FREQUENCY OF TET2 GENE MUTATION IN MYELOPROLIFERATIVE NEOPLASMS

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ABSTRACT

Objective: To determine the frequency of Ten-Eleven-Translocation-2 (TET2) gene mutation in Myeloproliferative Neoplasms (MPNs) and to generate a local data for evaluation of disease behavior in ten-eleven-translocation-2 positive and negative Patients.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, from Apr 2017 to Apr 2018.

Methodology: A total of 50 adult myeloproliferative neoplasms patients (>18 years of age), diagnosed according to WHO 2016 diagnostic criteria for myeloproliferative neoplasms were included in the study. Mutational screening for ten-eleven-translocation-2 gene was performed by flourescent in situ hybridization technique using Meta Systems XL-TET2 Deletion probe REF D-5038- 100-OG, LOT 18181, manufactured by Meta-Systems GmbH, Robert-Bosch - Germany. Bone marrow samples were used for Fluorescence in situ hybridization analysis. A total of 500 interphases were examined in each of the specimen. Data was entered and analyzed using SPSS-22. Distinctive demographic, haematological and molecular results are summarized by descriptive statistics.

Results: Total 50 cases of myeloproliferative neoplasms (14 CML, 10 PV, 12 essential thrombocythemia (ET) and 14 primary myelofibrosis (PMF) were analyzed. On fluorescent in situ hybridization studies, ten-eleven-translocation-2 mutation was detected in 8 (16%) patients. The mutational frequency among different myeloproliferative neoplasm entities was 4 (28.5%) in chronic myeloid leukemia (CML), 2 (20%) in Polycythemia Vera (PV), 1 (8.3%) in essential thrombocythemia and 1 (7.1%) in primary myelofibrosis respectively. Among the 15 cases, 36 (72%) patients were positive for JAK2V617F mutation while 14 (28%) were positive for BCR-ABL1 mutation. Ten-eleven-translocation-2 mutation observed in 4 (11.11%) of JAK2V617F positive and 4 (28.5%) of BCR-ABL1 positive patients.

Conclusion: Ten-eleven-translocation-2 mutation occur in both JAK2V617F positive and negative myeloproliferative neoplasms. They are found more in chronic myeloid leukemia as compared to with polycythemia vera, essential thrombocythemia, and primary myelofibrosis.

Keywords: Flourescent in situ hybridization, Ten-eleven-translocation-2, JAK2V617F, Myeloproliferative neoplasms, OS.

INTRODUCTION

The term "myeloproloferative disorders" (MPDs) was first introduced by William Dameshek in 1951 to describe 4 different diseases with clinical and biologic similarities: chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Later on, chronic neutrophil leukemia (CNL), hypereosinophilic syndrome, systemic mastocytosis, atypical chronic myeloid leukemia, and other rare chronic hematological disorders were added. Certain disorders that combine myeloproliferation and myelodysplasia, such as chronic myelomonocytic leukemia (CMML), are closely related to MPNs. In 2008, the World Health Organization recommended changing the term Myeloproliferative disorders to Myeloproliferative neoplasms.

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Received: 20 Jun 2018; revised received: 13 May 2019; accepted: 28 May 2019

The myeloproliferative neoplasms are clonal haematopoietic stem cell disorders. Over the years with better understanding of the biology of disease, many genetic molecular factors have been identified. Recently, mutations involving TET oncogene family member 2 (TET2) have been described to play a role not only in pathogenesis but may also exert an effect on disease phenotype, prognosis and response to treatment.

The enzyme TET2 (Ten-Eleven-Translocation-2) converts 5-methylcytosine (5-mC) to 5-hydro-xymethylcytosine (5-hmC) in DNA, thereby epigenetically regulates gene expression by altering methylation-driven gene silencing. This enzyme has a significant role in myelopoiesis and loss of function TET2 mutations have been found in several myeloid malignancies including myeloproliferative neoplasms[1]. However, the effect of this mutation on the clinical presentation and prognosis of disease is yet debatable and under study[2]. Recently, studies are also being conducted to see the influence of mutation order in patients with MPNs

who carry mutations in both JAK2 and TET2. A role of TET2 has been suggested in disease progression in MPNs[3].

Flourescent in situ hybridization (FISH) for the detection of TET2 abnormalities may become a potentially useful laboratory tool. The TET2 FISH probe hybridize to the TET2 gene and can be performed on both peripheral blood and bone marrow metaphase spreads and interphase nuclei. FISH provides accurate results in short period of time[4].

