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Frequency of PDGFRA, PDGFRB and FGFR1 Gene Rearrangements in Patients with *Eosinophilia* and Their Clinico-Haematologic Parameters

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ABSTRACT

Objective: To determine the frequency and clinico-haematological features of PDGFRA, PDGFRB and FGFR1 gene rearrangements in patients with persistent *Eosinophilia* using Fluorescence in situ hybridization.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Dec 2018 to Dec 2019.
Methodology: All Patients who presented to AFIP having absolute eosinophil count >1.5x10^9/L persistent for over six months or with Myeloid or Lymphoid neoplasms with persistent *Eosinophilia* were studied. Patients having reactive *Eosinophilia* and those on treatment were excluded. Interphase FISH studies were performed. In addition, 2.5ml of sodium heparin blood was taken. After the denaturation of DNA, slides were set up according to standard protocol. FIP1L1/CHIC2/PDGFRA dual colour probe was applied for PDGFRA, 5q32 PDGFRA break apart probe for PDGFRB and XL FGFR1 break apart probe for FGFR1 gene rearrangement.
Results: A total of 60 patients were included in the study. Of these, 50(83.3%) were males, and 10(16.7%) were females, with an average absolute *Eosinophilia* count of 5.92±7.10x10^9/L. The only rearrangement detected in patients with *Eosinophilia* was FIPILI-PDGFRA gene fusion, detected in 20% of the patients. No other rearrangement was found.
Conclusion: PDGFRA, PDGFRB and FGFR1 mutations are rare yet most prominent in patients with clonal *Eosinophilia*. About 80% of eosinophilic patients were found to have idiopathic *Eosinophilia*, which requires further consideration to address the disease prevalence.

Keywords: Clinico-haematologic features, *Eosinophilia*, FGFR1, PDGFRA, PDGFRB.

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INTRODUCTION

Eosinophilia is an absolute eosinophil count of >0.5x10^9/L in the peripheral circulatory blood.[1] Eosinophils are granulocytes associated with the cellular immune system. They are derived from haemopoietic stem cells with a half-life of 8-18 hours.[2] A finding of *Eosinophilia* in the peripheral blood film can be due to countless ailments, including mild to acute medical problems such as pollen allergies, drug allergies, parasitic infections, autoimmune disorders, endocrine disorders, blood disorders and cancers.[3,4] From a diagnostic standpoint, *Eosinophilia* is divided into two types; Clonal and Reactive.[5]

Reactive Eosinophilia (polyclonal) is caused by high levels of Interleukin 5 (IL-5) in tissues which is a mediator for *eosinophil* maturation and activation; produced by type-2 T helper cells and mast cells.[6] The WHO classification of tumours related to blood and lymphoid tissues, revised in 2008 (and upgraded in 2016).[7] sub-classifies primary *Eosinophilia* into Idiopathic *Eosinophilia* (without known cause and Asymptomatic) or *Idiopathic hypereosinophilic syndrome* (HES), *Chronic eosinophilic leukaemia* not otherwise specific (CEL-NOS) and Myeloid and lymphoid neop-lasms with *Eosinophilia* associated with chromosomal rearrangements of PDGFRA, PDGFRB and FIP1L1.[8,9]

This study was designed to determine the frequency and clinico-hematological features of PDGFRA, PDGFRB and FGFR1 gene rearrangements using FISH technology in patients with persistent *Eosinophilia*.

METHODOLOGY

The cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi Pakistan, from December 2018 to December 2019**.** The study was conducted after taking permission from the ERC (No: FC-HEM17-21/READ-IRB/18/664). The sample size was calculated using the WHO sample

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size calculator taking prevalence of Hypereosino-philia as 17%.[10]

Inclusion Criteria: Patients who presented to AFIP were examined with absolute eosinophil count >1.5x 10^9/L persistent for over six months or with Myeloid or Lymphoid neoplasms with persistent *Eosinophilia*, were included in the study.

Exclusion Criteria: Patients having reactive Eosinophilia with evidence of secondary causes and patients already taking any treatment were excluded.

Blood and bone marrow samples were collected for haematological and cytogenetic studies. About 2.5 ml venous blood in EDTA was collected and analyzed by a Sysmex analyzer, while the bone marrow aspirate samples (3ml each) were collected in a sodium heparin tube. The samples were cultured and incubated at 37°C without phytohaemagglutinin (PHA) for 24 hours and an hour after adding 2001-11 colchicine. After incubation, the sample was centrifuged at 1500rpm for 8-minutes. After discarding the supernatant, KCI was added, followed by repeat centrifugation.

