Original Article

Cocktail of Periodic Acid–Schiff and Papanicolaou: Novel staining technique for the identification of leukemic eosinophils – A pilot study

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Abstract

Background: Tissue eosinophilia may be caused due to reactive, neoplastic or idiopathic reasons. Reactive eosinophils in allergic and inflammatory conditions are transient and recruited from the circulation in response to various stimuli, whereas neoplastic eosinophils of leukemias and hematological malignancies are involved in the pathogenesis of the disease. The differentiation of reactive from neoplastic eosinophils has a serious implication on the treatment and prognosis of diseases. However, both these types of eosinophils display variation in morphology and staining characteristics in routine histopathology leading to a diagnostic dilemma.

Aim: The aim of this study is to evaluate the efficacy of special stains for the demonstration of eosinophils in normal/reactive lesions and leukemias.

Methodology: A retrospective study comprising twenty histologically diagnosed cases each of reactive oral lesions and leukemias were obtained from institutional archives. These tissue sections were subjected to staining with routine and special stains – Carbol chromotrope, Congo red, Leishman's stain, Periodic Acid–Schiff-papanicolaou (PAS-PAP) and PAS. Statistical analysis was performed using Pearson's Chi-square test to compare the various parameters in the evaluation of the staining efficacy.

Results: Carbol chromotrope and Congo red staining showed increased staining efficacy in normal/reactive eosinophils while PAS-PAP followed by PAS and Leishman's stain showed enhanced features such as homogenicity, specificity, increased staining intensity, enhanced nuclear and cytoplasmic details in leukemic eosinophils.

Conclusion: Combined PAS-PAP is a novel and cost-effective staining technique in differentiating reactive and leukemic eosinophils. It is significant in recognizing leukemic eosinophils of routine biopsies and alerts the clinician to rule out any underlying malignancies.

Keywords: Eosinophils, leukemia, reactive lesions

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INTRODUCTION

Eosinophils are multifunctional granulocytes presenting challenges in morphological identification and associated with various pathological conditions resulting from reactive, neoplastic or idiopathic causes. Increased number of eosinophils can be identified either in blood or body tissues and termed as eosinophilia. The conditions wherein eosinophilia occurs as a reactive phenomenon include allergic and inflammatory conditions, parasitic infections, metabolic and pulmonary diseases, immunodeficiency states, autoimmune disorders and malignancies. These eosinophils are secondary to antigenic stimuli, transient in nature and nonneoplastic. Conditions, in which eosinophils are pathogenic and form a part of the neoplastic clone include chronic and acute leukemias such as eosinophilic leukemias, chronic and juvenile myelomonocytic leukemias, chronic myeloid leukemia and myelodysplastic syndromes. However, idiopathic eosinophilia is a nonspecific condition and is a diagnosis of excluding reactive and neoplastic causes. According to Chowdhri *et al*., 2018, the early diagnostic features of leukemia that can be identified by a dentist is in the form of petechiae or bleeding (56%), ulceration (53%) and gingival enlargement (36%).[1-3]

Eosinophils were first introduced by Wharton Jones in 1846 as "coarse granule cells." Later, in 1879 Paul Ehrlich termed these cells as "eosinophils." These cells were also called as "end-stage degranulating effector cells" of the immune system. Eosinophils account for 2.3% of all white blood cells in peripheral blood. They have a bilobed nucleus with cytoplasm filled with large, coarse granules. These cells play a pivotal role in adhesion, locomotion, phagocytosis, secretion and also have physiological role in organ formation. Eosinophils are physiologically produced in bone marrow and controlled by T cells through the secretion of hematopoietic growth factors such as granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5.[1,2] Eosinophils are found in the peripheral blood as maturated cells for a short span of about 18 h, followed by migration into gastrointestinal tract or thymus where they reside under homeostatic conditions. The most astonishing morphological feature of eosinophil is the presence of specific/secretory/secondary granules in the cytoplasm. These granules contain basic proteins mainly cationic protein, major basic protein, eosinophilic-derived neurotoxin and eosinophilic peroxidase.[4,5]

