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Nasal Smear Eosinophilia as a Diagnostic Test for Allergic Rhiniti s in children: How Reliable?

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Abstract:

Background: *Allergic rhinitis is one of the most common chronic diseases in children. Developing countries such as ours lack standard diagnostic tests like Skin Prick Test, Radio Allegro Sorbent Test, or Enzyme-Link Immune Sorbent Assays. In their absence the need to confirm disease with a readily available tool such as nasal smear becomes necessary*

Aims*: To assess the reliability of nasal smear eosinophilia as an alternative tool for diagnosing allergic rhinitis.*

Subjects and Methods: A prospective, cross - sectional community based study of primary school children in ten (10) randomly selected Government primary schools. Children between 6 to 11years were recruited and evaluated for symptoms and signs of allergic rhinitis.

Nasal smear was collected, air dried and fixed with 95% alcohol. Staining was done with modified Wright's Giemsa solution and thereafter examined under light microscope. Blood samples were collected from each of the children for blood Absolute Eosinophil Count, and stool samples for helminthes. Data were analyzed using IBM Statistical Product and Service Solution version 26.0.

Results*: Participants that met inclusive criteria were 354; 176 (50.6%) were males and 175 (49.4%) females. 1 67 subjects (94.4%) were diagnosed with allergic rhinitis using nasal smear eosinophil count. The sensitivity, sp ecificity, positive and negative predictive values of nasal smear eosinophilia were 94.4%, 83%, 85%, and 100% respectively. The Spearman's correlation test showed a significant association between nasal smear eosinophili a and runny nose (p=0.001), excessive sneezing (p=0.025) and nasal itching / itching eyes (p =0.001) but not wi th nasal blockage (p=0.216).*

Conclusions*: Nasal smear eosinophilia is a reliable indicator or tool for diagnosis of allergic rhinitis. Being no n-invasive and inexpensive, it can be used as alternative specific test to diagnose allergic rhinitis.*

Key-words*: Allergic rhinitis: Nasal eosinophilia: Absolute eosinophil count*

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I. Introduction:

Allergic rhinitis (AR) is a symptomatic disorder of the nose induced by immunoglobulin E mediated in flammation following exposure of membranes lining the nose to allergens. It is an inflammatory disease of the nasal mucosa associated with nasal cellularity resulting in symptoms such as nasal congestion, watery rhinorhoe a, excessive sneezing, nasal and eye itching [1]. With detailed history and physical examination diagnosis can be made. However, common symptomatologies of AR significantly overlap with non-allergic rhinitis (NAR) [2]. The refore, children with AR symptoms have to be properly evaluate.AR can be confirmed by specific tests such as Skin Prick Test (SPT), Radio Allegro Sorbent Test (RAST), or Enzyme-Link Immune Sorbent Assays (ELISA). These facilities are either lacking or impracticable in poor resource settings. They require test allergen extracts f rom local allergens, which will be lacking in poor resource situations. Also test centers must have facilities to int ervene in cases of anaphylactic reactions during testing. Therefore, Nasal smear might be a valuable test for the diagnosis of allergic rhinitis [3]. Each technique has its own advantages and disadvantages and their applications v ary depending on the needs of the child.

The most direct method of studying the severity of inflammation in the nasal mucosa is the assessment of nasal cellularity; for example, nasal washing, nasal smears and nasal biopsy [4,5,6].

Nasal cytology was introduced in 1889, when Gollash highlighted the presence of numerous eosinophil s in the nasal secretion of asthmatic patient and suggested that these cells could be the key elements in the patho

genesis of the disease.[7] In 1927, Eyerman et al detected the presence of granulocytes and eosinophils in nasal se cretions of allergic patients and showed their importance in diagnosing the disease.[8]

This method of examining nasal mucosa was first described by Hansel et al[9] in 1934.With this method, the presence of eosinophils more than 5% per high power field in the patient's nasal smear (detected by Hansel stain) is suggestive, but not pathognomonic of allergic rhinitis. The first clinical examination in children were pe rformed by Matheson et al.[10]

Among these, nasal smear is the most simple, inexpensive and non – invasive procedure suitable for stu dying a large population such as school children.

Findings from nasal smear eosinophilia in a large population could help to determine its reliability as a n alternative diagnostic for AR in the absence of standard confirmatory test method.

II. Subjects and Methods:

This was a prospective cross-sectional community based study of 10 randomly selected Government Pri mary schools over a period of 14 (fourteen) months. Ethical permission for this study was obtained from Institut ion Review Board; approved number XXXX/HREC/33/320. Written approval was obtained from the Ministry o f Education and the principals of the schools. Also, written informed consent was obtained from the parents / car egivers and assent from the pupils before the commencement of this work. 36 participants were recruited into th e study from each school giving a total of 360 with sex and age matched subjects and control. Those with one or two symptoms of AR rhinitis were subjects. Those without symptoms constituted the controls. Those with purul ent nasal discharge or fever, medical therapy for rhinitis, previous nasal or paranasal surgical procedures, nasal t umors and structural causes of rhinitis were excluded from the study.

