being the most prevalent allergic disease globally. The affected population varies by region from 12% to 40%, with rates close to 20% in our country. Depending on the degree of severity, it has a negative impact not only on quality of life but also on impairment of work/
school performance and quality of sleep.\textsuperscript{4–7} Often considered a trivial disease, it causes great patient demand of medical resources and more effective therapies.\textsuperscript{8,9}

Sensitivity to environmental allergens is a hallmark of AR. House dust mites are the most common sensitizers in our indoor environment, followed by grass pollens (outdoors) and animal epithelia.\textsuperscript{10,11} The epithelial reaction to antigen exposure begins with the secretion of IL33, TSLP, and IL25, activating innate type 2 lymphoid cells (ILC2) in mucosa and the production of atopic T helper 2 lymphocyte profile cytokines (Th2), with the consequent progression of allergic inflammatory cascade due to its effects on mast cells, macrophages, eosinophils, and CD4 + T lymphocytes.\textsuperscript{12} In this context, IL33 has been reported having the greater chemotactic activity for ILC2 compared to IL25 and TSLP.

Detrimental effects of pollution have also been correlated with AR. Regarding indoor pollution, both active and passive tobacco smoking can alter the nasal microbiome, morphology, and inflammatory pattern not only in AR but also in bronchial asthma,\textsuperscript{13–18} or induce the development of asthma in patients with AR who actively smoked, in a dose-dependent manner.\textsuperscript{19}

In an experimental model of allergic asthma in mice, exposure to tobacco smoke caused a decrease in cytokines involved in IgE mediated atopic mechanisms, such as Interleukin 4 (IL 4), IL 5, IL 13, IL 25, and eotaxin, significantly reducing eosinophilic infiltration and the expression of Th2 cytokines, characteristics of allergic asthma.\textsuperscript{20} In an elegant mouse model study, a main compound (acrolein) of cigarette smoke exposure inhibited immediate hypersensitive reactions, probably due to atypical, anergic Foxp3+ T regulatory cells.\textsuperscript{21} Were these findings replicated in humans, it would suggest that smokers would show less allergy compared to non-smokers. A diminished prevalence of allergic sensitization was reported in a group of either second-hand or active smokers,\textsuperscript{22} and hypothetically potentially protective for the development of atopy when prenatally exposed.\textsuperscript{23}

A cross-sectional study of over one thousand adult patients with AR, comparing smokers versus non-smokers, found no significant differences in the severity of nasal symptoms or impairment of quality of life.\textsuperscript{24} Another evaluation analysing the joint effect of exposure to tobacco smoke and indoor allergens in children could not demonstrate any relevant influence of tobacco on allergen sensitization.\textsuperscript{25}

However, a systematic review and meta-analysis on the effect of exposure to tobacco smoke in children, found a significant increase in parameters of atopy such as levels of total IgE, allergen-specific IgE, and positive Skin Prick Test (SPT).\textsuperscript{26} Exposure to tobacco smoke can influence innate immunity towards a pattern of respiratory diseases of the characteristic Th2 type of atopy, and increase the risk of IgE mediated allergen sensitization; it can also cause a worsening in symptoms and severity of asthma and rhinitis.\textsuperscript{27}

A study of almost 15 000 European adolescents described a highly significant statistical association between active smoking and the presence of Allergic Rhino-Conjunctivitis (ARC), including severe ARC symptoms.\textsuperscript{28} The identification of ARC is the most reliable detection parameter for AR in epidemiological studies. In a cross-sectional study of adolescents in Argentina, it has been found that active and passive smoking was a significant risk factor to present current symptoms of Rhinitis and Asthma.\textsuperscript{29}

With the conflicting evidence currently available, we investigated the real-life effect of tobacco use in AR patients with objective evaluations of immuno-inflammatory parameters, lung function and quality of life.

MATERIAL AND METHODS

Population-study

A cross-sectional, comparative study of two groups of consecutive adult patients with clinical diagnosis of AR with positive SPT, case (Smokers) - control (Non-Smokers) 1:1.

