International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jms.htm
2015 Vol. 5 (1) January-April, pp. 291-297/ Vaidya et al.
Research Article

A STUDY ON UTILITY OF NASAL SMEAR EXAMINATION IN DIAGNOSING ALLERGIC RHINITIS AND ITS HISTOPATHOLOGICAL CORRELATION IN ALLERGIC NASAL POLYPS

***Kuladeepa Ananda Vaidya[1], Sunil Pai[2], T S Nagaraj[1] and Sukesh[1]**
[1]Department of Pathology, Srinivas Institute of Medical Science and Research Centre, Mukka, Mangalore - 575021
[2]Department of ENT, Srinivas Institute of Medical Science and Research Centre, Mukka, Mangalore - 575021
**Author for Correspondence*

ABSTRACT

One of the simple and economical diagnostic methods in detecting nasal allergic disorders is cytological examination of nasal smears. This study was conducted to evaluate the usefulness of nasal cytology in individuals suffering from allergic nasal disorders, including allergic nasal polyps with special interest on cytohistological correlation in case of allergic nasal polyps. Effort is also made to compare the nasal smear eosinophilia with that of peripheral blood absolute eosinophil count in allergic nasal disorder. Fifty patients with clinical features suggestive of nasal allergy were enrolled for this study. Twenty patients out of this showed nasal polyp on examination. Twenty five controls (group 2) were considered in this study without any history of allergy. Investigations like differential count for eosinophils, Absolute eosinophil count (AEC), and nasal smear examination were carried out for all patients including control group. Nasal smears were examined by light microscopy after staining with Leishman and H&E stain. Nasal polyps were surgically removed under general anaesthesia for histological examination. Nasal smear eosinophilia were compared with peripheral blood eosinophilia in all cases and also compared with eosinophilia in histological sections in cases with nasal polyps. Nasal eosinophilia was seen in 90% and Blood eosinophilia was seen in 94% of cases of clinically diagnosed allergic rhinitis. There was a good correlation of nasal smear eosinophilia with blood AEC in allergic rhinitis and with histological findings in cases of allergic nasal polyps. Nasal smear cytology is a simple, cost effective screening test to determine the presence or absence of allergic tissue response in conditions like allergic rhinitis and allergic nasal polyps. It has a good correlation with peripheral blood eosinophilia and histological findings in cases of allergic nasal polyps.

Keywords: *Allergic Rhinitis, Histopathology, Nasal Polyp, Eosinophilia*

INTRODUCTION: Nasal cytology was introduced in 1889. Gollash initially highlighted the presence of numerous eosinophils in the nasal secretions of an asthmatic patient, he suggested that these cells could be the key cells for the pathogenesis of the disease (Gollash, 1889). The nasal mucosa is formed by a ciliated pseudostratified epithelium, it represents the first line defense located in the airways. The diagnosis of nasal disorders through nasal cytology is based on the consideration that, in healthy subjects, the nasal mucosa is composed of normal subsets of cells, which commonly characterize the pseudo-stratified epithelium; besides neutrophils, no other cells are detected in healthy individuals (Gelardi *et al.*, 2012). The association between eosinophils and allergic disease has been known for many years. Nasal smear eosinophilia (NSE) is a valuable test for the diagnosis of allergic rhinitis (Sanli *et al.*, 2006). This study was conducted to evaluate the usefulness of nasal smear eosinophilia as a simple non-invasive & inexpensive method for confirming clinically diagnosed allergic rhinitis. Study has also evaluated the cytohistological correlation in cases of allergic nasal polyps.

MATERIALS AND METHODS

This study is accomplished by examining the consecutive, consenting patients who presented with allergic nasal symptoms at the ENT OPD of the tertiary referral hospital at coastal Karnataka, attending over a period of one year between Jan 2014 and Jan 2015.

