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FIP1L1-PDGFRA Gene Rearrangement by FISH Analysis in Pakistani Patients with Eosinophilia: Clinico-Haematologic Correlation

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ABSTRACT

Objective: To detect FIP1L1-PDGFRα gene rearrangement using Fluorescence in situ hybridization (FISH) in patients with eosinophilia and correlate the clinicohaematologic features.

Study design: Cross-sectional study.

Place and Duration of Study: Haematology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jul 2016 to Jun 2017.

Methodology: Patients having eosinophilia (absolute eosinophil count >1.5x109/l), both genders and any age group were recruited. Detailed history, signs and symptoms , blood counts and differential counts were recorded and peripheral films examined. FISH analysis was done for FIP1L1-PDGFRA gene rearrangement on peripheral blood/bone marrow samples using Metasystem XL PDGFRA probe.

Results: Sixty patients were enrolled in our study. Mean age was 44.0±8.53 years. There were 49(81.7%) males and 11(18.3%) female patients. Absolute eosinophil count ranged from 4x109/l to 38x109/l with a mean of 21x109/l ±10.47x109/l. Thirty two (53.3%) patients had underlying myeloid neoplasm while 28(46.7%) had lymphoid neoplasm. PDGFRA gene re-arrangement was detected in 7(11.7%) patients. There was no statistically significant difference in the frequency of PDGFRA gene re-arrangement across age (p=0.758), gender (p=0.456), absolute eosinophil count (p=0.903) and underlying hematological disorder (p=0.830) groups.

Conclusion: The PDGFRA gene re-arrangement was detected in 11.7% of patients, being more prevalent in Myeloid as compared to Lymphoid neoplasms.

Key words: Eosinophilia, Fluorescence In situ hybridization, Platelet-derived growth factor receptor alpha.

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INTRODUCTION

Eosinophils are white blood cells of granulocytic lineage accounting for 5-10% of leucocytes.[1] Morphologically, these cells are characteristically identified by their bilobed nucleus and bright orange granules in the cytoplasm.[2] Though seen in peripheral blood , these cells predominantly dwell in tissues .[3] Eosinophilia is defined as an absolute eosinophil count of 0.5x109/l or more. Eosinophils when activated, release granule products, cytokines and mediators, resulting in tissue damage.[4]

Eosinophilia may be seen in a wide range of disorders, which may be mild to life-threatening, reactive or clonal.[2] The most common causes being reactive, usually secondary to allergies and parasitic infestations.[5] Idiopathic cause includes the Hypereosinophilic syndrome, characterized by persistent eosinophilia (>1.5x109/l) for more than 6 months in the absence of any underlying cause.[6] These disorders present with organ dysfunction due to tissue infiltration by eosinophils.[7] Clonal disorders include the myeloproliferative neoplasm chronic eosinophilic leukemia (CEL).[8] Another unique category myeloid/ lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDFGRB or FGFR1, or with PCM1/JAK2' are a group of disorders presenting with eosinophilia and characteristic genetic alterations.[9]

Disorders having PDGFRA gene rearrangement include CEL and less commonly acute myeloid leukemia or T-lymphoblastic leukemia/lymphoma.[7] The most common molecular aberration is a cryptic deletion at 4q12, resulting in FIP1-like 1/platelet derived growth factor receptor alfa (FIP1L1-PDGFRA) gene fusion.[9] This fusion transcript enhances the tyrosine kinase activity of PDGFRA, thus resulting in increased proliferation and survival of hematopoietic cells, most commonly manifesting as an eosinophilicmyeloproliferative disorder (chronic eosinophilic leukemia).[10]

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Eosinophilic disorders usually are underdiagnosed. Armed forces Institute of Pathology offers tertiary care diagnostic facilities. Facility of FISH at our institute allows these disorders to be diagnosed. The aim of this study was to determine the frequency of eosinophilia with PDGFRA gene rearrangement andclinico-haematologic parameters of these patients. FIP1L1-PDGFRA is a therapeutic target for Tyrosine Kinase Inhibitors (TKI). Identification of this gene rearrangement will not only help clinicians to institute specific treatment but also to monitor treatment response. Early therapeutic intervention may prevent tissue damage, resulting in improved quality of life and survival.

METHODOLOGY

The study was conducted from 1 July 2016 to 30 June2017in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi after approval from the institutional Ethical Committee. The informed consent from all patients was taken and patient particulars were recorded in a performa. Patients were selected by non-probability consecutive sampling.

Inclusion Criteria: Patients of any age and gender having absolute eosinophil count>1.5x109/L persistent for more than six months or Myeloid or Lymphoid neoplasms with prominent eosinophilia were included in the study.