The samples were washed thrice by glacial acetic acid and methanol (3:1 ratio). For the FISH screening, prepared pellets were set on the slide. The slide was treated with ascending concentrations of alcohol followed by saline sodium citrate (SSC Solution). After Air-drying the slide, 101-11 Metasystems protocol of FIP1L1/CHIC2/PDGFRA dual colour probe was applied for detection of PDGFRA gene rearrangement, 5q32 PDGFRB break apart probe and XL FGFR1 break-apart probe for detection of PDGFRB gene rearrangement and FGFR1 gene rearrangement respectively. The slide was covered with a cover slip and fixed with elastic concrete.

The samples were hybridized trailed by the pattern of denaturation, hybridization and co-denaturation (at 74°C for 05 min, 37 C for 18hrs and 74°C for 5 min), respectively. The slide was taken out, washed, air-dried, and counterstained with DAPI following protocol 210-201-11. Then sample slide was frozen at-20°C for one day. Fluorescent microscopy assessment was done using an orange–green spectrum filter to analyze 500 interphases of nuclei per probe.

SPSS verses 23 was used for the data analysis. Mean±SD were calculated for quantitative variables, while the frequencies and percentages were calculated for qualitative variables. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

In our study, the total number of patients was 60. The mean TLC was 28.22±32.03x10^9/L, the mean Haemoglobin was 10.51±2.79x10^9/L, and the mean absolute eosinophil count was 5.92±7.10x10^9/L. A total of 32(53.3%) patients had idiopathic *Eosinophilia*, 26(43.3%) patients had *Eosinophilia* with myeloid neoplasm, while 2(3.3%)patients had *Eosinophilia* with lymphoid neoplasms as shown in Table-I. Table-II shows the PDGFRA, PDGFRB and FGFR1 gene arrangement frequency among the studied patients.

Table-I: Type of Hematological Disorder (n=60)

Type of Hematological Disorder	n(%)
Idiopathic *Eosinophilia*	32(53.3%)
Eosinophilia with myeloid neoplasms	26(43.3%)
Eosinophilia with lymphoid neoplasms	2(3.3%)

Table-II: Type of Gene Rearrangement (n=60)

Type of Gene Rearrangement	Detected	Not Detected
PDGFRA gene rearrangement	12(20.0%)	48(80.0%)
PDGFRB gene rearrangement	-	60(100%)
FGFR1 gene rearrangement	-	60(100%)

All 12 patients with PDGFRA gene rearrangement were males with a median age of 48 years. The clinical-haematologic features associated with FIPILI-PDGFRA rearrangement are summarized in Tables-III & IV.

Table-III: Hematologic Findings in PDGFRA Gene Rearrangement (n=60)

PDGFRA Gene Rearrangement	Absolute Eosinophil Count (10^9/L)	TLC (10^9/L)	Hb (g/L)
Detected	12.32±10.36	41.73±26.73	12.27±4.65
Not detected	4.32±5.01	20.68±15.33	10.07±1.92

Table-IV: Clinical features of Patients in PDGFRA Gene Rearrangement (n=60)

	PDGFRA Gene Rearrangement	
	Detected	Not detected
Pallor		
Present	6(50.0%)	30(62.5%)
Absent	6(50.0%)	18(37.5%)
Jaundice		
Present	0(0%)	2(4.2%)
Absent	12(100.0%)	46(95.8%)
Liver		
Present	4(33.3%)	10(20.8%)
Absent	8(66.7%)	38(79.2%)
Spleen		
Present	4(33.3%)	4(8.3%)
Absent	8(66.7%)	44(91.7%)
Lymph Nodes		
Present	06(10%)	54(90%)

In the present study, as per WHO categorization, 32(53.3%) patients had Idiopathic *Eosinophilia*, 26 (43.3%) had *Eosinophilia* with myeloid neoplasms, and 3.2% had *Eosinophilia* with lymphoid neoplasms. The only cytogenetic abnormality detected in 12(20%) patients was FIPILI-PDGFRA rearrangement, while no other gene rearrangement was found in our study. In patients with PDGFRA gene rearrangement, the mean absolute eosinophil count was 12.32±10.36x10^9/L, mean TLC was 41.73±26.73x10^9/L and mean Hb was 12.27±4.65 g/dl.

DISCUSSION

A detailed workup of clonal *Eosinophilia* in-volves the assessment of peripheral blood, bone marrow morphological features and cytogenetic analy-sis. The FIP1L1-PDGFRA is frequently karyotypically occult, which makes Fluorescence in situ hybridization (FISH) screening mandatory.[11,12] Although routine karyotyping can detect aberrations of PDGFRB and FGFR1 gene, FISH and molecular examinations are more helpful to affirm these modifications as different genes in the 5q31–33 and 8*p*11 region might be engaged with other translocations.[13,14] Therefore, the main objective of our study was to determine the frequ-ency and clinico-hematological features of PDGFRA, PDGFRB and FGFR1 gene rearrangement in patients with persistent *Eosinophilia* using Fluorescence in situ hybridization (FISH). Sixty patients who presented to AFIP with absolute eosinophil count >1.5x10^9/L persistent for six months were studied.