For serological evaluations only, a three-group comparison was performed between active smokers, passive smokers, and non-smokers. Passive smoker serum samples were obtained from a previously identified group of patients not having AR and being passive smokers, who had
previously given consent for samples to be used in subsequent studies.

Patients having at least 1 year of clinical history of AR, with no respiratory infection in previous month, were included. Patients with diagnosis of local AR, having received systemic steroids in last 6 months, allergen immunotherapy in previous 3 years, or anti-histamines in previous month were excluded. Participants' smoking status was based on self-report.30

Prior to any procedure, informed consent was obtained from each participant. The research study was approved by a local Ethics Committee where evaluations were carried out.

**Evaluations performed**

Demographic data of age and sex, weight and height

Self-reported cigarette consumption was determined by packages per year.

Saliva sample for cotinine quantification31,32

Taken spontaneously with disposable Pasteur plastic pipettes from the sublingual area 5 ml, with minimum requirements of the patient not having consumed drinks or meals for at least the previous 1 h before sampling, without rinsing or restricting previous smoking. Stored in Eppendorf tubes in refrigeration 0–2 °C, on average 1 h, prior to storage at −20 °C until analysis, using Elisa technique for cotinine determinations following strict supplier indications (www.salimetrics.com).

Nasal lavage

Technique reported by Bouloukaki and collaborators33 was followed to obtain the samples, with minimal modifications. The patients, in a sitting position, were instructed to flex the neck approximately 60° from the top, and not to breathe through the nose but through the mouth during the procedure. 5 ml of normal saline solution was applied at room temperature, gently instilled in each nostril using a disposable Pasteur pipette, with waiting time of about 5 s and a gentle massage to allow impregnation in the nostril. Next, the wash fluid was collected by repeated aspiration from both nasal cavities, using the same pipette, and placed in Eppendorf tubes. It was kept under refrigeration 0-2 °C for 1 h on average, prior to storage at −20 °C until analysis of IgE and cytokines.

**Venous blood sample**

Immediately after obtaining the above samples from each participant; centrifugation and serum collection were performed, and stored at −20 °C until analysis of IgE and cytokines could be performed. The third group of serum samples belonged to 13 well identified passive smokers having no personal background or current signs of any atopic disease, who had participated in an unrelated study (Chagas’ positive) and whose samples were ready to be discarded, with their previous consent for additional evaluations to their original purpose.34

**Functional respiratory tests**

Performed with a MIR Spirobank II spirometer with calibrated disposable turbine for the evaluation of all patients, with additional calibration control with a 3-l syringe. Measurements were made following American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations,35 avoiding smoking in previous hour (smokers). The 3 best reproducible maneuvers were selected, and the best values of FEV1, FVC, and FEV1/FVC% were taken for analysis.

**Sensitivity to inhalant allergens by SPT**

Assessed according to References.36,37 The sum of maximum and perpendicular diameters divided by 2 from the papules, resulting equal to or greater than 3 mm compared to the negative control were considered positive.

Allergens used were: *Dermatophagoides pteronyssinus* – *Dermatophagoides farinae*, *Blomia tropicalis*, Dog epithelium, Cat epithelium, Cockroach mix, Grass mix, Weeds mix, Trees mix, Alternaria sp, Aspergillus sp; glycerinated negative control and 10 mg/ml histamine positive control from Greer Labs through a local vendor. Lancets used were disposable metal tips 1 mm ALK type, also from local vendor.

In order to be considered atopic for inclusion, the patient had to demonstrate a positive test to at least 1 of the allergens listed, plus the positive histamine control.
Total IgE and cytokines

Levels of total IgE (kU/L) and IL 4, IL 5, IL 13, IL 17, and IL 33 (pg/ml) were obtained by Elisa strictly following instructions from provider (Peprotech USA, www.peprotech.com). All techniques were standardized accordingly.

Quality of life evaluation (Mini RQLQ)

Permission was obtained to use the abbreviated (and Spanish validated) Juniper’s questionnaire, being more efficient for cross-sectional studies, and which was self administered.

Statistical analysis

The calculation of sample size considered total population of city, with a prevalence of AR of 17% (16.9% of allergic rhinoconjunctivitis reported in Ait-Khaled et al) and 25% of them as smokers, resulting in a 1:1 comparison in 20 patients per group.