We have conducted this study in the Armed Forces Institute of Pathology, a tertiary care and referral center that caters to a large population from different parts of the country. There is no data available regarding the frequency of TET2 gene mutation in MPNs in our population. The rationale of our study was to determine the frequency of this novel mutation in our population, study clinico-haematologic parameters of MPN patients harbouring this mutation and to study its association with other mutations like JAK2V617F/ BCR-ABL1.

METHODOLOGY

This cross sectional study was performed at Department of Haematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, from April 2017 to April 2018. A total of 50 MPNs adult patients (>18 years old), diagnosed according to WHO 2016 revised criteria were recruited. Samples were collected by non probability consecutive sampling. By using the WHO sample size calculator with a confidence level of 95%, anticipated population proportion (P) of 0.85 and absolute precision (d) of 10. The sample size calculated was 50. Patients younger than 18 years old or patients with hepatic, renal failure or pregnant women were excluded from study. Informed written consent forms were signed by patients according to the Declaration of Helsinki (1964). All patients were subjected to a thorough assessment by history, clinical examination, baseline chemical investigations, complete blood count, bone marrow examination, immunophenotyping and conventional cytogenetic. All parameters were used for statistical analysis, except for those addressing prognosis (survival, leukemic and fibrotic transformation). A complete data set of all the major clinical characteristics were obtained.

Mutational screening for TET2 gene was performed by using Meta Systems XL TET2 Deletion probe REF D-5038-100-OG, LOT 18181, Manufactured by Meta Systems Gmb H, Robert-Bosch-Str. 6,68804 Altlussheim, Germany. Bone Marrow blood 2.5ml in sodium heparin tube was collected. Codenaturation of cells and FISH probes was carried out on a Thermobrite (Abbott Molecular) for three minutes at 74°C and hybridized at 37°C overnight. A total of 500 nuclei were scored in each of the 50 specimens. Interphase cells were obtained using standard cytogenetic methods.

All the collected data was entered and analyzed through SPSS-22 (IBM, NY, USA). Numerical variables; age, haemoglobin, WBC, and platelets count have been presented by numbers and percentages. Categorical variables; gender, presence of BCR-ABL1 and JAK2V617F have been presented by frequency and percentage. Data has been stratified for age, gender, haemoglobin, WBC, and platelets counts.

RESULTS

A total of 50 patients were recruited in this study. Mean age of participants was 43.88 ± 12.72 (range 18-70) with the most common age group being 41-50 years. There was a male preponderance, 27 (54%) were males and 23 (46%) were females with male to female ratio of 1.18. Among different entities of MPN male to female ratio was equal in PV and CML while PMF was found more common in 10 (72%) males and less in 4 (28%) females in comparison of ET which was more common in 7 (58%) females and less in 5 males (42%). Patients with CML and PMF were found more 14 (28%) each, in comparison of PV 10 (20%) and ET 12 (24%) while no patient of MPN-U, CNL and CEL was reported during our study period. Demographic results are summarized in fig-1 & 2.

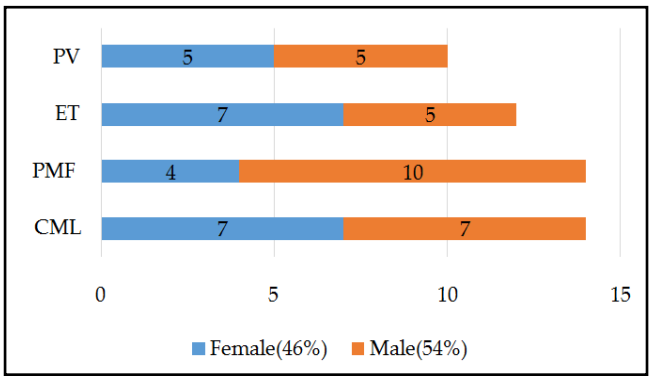

Figure-1: Distribution of MPNs according to gender (n=50).

We also studied the clinico-haematologic parameters of these patients. White blood cell count was found elevated in 100% cases of CML, 10% in PV, 50% in ET and 92.9% of PMF patients respectively. Haemoglobin level was found normal to low in all cases of MPNs except in PV patients where it was raised in

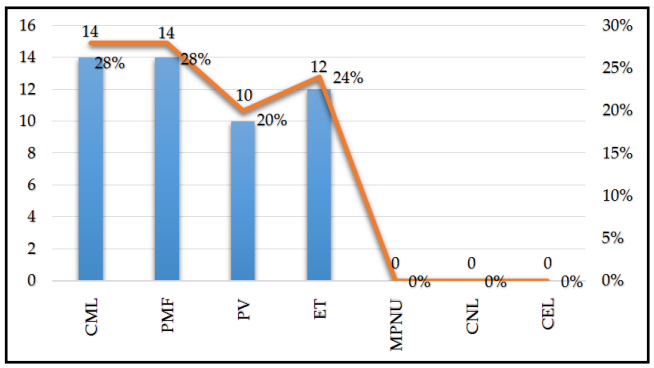

Figure-2: Frequency of MPNs (n=50).