Subjects: All the patients who were diagnosed with allergic rhinitis satisfying the inclusion criteria were included in the study after obtaining consent. A total of 50 patients were thus included in the study group. Detailed medical history was taken, in order to establish the diagnosis of allergic rhinitis. The patients were diagnosed based on the history & clinical features typical of allergic rhinitis which included sneezing, rhinorrhoea, nasal itching and nasal obstruction. Patient's demographic and clinical data including the presence or absence of a family history for atopic symptoms were also recorded on a standard questionnaire forms. Skin-prick tests were performed to determine the allergic state on the volar part of the forearm with a standard battery of common aeroallergens whenever required.

Criteria for selection of study group (group 1) are as follows.

Inclusion Criteria

-Patients with recurrent allergic symptoms like sneezing, rhinorrhoea, nasal itching and nasal obstruction since more than 6 months.

-Patients who were not treated with topical steroid in the past

- Patients with allergic symptoms with nasal polyps on examination.

Exclusion Criteria

-Patients with a history of acute respiratory tract infections in the past month.

-Known case of asthma on treatment.

-Snuff users.

-Patients who were prescribed anti-histaminics within the past two weeks.

- Previous surgical operations involving the nose and paranasal sinuses.

The X ray and CT of paranasal sinuses were done wherever required to diagnose the allergic nasal polyps.

Control group (group 2) consisted of 25 individuals, with age and sex match. The control group was recruited with their voluntary consent from the outpatient clinic of ENT departments who came for minor ailments or minor surgical procedures other than nasal symptoms and symptoms related to allergy. Detailed medical history, thorough physical examination were also done on group 2 as on group 1 before selection. Nasal secretion was collected by asking the patient to blow the nose into a plastic container then transferred onto a glass slide, teased out to make smears. Nasal samples were also collected by passing a sterile probe, from each nasal cavity, along the medial surface of the inferior turbinate 2 to 3 times and the specimen smeared on a clear glass slide. Minimum two smears are collected from each subject. Two ml of venous blood was collected from the subjects in EDTA vacutainers from median cubital vein for performing investigations like complete blood count, preparation of peripheral blood smears and absolute eosinophil count. Peripheral smears were prepared by wedge method by placing a small drop of blood from one end of a pre-cleaned slide and immediately spread using a spreader placed at an angle of 45 degrees and allowed to air dry. Complete blood counts were performed using Lab Life Premier automated hematology analyzer. Differential counts for eosinophils were noted by examining the peripheral smears. A reference range of 1-6 % is considered as normal. Absolute eosinophils count was performed by using Dungers fluid and counting was accomplished using modified Neubauer chamber. A reference range of 40 to 440 cells per microliter is considered as normal absolute count for eosinophils. Any value above this reference range is labelled as eosinophilia. Peripheral blood smears were studied using Leishman's stain whereas nasal smears were studied by both Leishman and Haematoxylin and Eosin stain. Smears were examined under a light microscope, using various magnifications. The grading of the nasal smear eosinophilia was done as shown in the table, incorporating information mentioned in publication works of Abhey (2005), Mygind (1979).

Table 1: Scale used to interpret nasal smear eosinophilia

No eosinophilia	< 5% eosinophils
Slight eosinophilia	5-10% eosinophils
Moderate eosinophilia	>10% but < 50% eosinophils
Severe/Marked eosinophilia	> 50% eosinophils

Nasal polyps were surgically removed under general anaesthesia. For histological examination, nasal polyp samples were fixed in 10 % formalin, embedded in paraffin, cut with the rotary microtome into 5-µm sections. Histological examination was performed using a light microscope after staining the sections with haematoxylin and eosin stain. Eosinophils in the surface layer in each section were counted and calculated the average number of eosinophils per HPF. In biopsy cellular grading was done as follow: Mild= grade 1=few cells (5-15 eosinophils) per HPF, Moderate= grade 2=moderate number of cells (16-49 eosinophils) per HPF, Severe = grade 3= many eosinophils (>50 eosinophils/HPF) and eosinophils in clumps.

The data was entered into excel sheet, & transferred to SPSS. "Statistical analysis was performed using the SPSS computer package version 20.0. The mean± SD was noted for quantitative variables. Independent samples t-test was used, to assess the differences in means of quantitative variables between patients and controls. P- value was calculated using chi square test analysis. P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Results

Of the 50 patients seen during the one year study, 30(60%) were males, 20(40%) were females. The ratio of male to female was3:2 in study group and 1.5:1in control group. The most common symptom was running nose, involving 96% of patients with allergic rhinitis. Majority of the patients with allergic rhinitis and nasal polyposis showed sensitivity to various common allergens such as pollens, fungi, house dust and mites.