Eosinophils are recognized as normal when their granules do not contain alkaline phosphatase, chloroacetate esterase, Astra blue and negative for Periodic Acid–Schiff (PAS) and toluidine blue staining, whereas pathological eosinophils show the presence of these components. In some cases, eosinophils display diverse morphologic features such as pelgeroid modification of nucleus (hypolobulated) which makes differentiation of pathological eosinophils from normal very difficult. Both neoplastic and reactive eosinophils show morphological changes which include variation in size, number of cells and granules.[6] Although eosinophils can be easily identified in tissue sections that are stained with Hematoxylin and Eosin (H&E), sometimes these granulocytes assume an uncommon morphology making their recognition difficult. In such situations, special stains such as Congo red, Carbol chromotrope, Sirius red and advanced techniques such as autofluorescence or immunohistochemistry are used to detect the presence of eosinophils. Currently, Carbol chromotrope and Congo red are the commonly used special stains to demonstrate the presence of tissue eosinophils.[1-5] Literature search provides meager evidence on the use of special stains for the differentiation of normal/reactive from leukemic eosinophils that may provide a diagnostic clue and affect the treatment and prognosis of the underlying disease. Moreover, there is a need to identify a special stain that is easy to use, rapid and economical to distinguish normal/reactive from leukemic eosinophils. Therefore, this study aims to evaluate the efficacy of special stains to demonstrate eosinophils in normal/reactive lesions and leukemias to facilitate diagnosis of the underlying diseased condition.

METHODOLOGY

A retrospective study was conducted on formalin-fixed paraffin-embedded tissue sections of reactive oral lesions and leukemias obtained from archives of Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences. The ethical clearance was obtained from the Institutional Review Board. A total number of forty histologically diagnosed cases of reactive oral lesions and leukemias with predominant eosinophils and adequate tissues were included in the study. Of forty cases, twenty were reactive oral lesions and twenty were leukemias (acute lymphoblastic leukemia).

Five tissue sections each of 4-μm thickness were obtained. The sections were subjected to special staining technique using Carbol chromotrope (Avistain-AMD labs), Congo red (Avistain-AMD labs), Leishman's stain (Chromospan diagnostics), PAS (combination of periodic acid [commercially available] and freshly prepared Schiff reagent), and PAS-papanicolaou (PAS-PAP) (Rapid PAP Kit-Bio lab diagnostics) combination. The staining protocol of all the special stains is summarized

in Figure 1. The stained slides were evaluated by two oral pathologists using certain parameters such as homogeneity, specificity, staining intensity, cytoplasmic and nuclear details that were specially devised for this study. Eosinophils within the vascular channels and in ulcerated areas were excluded for evaluation.

Statistical analysis

Pearson's Chi-square test was employed to compare various parameters for the evaluation of the staining efficacy. Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. released in 2013. Armonk, NY: IBM Corp., was used to analyze the data. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Eosinophils are represented as pleiotropic leukocytes that are involved in the initiation and propagation of different inflammatory responses, as well as modulators of innate and adaptive immunity. These cells are recruited from the circulation into inflammatory foci in response to various stimuli. Eosinophilic granulocytes are associated with allergy, myeloproliferative diseases, parasitic infections and different types of leukemias. In addition, these eosinophils exhibit different surface receptors for the ligands that support various biological functions such as growth, cell-to-cell interaction, adhesion, chemotaxis and degranulation.[7,8] Eosinophils are bilobed leukocytes with granular cytoplasm, consisting of four different types of secretory organelles namely secretory vesicles, crystalloid, primary and small granules. The morphologic feature responsible for diagnostic ambiguity in routine staining is their specific granules. Various cytological studies have been performed to demonstrate the pathological eosinophils in leukemias.[6] However, minimal literature is available till date to identify pathological eosinophils in the tissue sections of leukemias that provide diagnostic, management and prognostic significance. Therefore, the present study was performed to evaluate the staining efficacy of special stains in differentiating pathological from normal eosinophils in tissue sections of leukemias and normal/reactive lesions.

The stains employed were Carbol chromotrope, Congo red, Leishman, PAS and combination of PAS-PAP, H&E stain which were compared between reactive lesions and leukemias [Figures 2 and 3]. Comparison of all the six stains between normal/reactive and leukemic eosinophils was done with respect to homogeneity, specificity, staining intensity, cytoplasmic and nuclear details [Tables 1-3].

When Carbol chromotrope staining was compared between reactive and leukemic lesions, it was found that 20 (100%) reactive cases and 17 (85%) leukemias were homogeneously stained for eosinophils with $P = 0.072$. Eosinophils in 19 (95%) reactive cases appeared clear and distinct in comparison with 8 (40%) leukemic cases with a significant $P = 0.000$. Intense staining was observed in 17 (85%) reactive lesions and 4 cases (20%) of leukemias.