Average and Confidence Interval 95% plus Standard Deviation were obtained in the results with homogeneous samples without dispersion, and Median in the evaluations with evident dispersion or low level of detection.

Statistical analysis for continuous variables was performed with Anova and Student’s T-Test for homogeneous unpaired data, and Mann Whitney U for non-parametric samples due to the heterogeneity of the variances, plus Anova 2-way. Also Chi square was applied for comparison of proportions.

For the evaluation of serum samples from 3 groups, Kruskal Wallis (including Dunn’s post test) was used.

All results with \( P < 0.05 \) were accepted as significant.

RESULTS

Among the 49 patients invited to participate, 5 patients declined (3 smokers and 2 non-smokers), with 89.8% participation rate from patients meeting the necessary criteria. Thus, 44 patients...
were included in all, 22 smokers (case) and 22 non-smokers (control). Table 1.

There were no serious events associated with procedures performed. Local, mild, and self-limited symptoms in minutes (without the need for any treatment) were experienced in 6 samples of nasal washes (pruritus), 4 skin tests with allergens (pruritus), and 3 venous punctures for blood collection (pain).

There were no differences between groups in sensitivity to allergens (Table 1). More than half were mono-sensitized \(n = 28\); from these only 5 did not test positive to mites (2 to cat epithelium, 1 to dog epithelium, 1 to grass pool and 1 to Alternaria sp).

In the Non-smoking group there was 1 case with intermittent asthma as co-morbidity; in the Smoking group there were 2 cases of asthma and 2 cases with a diagnosis of COPD (Chronic Obstructive Pulmonary Disease). Table 1. No one received neither systemic steroids according to exclusion criteria nor inhaled cortico-steroids at the time of evaluation; the use of SABA on demand was permitted. No other active and treatment co-

|                              | Allergic Rhinitis, Smokers \((n = 21)\) | Allergic Rhinitis, Non Smokers \((n = 22)\) | Statistical difference (P) | Non Atopic, Passive Smokers \((n = 13)\) | Statistical difference (P) |
|------------------------------|---------------------------------------|--------------------------------------------|-----------------------------|------------------------------------------|-----------------------------|
| Serum IgE, in kU/L mean ± SD | 203,94 ± 163,45                       | 150,92 ± 85,96                             | \(P = 0.742^d\)             | 215,77 ± 118,25                         | \(P = 0.643^c\)             |
| Nasal Lavage IgE, in kU/L Mean ± SD | 2,94 ± 6,14 | 3,92 ± 7,29 | \(P = 0.088^d\) | b | |
| Serum IL 33, in pg/ml Mean ± SD | 181,94 ± 137,25                       | 609,58 ± 1080,56                          | \(P = 0.0028^d\)           | 127,31 ± 113,99                         | \(P = 0.0006^c\)           |
| Nasal Lavage IL 33, in pg/ml Mean ± SD | 16,18 ± 34,62 | 21,67 ± 43,92 | \(P = 0.088^d\) | NS | b |
| Nasal Lavage IL 17, in pg/ml Mean ± SD | 26,75 ± 26,22 | 29,3 ± 97,16 | \(P = 0.455^d\) | b | |
| Serum IL 4, in pg/ml Mean ± SD | 50,26 ± 25,85                          | 13.91 ± 12.24                             | \(P = 0.396^d\)            | 6.61 ± 0.41                             | \(P = 0.481^c\)            |
| Serum IL 13, in pg/ml Mean ± SD | 22.19 ± 6.06                          | 16.543 ± 6.18                             | \(P = 0.370^d\)            | 41.46 ± 0.87                            | \(P = 0.032^c\)            |

Table 2. Levels of IgE and cytokines on biological samples. For serological comparisons, a third group of non atopic, passive smokers is included. \(P<0.05\) is considered statistically significant. NS Non-Significant. a. Serological samples were obtained in 21 out of 22 Smoker AR patients, and nasal lavage and saliva in 22 of them. b. Not available. c. Anova Kruskal Wallis. d. Mann Whitney T test.
morbidities were registered at the time of the study.