Table 2: Patient profile

	Study group	**Control group**
Age/yr	30.5+/-10.5yrs	35.5+/- 12.5yrs
Male/Female	30/20	15/10

Table 3: Clinical features in the study group

Clinical features	**Number**
Sneezing	45
Itching in nose	25
Running nose	48
Nasal obstruction	25
Pale mucosa	40

Table 4: Number of patients with Allergic nasal polyps on clinical examination

Nasal polyps	**No. of Patients**	**Percentage**
Present	20	40%
Absent	30	60%

Table 5: Peripheral blood Absolute eosinophil count in study and control group

	No. of Patients	No. of Control
Present (>440/mm3)	45(90%)	0(0%)
Absent(<440/mm3)	05(10%)	25(100%)

Table 6: Nasal smear eosinophilia in study and control group

	No. of Patients	Percentage	No. of Control	Percentage
Present	47	94%	0	0%
Absent	03	6%	25	100%

Table 7: Severity of eosinophilia in nasal smears

Nasal eosinophilia	No of cases	%
Slight eosinophilia	10	20%
Moderate eosinophilia	26	52%
Severe eosinophilia	14	28%

Nasal smear eosinophilia is seen in 94% patients of study group but not seen in control group. Majority of them (52%) showed moderate type of eosinophilia. Blood eosinophil count in the study group was significantly higher than in control group.

Table 8: Histopathology of nasal polyps

Epithelium		
Normal	07	35%
Hypertrophied	10	50%
Metaplastic	03	15%
Edema		
Mild	08	40%
Moderate	10	50%
Severe	02	10%
Eosinophils		
Grade 1-Mild	04	20%
Grade 2-Moderate	14	70%
Grade 3-Severe	02	10%

Histopathological examinations of nasal polyps were done, out of 20 polyps 35% showed hypertrophied nasal mucosal epithelium with moderate edema. Out of these 20 polyps, majority (70%) showed moderate eosinophilia on histopathological examination. All the 20 cases of nasal polyps showed eosinophilia in nasal smears examination and had peripheral blood eosinophilia as well.

We found statistically significant correlation between eosinophil level in nasal secretions and peripheral blood AEC. We also found statistically significant correlation between eosinophilia in nasal secretions and histopathological sections in clinically diagnosed cases of allergic nasal polyps.

Discussion

Rhinitis is a disorder characterized by nasal symptoms such as rhinorrhoea, sneezing, nasal congestion and itching. Approximately 50% of all cases of rhinitis are caused by allergy. In the case of rhinitis caused by allergens, symptoms arise as a result of inflammation induced by a gamma globulin E (IgE) mediated immune response to specific allergens such as pollens, molds, dust etc. The immune response involves the release of inflammatory mediators and recruitment of cells to the nasal mucosa (Skoner, 2001). It is one of the most common chronic conditions with a significant impact on the quality of life (James, 1999). The diagnosis of nasal allergy is made based on typical history, combined with

characteristic symptoms and physical findings but Confirmation can be done by tests like skin prick tests (SPT), nasal smear examination (Takwoingi *et al.*, 2003), radio allegro sorbent test (RAST), enzyme linked immuno sorbent assay (ELISA). It is difficult in most of the clinics of developing countries to determine whether these cases are allergic or non-allergic type where access to SPT, RAST, or ELISA is not readily available (Patel and Nagpal, 2014). We observed a significant male preponderance of allergic rhinitis in this study. The correlation between clinical allergy and nasal smear eosinophilia was first emphasized by Eyermann (1927). The overall proportion of nasal smear eosinophilia was found 94% in study group. In the study of Takwoingi *et al.*, (2003) they found this rate as 76%.

The nasal smear for eosinophil appears to be a reliable diagnostic test with moderately high sensitivity and high specificity and it should be interpreted along with the information available by careful history and physical examination (Sood, 2005).