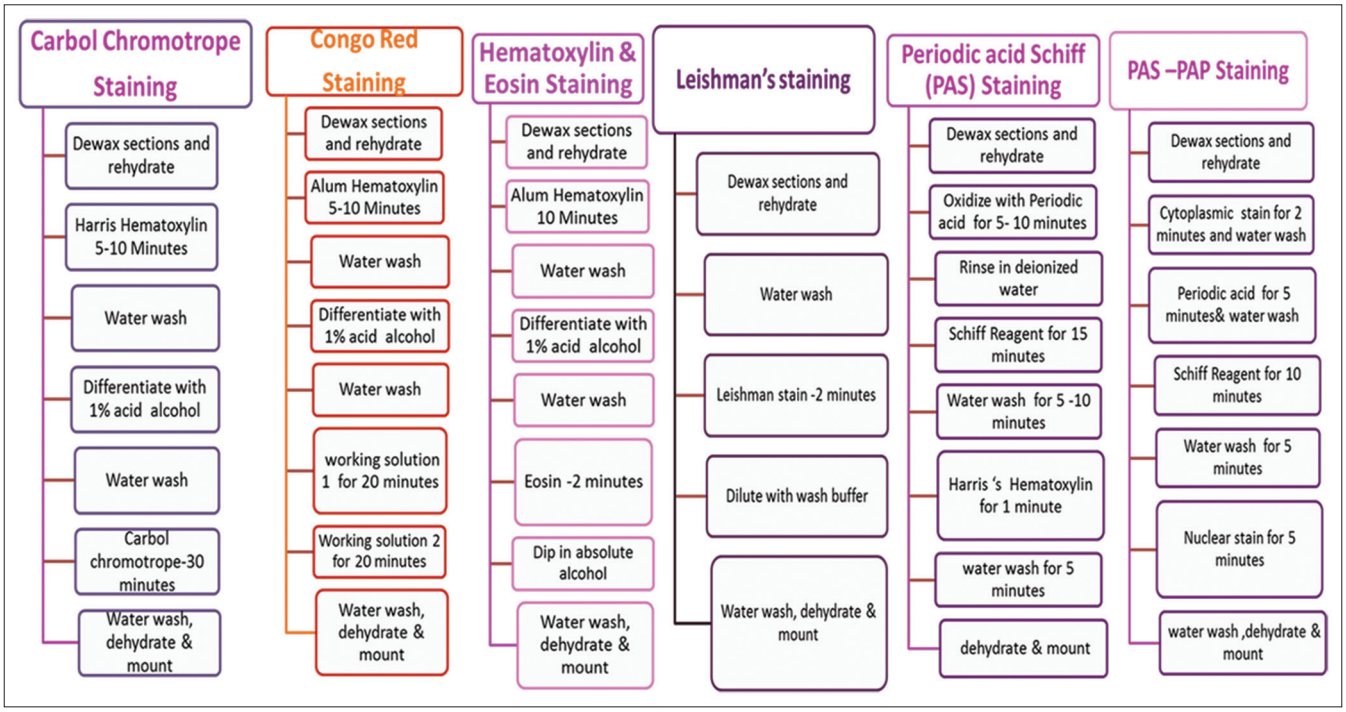

Figure 1: Methodology of staining employed using special stains

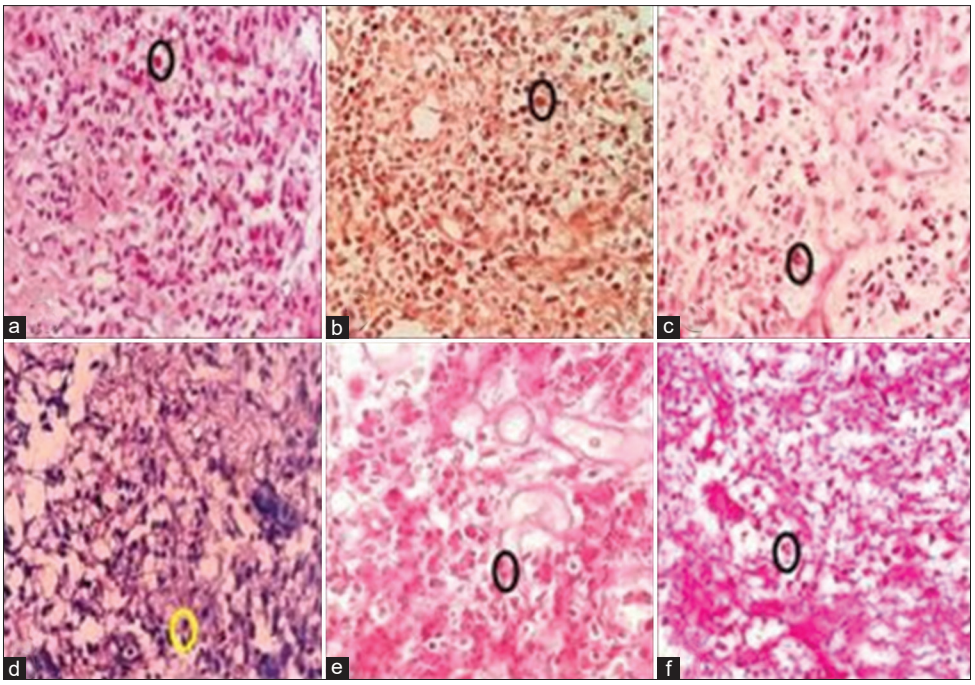

Figure 2: Photomicrograph of reactive lesions with special stains for eosinophils (×400) (a) carbol chromotrope; (b) Congo red; (c) hematoxylin and eosin; (d) Leishman's stain; (e) periodic acid–Schiff; (f) periodic acid–Schiff-papanicolaou stains

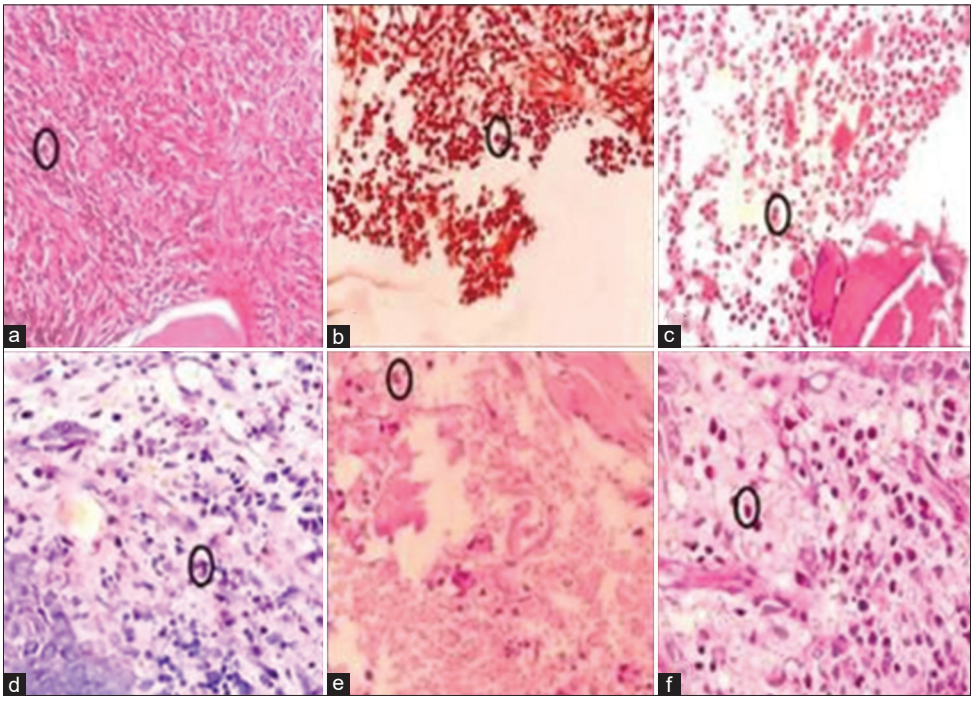

Figure 3: Photomicrograph of leukemias with special stains for eosinophils (×400) (a) carbol chromotrope; (b) Congo red; (c) hematoxylin and eosin; (d) Leishman's stain; (e) periodic acid –Schiff; (f) periodic acid–Schiff-papanicolaou stains

Cytoplasmic details such as granule staining and refractile properties were better appreciated in reactive cases 20 (100%) as compared to leukemias ($P = 0.000$).The nuclear details such as lobe architecture, size and chromatin condensation were noticed better in reactive lesions than leukemias using Carbol chromotrope staining ($P = 0.011$, 0.004 and 0.008, respectively). Statistically significant difference was observed in specificity, staining intensity, cytoplasmic and nuclear details when the efficacy of Carbol chromotrope staining was compared between reactive and leukemic cases. Debta *et al.* performed a study on tumor-associated eosinophils in oral squamous cell

Table 1: Comparison of reactive and leukemic eosinophils in various special stains