Initial pulmonary function analysis, demonstrated statistically significant differences in FEV1% and FEV1/FVC% correlation. However, when excluding the 2 patients with previous COPD diagnosis and adjusting for age-sex and BMI, these became non-significant. Table 1.

The impact of these results on quality of life parameters, measured by Mini RQLQ, showed no statistically significant differences in the comparison between patients with smoking and non-smoking AR. Table 1.

One patient from the Smoking group refused to undergo blood sampling. Thus, 21 serum samples were available from this group for all evaluations.

From serum samples, we obtained isolated positive results in the determinations of IL 4 (just one) and IL 5 (one detectable), and only 3 for IL17 in serum. The determinations of IL 5 and IL 13 were negative in all cases of Nasal Wash and only one detected for IL 4, even having been tested twice for this reason.

The cytokines most commonly identified besides IgE were IL 33 in serum and nasal lavage, and IL17 in nasal lavage. Statistical significant differences on systemic IL 33 levels are displayed in Fig. 1.

Relevant results are displayed in Table 2, with 3 groups in serum evaluations and 2 (smokers and non smokers) for the remaining procedures. Significant high IL 13 in control group was found.

DISCUSSION

As described in the results, there were no differences between groups in terms of demographic parameters or allergen sensitivity. There were also no differences in serum or nasal lavage concerning IgE. The true value of IgE as a marker of atopy has been debated, but has been re-evaluated following a demonstration of the effectiveness of the Anti-IgE treatment Omalizumab.\(^{40,41}\)

At the time of this report, no reference of IgE levels in Nasal Lavage from AR patients have been found. The mean nasal IgE levels (in our smoker and non smoker patientes) of 2.94 and 3.92 kU/L were not statistically different.

With regards to smoking status, there was a significant difference found in salivary cotinine measurements, with almost no detectable values in non-smokers (Table 1). Statistically significant differences were initially found in pulmonary function comparison, but after multiple logistic regression excluding 2 patients having COPD, it became non-significant. However, this tendency should be strongly remarked, as an anticipation of the increased incidence of asthma in patients with AR who smoke, in a dose-dependent way.\(^{19}\)

We faced the inability to detect certain cytokines; postulated reasons are: a) serum and nasal washes obtained were analyzed without stimulation or culture; b) patients were not required to be symptomatic at the time of sampling; c) ability to detect very low concentrations of these cytokines was limited by the performance of the testing kits, such as our IL 5 set with lower limit of 46 pg/ml, while detection limits of 2 pg/ml were reported in atopic subjects.\(^{42,43}\)

Despite this situation, it has been possible to obtain relevant findings, such as the case of IL 17 in nasal washes, an indicator of active inflammatory phenomenon, being described in different situations of AR at local level regarding symptoms and seasonality,\(^{44,45}\) and in nasal biopsies of patients with asthma.\(^{46}\)

IL 13 could not be detected in nasal washes but was positive in serum samples with no differences between groups; this cytokine is synthesized by mast cells (and other cellular sources) and stimulates the production of type I collagen by airway fibroblasts in an MMP-2 and TGFβ1-dependent manner in asthma.\(^{47}\) In AR (our study population) the presence of mast cells and an increase in type I and III collagen have also been identified in nasal biopsies, but remodeling in nasal mucosa is limited while in asthma it is extensive.\(^{48}\)

The most relevant finding of this work is evidenced by nasal and serum level of IL 33 in AR.\(^{49}\) Conflicting data on the correlation between serum and nasal levels have previously been reported, including being undetectable in serum but present in nasal specimens.\(^{44,50}\) Nevertheless, IL 33 in nasal secretions expresses an inflammation directed
towards a Th2 profile, alongside with IL 25 and TSLP, being released by tissue damage induced by a pathogen, an irritant or by allergen exposure. A murine experimental study demonstrated the fundamental role of IL 33 in comparison with IL 25 for the induction of AR sensitized to dust mites. This alarmin, IL 33, is a key factor to start defense mechanisms against infectious aggressors and allergens, in the epithelial - mesenchymal interaction, fundamentally collaborating with the IgE mediated phenomenon but also with the Th 17 inflammatory mechanism, also corroborated in our results. It has been previously reported that tobacco nicotine attenuates Th1 and Th17 responses, favoring a deviation towards Th2 in the neuro-inflammatory response. Furthermore, acrolein might also provoke an immunosuppressive response, with the remarkable finding of regulatory T cells (Foxp3+, CD4+ CD25+) both in tissue and bronchoalveolar lavage.

