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-haematologic 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 >1.5x10⁹/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. FIPIL1/CHIC2/PDGFRA dual colour probe was applied for PDGFRA, 5q32 PDGFRA break apart probe for PDGFRA 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±7.10x10⁹/L. The only rearrangement detected in patients with Eosinophilia was FIPIL1-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 >0.5x10⁹/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 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 neoplasms with Eosinophilia associated with chromosomal rearrangements of PDGFRA, PDGFRB and FIPIL1.⁸⁹

This study was designed to determine the frequency and clinico-haematologic 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 size calculator and the sample size was calculated using the WHO sample size calculator.
FGFR1 Gene Rearrangements

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 rearrangement frequency among the studied patients.

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<th>Table-I: Type of Hematological Disorder (n=60)</th>
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<td>Type of Hematological Disorder</td>
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<td>Idiopathic Eosinophilia</td>
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<td>Eosinophilia with myeloid neoplasms</td>
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<th>Table-II: Type of Gene Rearrangement (n=60)</th>
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<td>Type of Gene Rearrangement</td>
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<td>PDGFRA gene rearrangement</td>
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<td>PDGFRB gene rearrangement</td>
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<td>FGFR1 gene rearrangement</td>
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All 12 patients with PDGFRA gene rearrangement were males with a median age of 48 years. The clinical-haematologic features associated with FIPIL1-PDGFRA rearrangement are summarized in Tables-III & IV.

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<th>Table-III: Hematologic Findings in PDGFRA Gene Rearrangement (n=60)</th>
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<td>PDGFRA Gene Rearrangement</td>
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<th>Table-IV: Clinical features of Patients in PDGFRA Gene Rearrangement (n=60)</th>
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<td>PDGFRA Gene Rearrangement</td>
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<td>Lymph Nodes</td>
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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, KCl 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/PDGFR 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.
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 FIP1L1-PDGFRα rearrangement, while no other gene rearrangement was found in our study. In patients with PDGFRα gene rearrangement, the mean absolute eosinophil count was 12.32±10.36x10⁹/L, mean TLC was 41.73±26.73x10⁹/L and mean Hb was 12.27±4.65 g/dl.

**DISCUSSION**

A detailed workup of clonal Eosinophilia involves the assessment of peripheral blood, bone marrow morphological features and cytogenetic analysis. The FIP1L1-PDGFRα is frequently karyotypically occult, which makes Fluorescence in situ hybridization (FISH) screening mandatory. Although routine karyotyping can detect aberrations of PDGFRα and FGFR1 gene, FISH and molecular examinations are more helpful to affirm these modifications as different genes in the 5q31–33 and 8p11 region might be engaged with other translocations. Therefore, the main objective of our study was to determine the frequency and clinico-hematological features of PDGFRα, PDGFRβ 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 >1.5x10⁹/L persistent for six months were studied.

FIP1L1-PDGFRα gene rearrangement associated with clonal Eosinophilia is rare. In our study, FIP1L1-PDGFRα gene rearrangement was detected in only 20% of patients with Eosinophilia which is higher than that reported by Pardanani et al. (i.e. 14%). Rumor et al. reported the frequency of PDGFRα gene rearrangement in eosinophilic patients to be 17%, comparable to our results. In our study, all the patients with PDGFRα gene rearrangement were males, compared to the high male predominance reported in another study in patients with PDGFRα gene rearrangement. Rohmer et al. also reported a clear male predominance in PDGFRα rearrangement-positive patients. The haematological results showed that patients with FIP1L1-PDGFRα gene rearrangement had an average absolute eosinophilic count of 12.32±10.36x10⁹/L which is greater than that (5x10⁹/L) reported by Vega et al. A study reported average absolute eosinophil count in PDGFRα positive patients to be 10.3±5.9x10⁹/L which is also comparable to our results. In our study, we observed mean Hb in patients with PDGFRα gene arrangement to be 12.27±4.65 g/dl which was comparable to that reported in other published data.

Similarly, the fusion was found in only 40 of 376 individuals (11%) in a European trial of patients with persistent, unexplained Hyper Eosinophilia. In a Mayo series, 11 of 89 patients (12%) with moderate to severe Eosinophilia were FIP1L1-PDGFRα positive. These studies show that the incidence of FIP1L1-PDGFRα 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-PDGFRα gene rearrangement compared to those without this gene rearrangement. The signs and symptoms observed in our study in eosinophilic patients with the FIP1L1-PDGFRα gene rearrangement were nearly similar to the findings of Sreedharanunni et al. 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-PDGFRα gene rearrangement was found to be the most common gene rearrangement in clonal Eosinophilia. While the other types of gene rearrangements, i.e., PDGFRβ and FGFR1, had not been detected. The rarity of PDGFRβ 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.

**CONCLUSION**

Eosinophilia is a common haematological disorder. PDGFRα 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.
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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