Stains	Lesions	Homogenicity		Specificity		Intensity of staining			Cytoplasmic details			Nuclear details				
		Homogeneous, *n* (%)	Non-homogeneous, *n* (%)	Clear and distinct, *n* (%)	Variable, *n* (%)	Mild, *n* (%)	Moderate, *n* (%)	Intense, *n* (%)	Granule staining property		Refractile property	Lobe architecture		Size		Chromatin condensation	
									Distinct, *n* (%)	Indistinct, *n* (%)	Present, *n* (%)	Absent, *n* (%)	Well defined, *n* (%)	Ill defined, *n* (%)	Uniform, *n* (%)	Varied, *n* (%)	Condensed, *n* (%)	Dispersed, *n* (%)
Carbol chromotrope	Reactive	20 (100)	0	19 (95)	1 (5)	1 (5)	2 (10)	17 (85)	20 (100)	0	20 (100)	0	13 (65)	7 (35)	16 (80)	4 (20)	20 (100)	0
	Leukemic	17 (85)	3 (15)	8 (40)	12 (60)	8 (40)	8 (40)	4 (20)	5 (25)	15 (75)	9 (45)	11 (55)	5 (25)	15 (75)	7 (35)	13 (65)	14 (70)	6 (30)
	P	0.072		0.000		0.000			0.000		0.000		0.011		0.004		0.008	
Congo red	Reactive	20 (100)	0	18 (90)	2 (10)	0	5 (25)	15 (75)	15 (75)	5 (25)	20 (100)	0	20 (100)	0	20 (100)	0	20 (100)	0
	Leukemic	16 (80)	4 (20)	10 (50)	10 (50)	15 (75)	3 (15)	2 (10)	7 (35)	13 (65)	6 (30)	14 (70)	9 (45)	11 (55)	8 (40)	12 (60)	13 (65)	7 (35)
	P	0.035		0.006			0.000		0.011		0.000		0.000		0.000		0.004	
H&E	Reactive	20 (100)	0	6 (30)	14 (70)	3 (15)	13 (65)	4 (20)	12 (63.2)	7 (32.8)	20 (100)	0	20 (100)	0	20 (100)	0	16 (80)	4 (20)
	Leukemic	20 (100)	0	1 (5)	19 (95)	13 (65)	7 (35)	0	3 (15)	17 (85)	9 (45)	11 (55)	8 (40)	12 (60)	12 (60)	8 (40)	16 (80)	4 (20)
	P	Remains constant		0.037			0.002		0.002		0.000		0.000		0.002		1.000	
Leishman	Reactive	20 (100)	0	16 (80)	4 (20)	17 (85)	2 (10)	1 (5)	14 (70)	6 (30)	16 (80)	4 (20)	14 (70)	6 (30)	20 (100)	0	17 (85)	3 (15)
	Leukemic	14 (70)	6 (30)	8 (40)	12 (60)	14 (70)	6 (30)	0	0	20 (100)	6 (30)	14 (70)	2 (10)	18 (90)	10 (50)	10 (50)	9 (45)	11 (55)
	P	0.008		0.010			0.193		0.000		0.001		0.000		0.000		0.008	
PAS	Reactive	9 (45)	11 (55)	4 (20)	16 (80)	10 (50)	10 (50)	0	3 (15)	17 (85)	8 (40)	12 (60)	4 (20)	16 (80)	17 (85)	3 (15)	19 (95)	1 (5)
	Leukemic	20 (100)	0	13 (65)	7 (35)	0	5 (25)	15 (75)	16 (80)	4 (20)	20 (100)	0	14 (70)	6 (30)	5 (25)	15 (75)	3 (15)	17 (85)
	P	0.000		0.004			0.000		0.000		0.000		0.001		0.000		0.000	
PAS-PAP	Reactive	7 (35)	13 (65)	4 (20)	16 (80)	12 (60)	8 (40)	0	6 (30)	14 (70)	0	20 (100)	5 (25)	15 (75)	18 (90)	2 (10)	20 (100)	0
	Leukemic	20 (100)	0	20 (100)	0	0	0	20 (100)	20 (100)	0	20 (100)	0	17 (85)	3 (15)	0	20 (100)	0	20 (100)
	P	0.000		0.000			0.000		0.000		0.000		0.000		0.000		0.000	

PAS: Periodic Acid-Schiff, PAP: Papanicolaou, H&E: Hematoxylin and eosin

carcinoma (OSCC) using special stains and revealed that Carbol chromotrope staining method is better to determine tissue eosinophils.[9] Astekar *et al.* evaluated staining quality of carbol chromotrope and congo red for mast cells and eosinophils in odontogenic cystic linings and found that carbol chromotrope was better than Congo red.[10]