Nasal smear were taken from each nostril and smeared on glass slides, which were immediately fixed with 95% ethyl alcohol by the ORL researchers. Slides were stained with Wright's Giemsa stain, viewed at high power oil immersion x 1000 and reported by the consultant pathologist single blinded to the clinical status of the participants. A finding of > 5 eosinophils in 3 -5 fields of view or a single finding of 20 to 30 cells was considered positive for nasal eosinophilia.[11] The grading of the nasal smear eosinophilia (NSE) was done according to Abhey et al.[12,13,14,] using semi – quantitative scale..

3mls of venous blood was equally collected into anti-coagulant (EDTA) bottle. These were examined fo r absolute eosinophil count (AEC). Blood eosinophilia was considered present if > 600cells per micro – litre and graded as mild (600 -1500 cells), moderate (1500 -5000cells) and severe (>5000cells) per micro – litre. [1516,17,18]

Universal sample bottle were distributed to participants to collect and bring stool the next day. The micr obiologist examined theses for helminthes.

Statistical analysis of the data were done using IBM Statistical Product and Service Solution version 26. 0. Chi-square or Fisher's Exact Test and Spearman's rank correlation coefficient were used for correlation.

III. Results:

354 out of 360 subjects recruited completed the study; 179 (50.6%) males and 175 (49.4%) females. 2 p arents refused consent for their children to take part in the study. 2 pupils did not want blood samples taken from them. The other 2 did not turn up with stool samples.

Table1: Socio-demographic characteristics of study participants

Biodata	Item	Subjects N (%)	Controls N (%)
Age group (years)	6 – 8	126 (71.2)	106 (59.9)
	9 – 11	51 (28.8)	71 (40.1)
Gender	Female	96 (54.2)	79 (44.6)
	Male	81 (45.8)	98 (55.4)
Class in School	Primary 1 – 3	93 (52.5)	95 (53.7)
	Primary 4 – 6	84 (47.5)	82 (46.3)

Age ranged from 6 - 11 years with mean age of 10.5 ± 1.9 for AR group and 10.3 ± 1.9 for controls. 12 6 (71.2%) AR and 106 (59.9%) for control were in the age group 6 – 8 years. 93 (52.5%) AR and 95 (53.7%) co ntrol were in primary 1 – 3 classes. 179 (50.6%) males and 175 (49.4%) females; with male: female ratio = 1:1. There was no statistically significant difference between the mean ages of allergic rhinitis group and controls.

Table 2: Distribution of severity of allergic symptoms among subjects and controls

Symptom Severity	Subjects N (%)	Controls N (%)	Total	X^2	P–value	Remarks
No symptom	10 (5.6)	147 (83.1)	157	276.548	≤0.0001	Using Fisher exa ct value
Mild	15 (8.5)	30 (16.9)	45			
Moderate	125 (70.6)	0	125			
Severe	27 (15.3)	0	27			
Total	177	177	354			

125 (70.6%) were moderately affected while 27 (15.3%) had severe symptoms in AR Only 15 (8.5%) of the cas es were in the mild category. For the control group, no cases were seen in the moderate and severe categories ex cept 30 (16.9%) in the mild category. There was a statistically significant difference between the AR and the co ntrol group in the distribution of severity of symptoms (p = ≤0.0001).

Table 3: Blood levels of eosinophilia among subjects and controls

Level of eosinophilia	Subjects N (%)	Controls N (%)	Total	X^2	p-value	Remarks
No eosinophilia	108(61.0)	124 (70.1)	232	10.468	0.005	Using Fisher exact value
Mild eosinophilia	40 (22.6)	43 (24.3)	83			
Moderate eosinophilia	29 (16.4)	10(5.6)	39			
Total	177	177	354			

In the AR subjects, 40 (22.6%) and 29 (16.4%) had mild and moderate blood eosinophilia respectively. There was no blood eosinophilia in 108 (61%) subjects. In the control group 43 (24.3%) and 10 (5.6%) of blood eosinophilia were seen in the mild and moderate categories while there were no blood eosinophilia in 124 (70.1 %). There was a statistically significant difference between the distribution of blood level of eosinophilia in the subjects and the controls (p = 0.005).

Table 4: Allergic symptoms and Nasal Smear Eosinophilia of Subjects using Spearman Correlation Test

Allergic symptoms of subjects	Nasal Smear Eosinophilia (NSE) r (p – value)
Nasal blockage	0.102 (0.216)
Runny nose	0.342 (0.001)*
Sneezing	0.232 (0.025)*
Nasal Itching / itching eyes	0.362 (0.001)*

*Significant association

The Spearman correlation test showed a significant association between nasal smear eosinophilia (NSE) and runny nose (p = 0.001), excessive sneezing (p = 0.025) and nasal itching / itching eyes (p =0.001). Howev er, there was no significant association with nasal blockage (p = 0.216).