Our evaluations found that tobacco causes a decrease in IL 33, necessary to initiate these natural defense mechanisms, and attenuates the IgE mediated mechanism. In an experimental murine model, tobacco smoke did not generate active secretion of IL 33, and even demonstrated a decrease in the expression of its ST2 receptor in ILC2 cells.

Amazingly, levels of serum IL33 on the third group of passive smokers were comparable to smokers (and also IgE); unfortunately we do not have quantification on the exposure load in this group. Their elevated mean level of IgE could be explained both by the passive exposure to tobacco and/or by Chagas’ infection condition.

Also in the Chagas’ passive smoker group, significantly elevated IL 13 levels were found. Even sharing many biological activities with IL 4, this last one was not increased in the same way as the former, a dissociation that was precisely described over 2 decades ago.

In order to demonstrate the clinical relevance of inflammatory markers in real life and to quantify this impact, we searched for a validated instrument both in clinical research and in daily practice. The Mini RQLQ developed by Prof. E. Juniper was implemented (under authorization) and no statistical difference was found between smokers and non-smokers. Similar evidence and also reduced allergic symptoms on tobacco smoke exposed individuals was previously mentioned.

CONCLUSIONS

Our group of patients with AR who smoke do not show a worsening in their quality of life or an increase in measured inflammatory parameters. Both groups of patients showed evidence of allergic inflammation at the nasal level with the presence of IgE and IL 17. However, smokers evidenced a significant decrease of IL 33 systemically and a remarkable reduced tendency at local level.

This reduction of the alarmin signalling fails to alert smokers of the damaging effects of smoking; thus patients are less likely to be persuaded to quit smoking, with subsequent increase in incident asthma and COPD.

Abbreviations
ALK: Company’s brand name; AR: Allergic Rhinitis; ARC: Allergic Rhino-Conjunctivitis; ATS: American Thoracic Society; BMI: Body Mass Index; °C: Celsius centigrades; CD4: Cluster of Differentiation 4 (Lymphocyte); COPD: Chronic Obstructive Pulmonary Disease; ERS: European Respiratory Society; FEV1: Forced Expiratory Volume in 1 (first) second; FVC%: Forced Vital Capacity in average (%); IgE: Immunoglobulin E; IL: Interleukin; ILC2: Innate Lymphoid Cells – type 2; MIR: Spirometer brand’s name; MMP-2: Matrix Metalloproteinase 2; P (as P-value): Probability of test result; Pg: picograms; RQLQ: Rhinitis’ Quality of Life Questionnaire; SABA: Short Acting Beta Agonist; SD: Standard Deviation; SPT: Skin Prick Test; ST2: IL33 receptor; TGFβ1: Transforming Growth Factor Beta 1; Th: T helper (Lymphocyte); TSLP: Thymic Stromal Lymphopoietin.

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Consent for publication
RM Gómez, VH Croce, ME Zernotti and JC Muiño give their consent for the publication of this work in the World Allergy Organization Journal.

Author’s contribution
RMG contributed on the design of study, execution of protocol, interpretation of results, discussion and writing of manuscript.
VHC contributed on evaluation of study's design, checking of protocol procedures, interpretation and discussion of results.

MEZ contributed on evaluation of study's design, checking of protocol procedures, interpretation and discussion of results.

JCM contributed on evaluation of study's design, statistical analysis of data, interpretation and discussion of results.

Availability of data and materials
All material used on study's procedures were obtained through Fundación Ayre specific and personal grant to RMG, except spirometer which belongs to RMG.

Ethics approval
The protocol for this study was approved by Bioethics Committee of the Medical Association of Salta (Argentina), where all subjects' procedures were performed.

Declaration of competing interest
RM Gómez, VH Croce, ME Zernotti and JC Muño declare to have no conflict of interest regarding present study.

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