Comparison of Congo red staining in the present study between reactive and leukemic cases showed homogeneously stained eosinophils in 20 (100%) reactive and 16 (80%) leukemias ($P = 0.035$). Similarly, eosinophils in 18 (90%) reactive and 10 (50%) leukemic cases were clear and distinct with a statistically significant $P = 0.006$. Reactive lesions (15 cases) showed increased staining intensity as compared to leukemias (2 cases) ($P = 0.000$). Cytoplasmic details such as granule staining and refractile properties were better appreciated in reactive lesions than leukemias ($P = 0.011$ and 0.000, respectively). Significant difference was observed in the lobe architecture ($P = 0.000$), size ($P = 0.000$) and chromatin condensation ($P = 0.004$) on comparing reactive and leukemic cases. Joshi and Kaijkar assessed the efficacy of Congo red staining to differentiate eosinophils in OSCC and found that Congo red was better than routine H&E staining.[11]

H&E-stained eosinophils were compared between reactive lesions and leukemias in the present study. It was found that eosinophils in 20 (100%) cases each of reactive lesions and leukemias were homogeneously stained. The specificity of H&E staining was variable in 14 (70%) of reactive cases as against 19 (95%) of leukemias. Moderate staining intensity was observed in 13 (65%) and 7 (35%) cases of reactive lesions and leukemias, respectively ($P = 0.002$). On comparison of granule staining and refractile properties of eosinophils between reactive and leukemic cases, a statistically significant difference of $P = 0.002$ and 0.000, respectively, was observed. The nuclear details such as lobe architecture, size and chromatin condensation were appreciated better in reactive than leukemic cases using H&E stain ($P = 0.000$, 0.002 and 1.000, respectively).

In the present study, 20 (100%) reactive lesions were homogeneously stained with Leishman's stain in contrast to 14 (70%) cases of leukemias ($P = 0.008$). Similarly, 16 (80%) reactive and 8 (40%) leukemic cases were clear and distinctly specific for eosinophils with $P = 0.010$. Mild staining intensity ($P = 0.193$) was observed for eosinophils in 17 (85%) and 14 (70%) cases of reactive lesions and leukemias, respectively. Cytoplasmic details such as granule staining and refractile properties were found to be better in reactive lesions in comparison to leukemias ($P = 0.000$, 0.001, respectively). Lobe architecture ($P = 0.000$),

Table 2: Comparison of staining efficacy of special stains in normal/reactive lesions

Criteria	Comparison of staining efficacy of special stains in normal/reacitve lesions					
	PAS-PAP, *n* (%)	Carbol chromotrope, *n* (%)	Congo red, *n* (%)	H&E, *n* (%)	Leishman, *n* (%)	PAS, *n* (%)
Homogeneity						
Homogeneous	7 (35)	20 (100)	20 (100)	20 (100)	20 (100)	9 (45)
Nonhomogeneous	13 (65)	0	0	0	0	11 (55)
Specificity						
Clear and distinct	4 (20)	19 (95)	18 (90)	6 (30)	16 (80)	13 (65)
Variable	16 (80)	1 (5)	2 (10)	14 (70)	4 (20)	7 (35)
Intensity of staining						
Mild	12 (60)	1 (5)	0	3 (15)	17 (85)	0
Moderate	8 (40)	2 (10)	5 (25)	13 (65)	2 (10)	5 (25)
Intense	0	17 (85)	15 (75)	4 (20)	1 (5)	15 (75)
Granule staining property						
Distinct	6 (30)	20 (100)	15 (75)	12 (63.2)	14 (70)	3 (15)
Indistinct	14 (70)	0	5 (25)	7 (36.8)	6 (30)	17 (85)
Refractile property						
Present	0	20 (100)	20 (100)	20 (100)	16 (80)	20 (100)
Absent	20 (100)	0	0	0	4 (20)	0
Lobe architecture						
Well defined	5 (15)	13 (65)	20 (100)	20 (100)	14 (70)	4 (20)
Ill defined	15 (75)	7 (35)	0	0	6 (30)	16 (80)
Size						
Uniform	18 (90)	16 (80)	20 (100)	20 (100)	20 (100)	17 (85)
Varied	2 (10)	4 (20)	0	0	0	3 (15)
Chromatin condensation						
Condensed	20 (100)	20 (100)	20 (100)	16 (80)	17 (85)	19 (95)
Dispersed	0	0	0	4 (20)	3 (15)	1 (5)