FIP1L1-PDGFRA gene rearrangement associated with clonal *Eosinophilia* is rare.[14] In our study, FIP1L1-PDGFRA gene rearrangement was detected in only 20% of patients with *Eosinophilia* which is higher than that reported by Pardanani *et al.*(i.e. 14%).[15] Rumor *et al.* reported the frequency of PDGFRA gene rearrangement in *eosinophilic* patients to be 17%,[13] comparable to our results. In our study, all the patients with PDGFRA gene rearrangement were males, compared to the high male predominance reported in another study in patients with PDGFRA gene rearrangement.[7] Rohmer *et al.*also reported a clear male predominance in PDGFRA rearrangement-positive patients.[13] The haematological results showed that patients with FIP1L1-PDGFRA gene rearrangement had an average absolute eosinophilic count of 12.32±10.36x10^9/L which is greater than that (5x10^9/L) reported by Vega *et al.*[16] A study reported average absolute *eosinophil* count in PDGFRA positive patients to be 10.3±5.9x 10^9/L which is also comparable to our results.[17] In our study, we observed mean Hb in patients with PDGFRA gene arrangement to be 12.27±4.65 g/dl which was comparable to that reported in other published data.[13, 7]

Similarly, the fusion was found in only 40 of 376 individuals (11%) in a European trial of patients with persistent, unexplained Hyper*Eosinophilia*.[18] In a Mayo series, 11 of 89 patients (12%) with moderate to severe *Eosinophilia* were FIP1L1–PDGFRA positive.[15] These studies show that the incidence of FIP1L1–PDGFRA fusion is approximately 10–20% among patients presenting with idiopathic Hyper*Eosinophilia* in developed countries.

Gotlib *et al.* reported splenomegaly as a more frequent finding in patients with FIP1L1-PDGFRA gene rearrangement compared to those without this gene rearrangement.[19] The signs and symptoms observed in our study in eosinophilic patients with the FIP1L1-PDGFRA gene rearrangement were nearly similar to the findings of Sreedharanunni *et al.*[20] 6 out of 12 patients had pallor, while organomegaly was observed in 8 patients; of which four patients presented with splenomegaly and four patients had hepatomegaly while six patients had lymphadenopathy.

FIP1L1-PDGFRA gene rearrangement was found to be the most common gene rearrangement in clonal *Eosinophilia*. While the other types of gene rearrangements, i.e., PDGFRB and FGFR1, had not been detected. The rarity of PDGFRB and FGFR1 mutations was comparable to a study by Gotlib *et al.* who reported the frequency of these mutations to be less than 1% in patients with primary *Eosinophilia*.[19]

CONCLUSION

Eosinophilia is a common haematological disorder. PDGFRA gene rearrangement was found in about 20% of patients with clonal *Eosinophilia*; the other two were not detected in our set of patients. All the patients had a roughly similar spectrum of symptoms. About 80% of the patients had idiopathic *Eosinophilia*, which must be considered to address the disease prevalence, diagnosis and treatment.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SJ: & HMR: Conception, data acquisition, data analysis, approval of the final version to be published.

HS & AMA: Study design, drafting the manuscript, data interpretation, approval of the final version to be published.