Exclsuion Criteria: Patients having reactive eosinophilia with evidence of secondary cause and patients already on treatment were excluded from our study.

Detailed history was taken and all reactive causes were ruled out. Complete physical examination was done and all positive findings including splenomegaly were noted. Complete blood counts (CBC) was performed on sysmex XE 5000 automated haematology analyzer. Peripheral films were examined, Differential leucocyte counts (DLC) performed and absolute eosinophil counts were calculated. ESR, CRP and baseline biochemical tests were performed. Bone marrow aspirate and trephine were done to establish any myeloid or lymphoid neoplasm associated with the eosinophilia.

FISH analysis for PDGFRA gene rearrangement was performed on all patients. 3 ml venous blood in sodium heparin was collected. FISH studies were performed on blood or bone marrow specimens processed by standard methods. Sample were cultured at 37°C without phytohaemagglutinin (PHA) for 24 hrs and then after adding 200µl colchicine for 1 hr. Following this centrifugation was done at 1500rpm for 8 min. After discarding the supernatant KCL was added and the samples were centrifuged. This was followed by minimum of three washings with 3:1 mix of glacial acetic acid and methanol. The clear pellet obtained was placed on a slide. Slides were then treated with ascending concentrations of alcohol and later by saline sodium citrate (SSC) solution. After air drying the slide 10µl of Metasystem XL PDGFRA probe was applied to the target. A total of 500 nuclei were analyzed per probe set by using a fluorescent microscope with an orange green spectrum filter. The XL FIP1L1/CHIC2/PDGFRA break apart probe contains an orange probe spanning the CHIC2 gene which is flanked by two green probes. One green probe hybridizes proximal to FIP1L1 gene and another green probe hybridizes distal to PDGFRA gene. Thus, normal results are an orange and green fusion signal. Deletion of CHIC2 region results in FIP1L1-PDGFRA gene rearrangement, showing green signals with loss of orange signal.

Data was analyzed by SPSS version 22. Numerical variables; age and eosinophil count were presented by Mean±SD. Categorical variables presented by frequency and percentage. Data has been stratified for age, gender, eosinophil count and type of hematologic disorder to address affect modifiers. Post-stratification chi-square test has been applied taking $p \leq 0.05$ as significant.

RESULTS

A total of sixty patients were included in the study. The age of the patients ranged from 28 year to 60 years with a mean of 44.0±8.53 years. Majority of the patients were male with a male to female ratio of 4.5:1. Absolute eosinophil count ranged from 4x109/l to 38x109/l with a mean of 21x109/l±10.47x109/l. The clinicohaematological characteristics of the study population are shown in Table-I.

Table-I: Clinicohaematological Characteristics of Study Participants (n=60)

Characteristics	n(%)
Age (years)	44.0±8.53
28-44 years	31(51.7%)
45-60 years	29(48.3%)
Gender	
Male	49(81.7%)
Female	11(18.3%)
Absolute Eosinophil Count (/µl)	21000±10478
4x109/L – 20x109/L	33(55.0%)
21x109/L – 38x109/L	27(45.0%)
Type of Hematological Disorder	
Eosinophilia with Myeloid Neoplasm	32(53.3%)
Eosinophilia with Lymphoid Neoplasm	28(46.7%)

PDGFRA gene re-arrangement was detected in 7(11.7%) of our patients.There was a predominance of patients who had underlying myeloid neoplasm seen in 32(53.3%)while eosinophilia with lymphoid neoplasms were seen in 28(46.7%) of the study population.

We then stratified the patients according to age, gender, absolute eosinophil count and underlying myeloid or lymphoid neoplasm. No statistically significant difference was found in the frequency of PDGFRA gene re-arrangement across age (p=0.758) or gender (p=0.456) as shown in Table-II and Table-III. On statistical analysis, there was no significant association of PDGFRA with absolute eosinophil count (p=0.903) or underlying hematological disorder (p=0.830) groups as shown in following Table-IV & V.