PAS: Periodic Acid Schiff, PAP: Papanicolaou, H&E: Hematoxylin and eosin

Table 3: Comparison of staining efficacy special stains in leukemias

Criteria	Comparison of staining efficacy of special stains in Leukemias					
	Carbol chromotrope, *n* (%)	PAS-PAP, *n* (%)	Congo red, *n* (%)	H&E, *n* (%)	Leishman, *n* (%)	PAS, *n* (%)
Homogeneity						
Homogeneous	17 (85)	20 (100)	16 (80)	20 (100)	14 (70)	20 (100)
Nonhomogeneous	3 (15)	0	4 (20)	0	6 (30)	0
Specificity						
Clear and distinct	8 (40)	20 (100)	10 (50)	1 (5)	8 (40)	4 (20)
Variable	12 (60)	0	10 (50)	19 (95)	12 (60)	16 (80)
Intensity of staining						
Mild	8 (40)	0	15 (75)	13 (65)	14 (70)	10 (50)
Moderate	8 (40)	0	3 (15)	7 (35)	6 (30)	10 (50)
Intense	4 (20)	20 (100)	2 (10)	0	0	0
Granule staining property						
Distinct	5 (25)	20 (100)	7 (35)	3 (15)	0	16 (80)
Indistinct	15 (75)	0	13 (65)	17 (85)	20 (100)	4 (20)
Refractile property						
Present	9 (45)	20 (100)	6 (30)	9 (45)	6 (30)	8 (40)
Absent	11 (55)	0	14 (70)	11 (55)	14 (70)	12 (60)
Lobe architecture						
Well defined	5 (15)	17 (85)	9 (45)	8 (40)	2 (10)	14 (70)
Ill defined	15 (75)	3 (15)	11 (55)	12 (60)	18 (90)	6 (30)
Size						
Uniform	7 (35)	0	8 (40)	12 (60)	10 (50)	5 (25)
Varied	13 (65)	20 (100)	12 (60)	8 (40)	10 (50)	15 (75)
Chromatin condensation						
Condensed	14 (70)	0	13 (65)	16 (80)	9 (45)	3 (15)
Dispersed	6 (30)	20 (100)	7 (35)	4 (20)	11 (55)	17 (85)

PAS: Periodic Acid Schiff, PAP: Papanicolaou, H&E: Hematoxylin and eosin

size ($P = 0.000$) and chromatin condensation ($P = 0.008$) was better appreciated in reactive lesions.

When PAS staining was compared between reactive and leukemic lesions, it was found that 9 (45%) reactive cases and 20 (100%) leukemias were homogeneously stained for eosinophils with $P = 0.000$. Similarly, eosinophils in 4 (20%) reactive and 13 (65%) leukemic cases showed clear and distinctly specific staining ($P = 0.004$). Fifteen (75%) leukemic cases showed intense staining, whereas none

of the reactive lesions displayed intense staining for eosinophils which was statistically significant ($P = 0.000$). Cytoplasmic details such as granule staining and refractile properties were well appreciated in leukemic cases as compared to reactive ($P = 0.000$). The nuclear details such as lobe architecture, size and chromatin condensation were noticed better in leukemias than reactive lesions using PAS staining with significant $P = 0.001, 0.000, 0.000$ respectively.

In the present study, combined PAS-PAP staining technique showed homogeneously stained eosinophils in 20 (100%) leukemias and 7 (35%) reactive lesions with significant difference ($P = 0.000$). Similarly, eosinophils in 20 (100%) leukemic cases showed clear and distinctly specific staining in contrast to 4 (20%) reactive cases with a significant $P = 0.000$. Twenty (100%) cases of leukemias showed intense staining as against none in reactive lesions ($P = 0.000$). Granule staining and refractile properties were better observed in leukemic lesions in comparison to reactive lesions ($P = 0.000$). A significant difference ($P = 0.000$) was observed in nuclear details such as lobe architecture, size and chromatin condensation in leukemias in as compared to reactive lesions.

In the present study, Carbol chromotrope and Congo red staining showed increased specificity and staining intensity, better nuclear and cytoplasmic details in normal/reactive eosinophils in comparison to leukemias. Leukemic eosinophils in our study showed superior features such as homogenicity, specificity, increased staining intensity, enhanced nuclear and cytoplasmic details in PAS-PAP followed by PAS and Leishman's stain.