AL & AS: Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. 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. doi: 10.1155/2018/9095275.
2. Kovalszki A, Weller PF. *Eosinophilia*. Prim Care 2016; 43(4): 607-617. doi: 10.1016/j.pop.2016.07.010.
3. Falchi L, Verstovsek S. *Eosinophilia* in hematologic disorders. Immunol Allergy Clin North Am 2015; 35(3): 439-452. doi: 10.10 16/j.iac.2015.04.004.
4. Tefferi A. Blood *Eosinophilia*: a new paradigm in disease classification, diagnosis, and treatment. Mayo Clin Proc 2005; 80(1): 75-83. doi: 10.1016/S0025-6196(11)62962-5.
5. Baer C, Muehlbacher V, Kern W, Haferlach C, Haferlach T. Molecular genetic characterization of myeloid/lymphoid neoplasms associated with *Eosinophilia* and rearrangement of PDGFRA, PDGFRB, FGFR1 or PCM1-JAK2. Haematologica 2018; 103(8): e348-e350. doi: 10.3324/haematol.2017.187302.
6. Jain N, Khoury JD, Pemmaraju N, Kollipara P, Verstovsek S. Imatinib therapy in a patient with suspected chronic neutrophilic leukemia and FIP1L1-PDGFRA rearrange-ment. Blood 2013; 122(19): 3387-3388. doi: 10.1182/blood-2013-07-516500.
7. Pardanani A, Ketterling RP, Li CY, Patnaik MM, Wolanskyj AP, Elliott MA, et al. FIP1L1-PDGFRA in eosinophilic disorders: prevalence in routine clinical practice, long-term experience with imatinib therapy, and a critical review of the literature. Leuk Res 2006; 30(8): 965-970. doi: 10.1016/j.leukres.2005.11.011.
8. Kawagishi J, Kumabe T, Yoshimoto T, Yamamoto T. Structure, organization, and transcription units of the human alpha-platelet-derived growth factor receptor gene, PDGFRA. Genomics 1995; 30(2): 224-232. doi: 10.1006/geno.1995.9883.
9. Heldin CH, Lennartsson J. Structural and functional properties of platelet-derived growth factor and stem cell factor receptors. Cold Spring Harb Perspect Biol 2013; 5(8): a009100. doi: 10.1101/cshperspect.a009100.
10. Helsten T, Schwaederle M, Kurzrock R. Fibroblast growth factor receptor signaling in hereditary and neoplastic disease: biologic and clinical implications. Cancer Metastasis Rev 2015; 34(3): 479-496. doi: 10.1007/s10555-015-9579-8.
11. Ren M, Qin H, Wu Q, Savage NM, George TI, Cowell JK, et al. Development of ZMYM2-FGFR1 driven AML in human CD34+ cells in immunocompromised mice. Int J Cancer 2016; 139(4): 836-840. doi: 10.1002/ijc.30100.
12. Tefferi A, Gotlib J, Pardanani A. Hypereosinophilic syndrome and clonal *Eosinophilia*: point-of-care diagnostic algorithm and treatment update. Mayo Clin Proc 2010; 85(2): 158-164. doi: 10.4065/mcp.2009.0503.
13. Rohmer J, Couteau-Chardon A, Trichereau J, Panel K, Gesquiere C, Ben Abdelali R, et al. CEREO and GBMHM collaborators. Epidemiology, clinical picture and long-term outcomes of FIP1L1-PDGFRA-positive myeloid neoplasm with *Eosinophilia*: Data from 151 patients. Am J Hematol 2020 ; 95(11): 1314-1323. doi: 10.1002/ajh.25945.
14. Anwar N, Nadeem M, Shan S, Shamsi T. *Eosinophilia* with PDGFRB gene rearrangement and isolated del 5q; an extremely rare presentation of myeloid neoplasm. Acta Medica Int 2017; 4(1): 7.doi: 10.5530/ami.2017.4.2.
15. Pardanani A, Brockman SR, Paternoster SF, Flynn HC, Ketterling RP, Lasho TL, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe *Eosinophilia*. Blood 2004; 104(10): 3038-3045. doi: 10.1182/blood-2004-03-0787.
16. Vega F, Medeiros LJ, Bueso-Ramos CE, Arboleda P, Miranda RN. Hematolymphoid neoplasms associated with rearrangements of PDGFRA, PDGFRB, and FGFR1. Am J Clin Pathol 2015; 144(3): 377-392. doi: 10.1309/AJCPMORR5Z2IKCEM.
17. Baccarani M, Cilloni D, Rondoni M, Ottaviani E, Messa F, Merante S, et al. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRα-positive hypereosinophilic syndrome. Results of a multicenter prospective study. Haematologica 2007; 92(9): 1173-1179. doi:10.3324/haematol.11420.
18. Jovanovic JV, Score J, Waghorn K, Cilloni D, Gottardi E, Metzgeroth G, et al. Low-dose imatinib mesylate leads to rapid induction of major molecular responses and achievement of complete molecular remission in FIP1L1-PDGFRA-positive chronic eosinophilic leukemia. Blood 2007; 109(11): 4635-4640. doi: 10.1182/blood-2006-10-050054.
19. Gotlib J, Cools J. Five years since the discovery of FIP1L1-PDGFRA: what we have learned about the fusion and other molecularly defined *Eosinophilia*s. Leukemia 2008; 22(11): 1999-2010. doi: 10.1038/leu.2008.287.
20. Sreedharanunni S, Varma N, Sachdeva MUS, Naseem S, Malhotra P, Bansal D, et al. The Spectrum of Hyper*Eosinophilia* and Associated Clonal Disorders - A Real-World Data Based on Combined Retrospective and Prospective Analysis from a Tropical Setting. Mediterr J Hematol Infect Dis 2018 ; 10(1): e2018052. doi: 10.4084/MJHID.2018.052