Table-II: Distribution of Patients according to Gender (n=60)

Gender	PDGFRA Gene Re-Arrangement		*p*-value
	Detected (n=7)	Not-Detected (n=53)	
Male(n=49)	5	44	0.456
	10.2%	89.8%	
Female(n=11)	2	9	
	18.2%	81.8%	
Total(n=60)	7	53	
	11.7%	88.3%	

Table-III: Distribution of patients with and without PDGFRA Gene rearrangement according to Age (n=60)

Age	PDGFRA Gene Re-Arrangement		*p*-value
	Detected (n=7)	Not-Detected (n=53)	
28-44 years (n=31)	4	27	0.758
	12.9%	87.1%	
45-60 years (n=29)	3	26	
	10.3%	89.7%	
Total(n=60)	7	53	
	11.7%	88.3%	

Table-IV: Absolute Eosinophil Count of the Study Population (n=60)

Absolute Eosinophil Count	PDGFRA Gene Re-Arrangement		*p*-value
	Detected (n=7)	Not-Detected (n=53)	
4x109/L–20x109/L (n=33)	4	29	0.903
	12.1%	87.9%	
21x109/L–38x109/L (n=27)	3	24	
	11.1%	88.9%	
Total (n=60)	7	53	
	11.7%	88.3%	

DISCUSSION

PDGFRA associated eosinophilic syndromes are unique in the sense that these disorders show a good response to TKI therapy. Early recognition of PDFRA gene rearrangement does not only identify the diagnosis but also guides treatment strategies and is a marker for monitoring minimal residual disease.

The identification of FIP1L1-PDGFRA gene fusion has not only helped in diagnosis, but its significance lies in the fact that these disorders are more responsive to imatinib.[11] Considering that FIP1L1-PDGFRA associated neoplasms are multisystem disorders, early detection of this gene rearrangement helps to institute treatment early in disease before cardiac and other tissue damage is manifested.[12]

This genetic alteration is usually in the form of deletion of a part of chromosome 4q in case of PDGFRA, that makes it difficult to be picked up on conventional cytogenetic study that often yields a normal karyotype.[13] This fusion gene is often karyotypically occult which makes fluorescence in situ hybridization (FISH) screening mandatory.[14] FISH has >95% sensitivity and specificity in diagnosing such a potentially treatable genetic lesion.[15]

In this study, the mean age of the patients was 44.0±8.53 years. Pardanani *et al*.[16] in his study reported similar mean age of 43.6±9.3 years in American patients undergoing treatment for eosinophilia whileVandenberghe *et al* reported it to be 48.8±4.2 years among such patients in Belgium.[17]

Table-V: Patients according to underlying Haematological Disorder (n=60)

Diagnosis	PDGFRA Gene Re-Arrangement		*p*-value
	Detected (n=7)	Not-Detected (n=53)	
Eosinophilia with Myeloid Neoplasm (n=32)	4	28	0.830
	12.5%	87.5%	
Eosinophilia with Lymphoid Neoplasm (n=28)	3	25	
	10.7%	89.3%	
Total (n=60)	7	53	
	11.7%	88.3%	

We observed that there were 49(81.7%) male and 11(18.3%) female patients with a male to female ratio of 4.5:1. Starza *et al*.[18] also reported similar male predominance with a male to female ratio of 4.2:1 in Italian patients presenting with eosinophilia. A similar male predominance was also observed by Roche

Lestienne *et al*.[19] who reported male to female ratio of 2.8:1 in such patients in France. A much higher male predominance has been reported by Vandenberghe *et al*.[17] and Cools *et al*.[20] with male to female ratio of 6.7:1 and 7:1 respectively in European and American such patients.

PDGFRA gene re-arrangement was detected in 7(11.7%) patients. There was no statistically significant difference in the frequency of PDGFRA gene re-arrangement across age (p=0.758), gender (p=0.456), absolute eosinophil count (p=0.903) and underlying hematological disorder (p=0.830) groups. Our results showed similarities to those of Pardanani *et al*. (2004) who reported the frequency of PDGFRA gene re-arrangement to be 12.36% in USA and Loules *et al*.[21] 13.3% in such patients in Greece.

The present study has found that 11.7% patients presenting with eosinophilia in local population have PDGFRA gene re-arrangement irrespective of patient's age, gender, absolute eosinophil count and underlying hematological disorder and that it can be detected by fluorescent in situ hybridization (FISH). The detection of this abnormality explains the causative role of tyrosine kinase in clonal proliferation of eosinophils implying a potential target for treatment and thus determines the management plan and prognosis of these patients.[22]

A limitation of our study was that we didn't compare the treatment response of patients with eosinophilia with and without PDGFRA gene rearrangement which could have enabled risk stratification of such patients. In order to discover the disease spectrum in this disorder, such kind of a study is highly recommended in future research.

CONCLUSION

The frequency of PDGFRA gene re-arrangement was 11.7% in patients presenting with eosinophilia. It was not affected by patient's age, gender, absolute eosinophil count and underlying hematological disorder.

Conflict of Interest: None.

Author's Contribution

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

SA: & RM: Conception, study design, drafting the manuscript, approval of the final version to be published.

AM: & NK: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AY: & SAK: Critical review, data acquisition, 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.

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