Table 5: Correlation of NSE Vs. Blood AEC and Stool helminthes

	Nasal Smear (%)	Blood AEC (%)	Stool Helminthes (%)
SENSITIVITY	94.4	39	7
SPECIFICITY	83	70	81
PPV	85	56	25
NPV	100	53	46
ACCURACY	91.2	54.5	43.2

The sensitivity and accuracy of nasal smear eosinophil count was higher in AR subjects compared to pe ripheral blood eosinophil counts and stool helminthes (94.4 vs. 39 and 7) respectively (Table5).

IV. Discussion:

AR is a common condition in childhood, though not life threatening but causes significant morbidity in terms of quality of life. Though a thorough history and physical examination are required to establish the clinica l diagnosis, allergy testing is essential to confirm the underlying causes. Aetiology remains idiopathic in most p atients.

SPT is considered the golden standard. It may be deferred in those on oral antihistamine and cannot be done also in those with extensive atopic eczema or dermatographism. In cases where skin testing is done, import ed allergens are used. These are specific and non – identical to the common allergens in our environment. Thus, the need to have simple, economical and non-invasive tests like nasal smear eosinophil count and peripheral sme ar eosinophil count for establishing the diagnosis of allergic rhinitis in children.

The inclusion of nasal smear, blood and stool examination in the present study was to exclude helminth iasis and NAR with eosinophilia syndrome. In AR, eosinophils are found at higher levels both in peripheral bloo d and nasal tissues.[19] Elevated total IgE levels and eosinophils are not only associated with allergies but also wit h helminthic infections.[20]

Majority of the AR subjects 126 (71.1%), in this study were in the age group 6 - 8 years followed by 9 – 11 years 51(28.8%). This was in agreement with the findings of Skoner et al[1] who stated that AR was more pr evalent during school age and Sanli et al[3] that it frequently affects teenagers and young adults. Also, the greater proportion of the AR subjects were seen in primary 1 – 3 classes 93 (52.5%) as well as 95 (53.7%) of the control s.

The correlation between clinical allergy and nasal smear eosinophilia was first emphasized by Eyerman who reported a series of 92 cases with AR in which 72% had positive Eosinophilia.[8] Eosinophils in the nasal sm ear was shown to display the best correlation with clinical allergic rhinitis[21] and thus can be used not only to esta blishes the diagnosis of AR but also useful in the follow up of patients with this condition.[5]

This study revealed the distribution of severity of AR among the subjects as moderate category in 125 (70.6%) of the participants and severe category in 27 (15.3%).

There was statistically significant association in the distribution of severity of allergic rhinitis between t he subjects and controls with a p – value of ≤ 0.0001. This was in accord with

Ologe et al[22]study where 71.6% of their cases with allergic rhinitis were in the moderate category and 2 8.4% were in the severe category. Similar findings were seen in the works of Lasisi et al[23] Olusesi et al[24] and Pa tel et al.[17]

In the present study, nasal smear of 177 AR subjects were examined. However, 167 (94.4%) were nasal smear positive showing that nasal smear eosinophilia is more reliable in this group. Meanwhile, 30 (16.9%) of 1 77 children in the control group were also nasal smear positive. The fact that 16.9% of the control had positive n asal smear eosinophilia might have actually had an asymptomatic allergic condition or they might have come int o contact with non-specific irritants or stimuli. Furthermore, the possibility that some of the subjects or controls had non-allergic rhinitis with eosinophilia syndrome must be acknowledged. However, this condition is rare; it i s doubtful that it played any role in the overall results.[25,26]

The Spearman's Correlation test showed a significant association between nasal smear and runny nose ($p = 0.001$), excessive sneezing ($p = 0.025$) and nasal itching / itching eyes ($p = 0.001$). No association with nasal blockage observed ($p = 0.216$). This implies the degree of nasal smear may be useful in predicting the severity of AR[27,.] There was a good correlation of nasal smear eosinophilia with the severity of symptoms of allergic rhi nitis in this study. No correlation of blood absolute eosinophil count with severity of symptoms of allergic subje cts was observed, as majority 108 (61%) of them had no eosinophilia. These findings were similar to studies of Kumar et al[15] and Patel et al.[17]

In addition, a study by Malmberg et al[28] a significant correlation was obtained between nasal eosinophil ia and allergy. Also, Urmil et al[29] found nasal eosinophilia in 90% of the cases and none in the controls. High de gree of correlation between nasal allergy and eosinophilia was similarly demonstrated by Sasaki et al.[30]

This study showed nasal smear examination had Sensitivity of 94.4% and Specificity of 83%. This is at variance with sensitivity and specificity observed in other studies. Kumar et al[15]reported 73.5% and 72.4% resp ectively. Ahmadiafshar et al[11] had 74% sensitivity and 90% specificity. While Abhey et al[12] reported that positiv e nasal smear eosinophilia, graded quantitatively was found in 80% of the cases and in 5% of the controls.

V. Conclusion:

Nasal smear eosinophilia is simple, cost effective and non-invasive diagnostic test to determine the pres ence or absence of allergy. A good correlation between nasal smear and AR symptoms was observed in this stud y. Thus, it can be applied as a reliable tool in diagnosing AR in children where other sophisticated laboratory inv estigations are not readily available.

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