The combination of PAS staining with PAP is based on the acid-base reaction in which acidic dye of PAP (eosin azure) binds with the cytoplasm and connective tissue components of the cell which are basic, whereas the basic dye (Harris' hematoxylin) binds with acidic (nuclei) component of the cell. The leukemic eosinophils, therefore, assume a pink granular cytoplasm suggesting the presence of PAS-positive granules with dark blue nuclei which is absent in normal/reactive eosinophils. This occurs due to oxidation of carbon-to-carbon bond-forming aldehydes which react with fuchsin – sulfurous acid present in Schiff's reagent rendering dark magenta colored granular cytoplasm to the leukemic eosinophils.[7]

Very minimal studies have been performed till date to evaluate the pathological/leukemic eosinophils in tissue sections. However, numerous cytological studies have been reported earlier. Liso *et al*. demonstrated the presence of abnormal eosinophils cytologically in leukemias using special stains such as Giemsa, PAS, toluidine blue, Sudan black and Astra blue and stated that the leukemic eosinophils showed alkaline phosphatase, chloroacetate esterase, specific PAS-positive granules which were absent in normal/reactive eosinophils.[6] Similarly, Yam *et al*. (1971) performed a cytological study using chloroacetate esterase-cyanide-resistant peroxidase and chloroacetate esterase-chlorazol fast pink staining techniques and showed that leukemic eosinophils possess peculiar features like abnormal and uneven distribution of large granules with vacuoles in the cytoplasm.[12-15]

Further studies involving larger sample size with clinicopathologic correlation and comparison of cytology with histopathology may help in providing definitive diagnosis by the use of simple and feasible differential stains.

CONCLUSION

To our knowledge, the present study is the first of its kind to differentiate the staining properties of eosinophils in normal/reactive and leukemias in tissue sections. Carbol chromotrope and Congo red stains may be efficiently used to identify normal/reactive eosinophils. However, leukemic eosinophils may be demonstrated using a cocktail of PAS-PAP followed by PAS and Leishman's stains. As leukemic eosinophils showed superior cellular and nuclear details in combined PAS-PAP staining technique, it may be considered as a novel stain for the detection of leukemic eosinophils. This simple, yet economical staining technique (cocktail PAS-PAP) may provide diagnostic clue for any underlying malignancies by the recognition of pathological/leukemic eosinophils in tissue eosinophilia cases of routine biopsies, thereby enables the clinician to tailor his therapies.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: Changing perspectives in health and disease. Nat Rev Immunol 2013;13:9-22.
2. McEwen BJ. Eosinophils: A review. Vet Res Commun 1992;16:11-44.
3. Chowdhri K, Tandon S, Lamba AK, Faraz F. Leukemic gingival enlargement: A case report and review of literature. J Oral Maxillofac Pathol 2018;22:S77-81.
4. Silberstein DS. Eosinophil function in health and disease. Crit Rev Oncol Hematol 1995;19:47-77.
5. Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, Saporiti N, *et al*. Eosinophils from physiology to disease: A Comprehensive review. Biomed Res Int 2018;2018:9095275.

6. Liso V, Troccoli G, Specchia G, Magno M. Cytochemical "normal" and "abnormal" eosinophils in acute leukemias. Am J Hematol 1977;2:123-31.
7. Weller PF, Spencer LA. Functions of tissue-resident eosinophils. Nat Rev Immunol 2017;17:746-60.
8. Wen T, Rothenberg ME. The regulatory function of eosinophils. Microbiol Spectr 2016;4. doi: 10.1128/microbiolspec.MCHD-0020-2015.
9. Priyanka D, Debta FM, Minal C, Anurag D. Evaluation of infiltration of immunological cells (tumour associated tissue eosinophils and mast cells) in oral squamous cell carcinoma by using special stains. Br J Med Med Res 2012;2:75-85.
10. Astekar M, Ahmed J, Metgud R, Phull K, Sowmya GV. Evaluation of mast cells and eosinophils in odontogenic cysts: A histochemical approach. J Dr. NTR Univ of Health Sci 2014;3:243-48.
11. Joshi PS, Kaijkar MS. A histochemical study of tissue eosinophilia in oral squamous cell carcinoma using Congo red staining. Dent Res J (Isfahan) 2013;10:784-9.
12. Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 1971;55:283-90.
13. Rezamand A, Ghorashi Z, Ghorashi S, Nezami N. Eosinophilic presentation of acute lymphoblastic leukemia. Am J Case Rep 2013;14:143-6.
14. Fletcher S, Bain B. Eosinophilic leukaemia. Br Med Bull 2007;81-82:115-27.
15. Boyer DF. Blood and bone marrow evaluation for eosinophilia. Arch Pathol Lab Med 2016;140:1060-7.