There is direct proportional relationship between eosinophilia and severity of nasal obstruction in allergic rhinitis patients (Chanda *et al.*, 2002). General belief of that severity of allergic rhinitis is associated with blood AEC also correlated with our findings but the study conducted by Patel and Nagpal (2014) showed blood AEC does not contribute in diagnosis of allergic rhinitis with present standardization of grading and recommended to revise the normal standard value and grading of blood AEC.

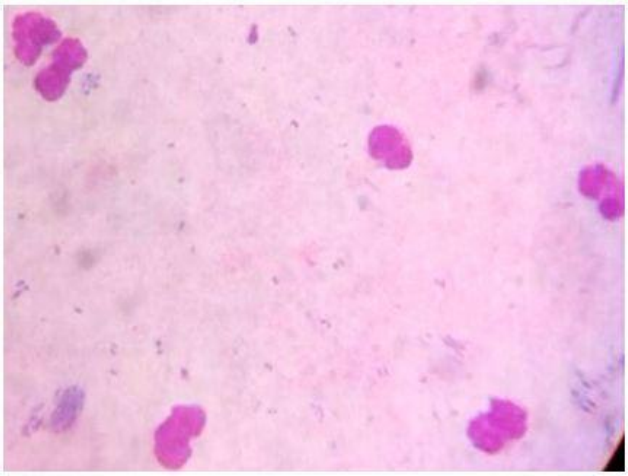

Figure 1: Nasal smear showing the presence of numerous eosinophils in a patient with nasal polyp and symptoms of allergic rhinitis (Leishman stain, Higher magnification)

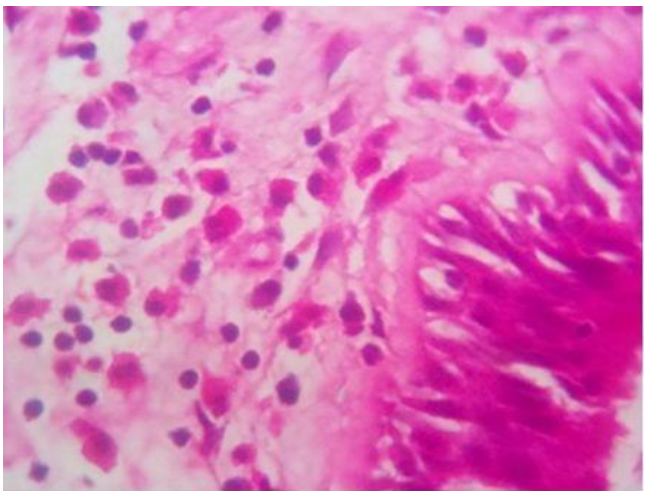

Figure 2: Histopathological section from the nasal polyp showing pseudostratified ciliated columnar epithelium and underlying edematous stroma with inflammatory cells predominantly composed of eosinophils (H&E stain, higher magnification)

Nasal polyps originate in the upper part of the nose and extend into the nasal cavity from the middle meatus, resulting in nasal blockage. The polyp stroma is highly oedematous with a varying density of inflammatory cells (Settipane *et al.*, 1997; Mygind and Lildholdt, 1997). Jovićević and Kljajić (2005) found that about 30 % of patients with allergic rhinitis had nasal polyposis. The stromal layer of the polyp tissue includes mixed inflammatory cells. The predominant histological form of nasal polyposis is the eosinophilic type, with an incidence of about 90 % (Rostkowska-Nadolska *et al.*, 2008). T-cell-secreted IL-5 and autosecretion of IL-5 from activated eosinophils may be the reasons for persistent and growing eosinophil inflammation in the nasal polyp tissue (Fan *et al.*, 2007). Study by Chanda *et al.*, (2002) showed that nasal biopsies proved to be better than smears for the detection of eosinophils though smears were also significant (Chanda *et al.*, 2002). Allergic nasal polyposis patients in our study showed a higher level of eosinophilic inflammation than patients with just allergic rhinitis in nasal smear preparation which is in accordance with the study conducted by Perić *et al.*, (2011). Majority had moderate degree of eosinophils in histological sections. Which is comparable with study published by Garín *et al.*, (2008) as they also found that majority of their patients had eosinophilia in polyp tissue and the majority had number of eosinophil between 20 and 50 per field ×400.

Conclusion

Nasal smear cytology is a simple, cost effective screening test to determine the presence or absence of allergic tissue response in allergic rhinitis and allergic nasal polyps. It has a good correlation with blood AEC and histological findings in allergic nasal polyps. Hence can be applied routinely as a reliable tool in diagnosing nasal allergic conditions where other sophisticated laboratory investigations are not readily available.

REFERENCES

Chanda R, Kumar A, Aggarwal GS, Kohli GS, Jaswal TS and Gupta KB (2002). Comparative study of nasal smear and biopsy in patients of allergic rhinitis. *Indian Journal of Allergy Asthma and Immunology* **16** 27-31.

Eyermann CH (1927). Nasal manifestations of allergy. *Annals of Otology, Rhinology & Laryngology* **36** 808-15.

Fan GK, Wang H and Takenaka H (2007). Eosinophil infi ltration and activation in nasal polyposis. *Acta Oto-laryngologica* **127** 521- 6.

Garín L, Miguel Armengot, José Ramón Alba and Carmen Cardab (2008). Correlations between Clinical and Histological Aspects in Nasal Polyposis. *Acta Otorrinolaringológica Española* **59**(7) 315-20.

Gelardi *et al.*, (2012). *Italian Journal of Pediatrics* **38** 51.

Gollash H (1889). *Fortschritte der Medizin* **7** 361–5.

James A Hadley (1999). Otolaryngology for the internist: Evaluation andmanagement of Allergic rhinitis. *Medical Clinics of North America* **83**(1) 13-25.

Jovićević J and Kljajić V (2005). Nasal polyposis and allergic rhinitisour experience, in Serbian. *Srpski Arhiv za Celokupno Lekarstvo* **133**(Suppl 2) 105-7.

Mygind N (1979). Eosinophil leucocytes. In: *Nasal Allergy*, 2nd edition (blackwell scientific publications) 170-81.

Mygind N and Lildholdt T (1997). *Nasal Polyposis: An Inflammatory Disease and its Treatment* (Copenhagen: Munksgaard).

Patel AK and Nagpal TP (2014). Comparison of blood absolute eosinophil count and nasal smear eosinophils with symptoms and severity of clinical score in patients of allergic rhinitis. *Indian Journal of Allergy Asthma and Immunology* **28** 74-7.

Perić A *et al.*, (2011). Inflammatory mediators in allergic rhinitis and nasal polyposis. *Arhiv za Higijenu Rada i Toksikologiju* **62** 341-8.

Rostkowska-Nadolska B, Pazdro-Zastawny, Ziołkowski P, Gamian E, Jaworska M, Bochnia M and Jabłonka A (2008). Expression of basic fi broblast growth factor in nasal polyps. *Advances in Clinical and Experimental Medicine* **17** 33-40.

Sanli A, Aydin S, Ates G, Eken M and Celebi O (2006). Comparison of nasal smear eosinophilia with skin prick test positivity in patients with allergic rhinitis. *Kulak Burun Bogaz Ihtisas Dergisi* **16** 60-3.

Settipane GA, Lund VJ, Bernstein JM and Tos M (1997). Nasal polyps: epidemiology, pathogenesis and treatment. *Providence, Rhode Island: The New England and Regional Allergy Proceedings*.

Skoner DP (2001). Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *Journal of Allergy and Clinical Immunology* **108**(1 Suppl) 2-8.

Sood A (2005). Diagnostic significance of nasal eosinophilia in allergic rhinitis. *Indian Journal of Otolaryngology and Head and Neck Surgery* **57**(1) 13-6.

Takwoingi Y, Akang E, Nwaorgu G and Nwawolo C (2003). Comparing nasal secretion eosinophil count with skin sensitivity test in allergic rhinitis in Ibadan, Nigeria. *Acta Oto-laryngologica* **123** 1070-4.