100% cases, 42.9% CML patients were found to have thrombocytosis and 14.3% presented with thrombocytopenia. Twenty percent PV and 50% PMF patients were found to have thrombocytopenia while 100% patients of ET were presented with thrombocytosis. The distribution of haematological parameters is shown in table-I.

Forty two (84%) patients including 14 of CML, 12 of ET, 03 of PV and 13 of PMF were had splenomegaly. had JAK2V617F mutation positive while patients 8 (16%) including 4 (28.5%) of CML, 2 (20%) of PV, 1 (8.3%) of ET and 1 (7.1%) of PMF were positive for TET2 gene mutation. Most of the PV patients did not present with splenomegaly while approximate 100% of CML, ET and PMF patients were presented with mild to moderate splenomegaly. Results are summarized in table-II.

On FISH analysis of 50 MPNs patients, 8 (16%) patients were positive for TET2 gene mutation. Of these, 5 (62.5%) patients presented with increase white blood cell count while 3 (37.5%) patients with normal TLC. Two (25%) with high haemoglobin, another six (75%) with decrease haemoglobin. Four (50%) patients reported with thrombocytopenia while 1 (12.5%) with normal platelets count and 3 (37.5%) with thrombocytosis. One (12.5%) patient reported with diffuse fibrosis, 2 (25%) with focal fibrosis while no any fibrosis observed in another 5 (62.5%) patients. Out of 8 TET2 positive MPNs 4 (50%) patients were positive for JAK2 V617F mutation, while another 4 (50%) were positive

Table-I: Haematological characteristics of MPNs patients (n=50).

MPNs	CML			PV			ET			PMF		
	Total		Disease %	Total		Disease %	Total		Disease %	Total		Disease %
Patient Count	14		28%	10		20%	12		24%	14		28%
CBC	**High**	**Normal**	**Low**	**High**	**Normal**	**Low**	**High**	**Normal**	**Low**	**High**	**Normal**	**Low**
Wbc	100%	-	-	10%	70%	20%	50%	50%	-	92.9%	-	7.1%
Hb	-	21.4%	78.6%	100%	-	-	-	50%	50%	-	7.1%	92.9%
Plt	42.9%	42.9%	14.3%	00	80%	20%	100%	-	-	-	50%	50%
Fibrosis	Absent	Focal	Diffuse	Absent	Focal	Diffuse	Absent	Focal	Diffuse	Absent	Focal	Diffuse
	64.3%	28.6%	7.1%	80%	20%	-	100%	-	-	-	14.3%	85.7%

Table-II: Frequency of spleen and molecular genetics in MPNs (n=50).

S. No.	Results	Diseases				Positive Results	Negative Results
		CML	ET	PV	PMF		
1	Splenomegaly	14	12	03	13	42 (84%)	08 (16%)
2	BCR-ABL1	14	00	00	00	14 (28%)	36 (72%)
3	JAK2V617F	00	12	10	14	36 (72%)	14 (28%)
4	Frequency of TET2 gene mutation	04	01	02	01	08 (16%)	42 (84%)

Table-III: Frequency of haematological and molecular characteristics of TET2 positive MPNs (n=8).

Findings	High	Normal	Low
Wbc	5 (62.5%)	3 (37.5%)	-
Hb	2 (25%)	-	6 (75%)
Platelets	3 (37.5)	1 (12.5%)	4 (50%)
Fibrosis	**Absent**	**Focal**	**Diffuse**
	5 (62.5%)	2 (25%)	1 (12.5%)
Molecular	Positive	Negative	-
JAK2V617F	4 (50%)	4 (50%)	-
BCR-ABL1	4 (50%)	4 (50%)	-

All 14 (28%) patients of CML were positive for BCR-ABL1 gene mutation. Thirty six (72%) patients including 12 of ET, 10 of PV and 14 of PMF patients were for BCR-ABL1 mutation simultaneously. Results are summarized in table-III.

DISCUSSION

TET2 acts as a tumor-suppressor gene at critical loci important for myelopoiesis and leukemogenesis[3]. TET2 is frequently occurring mutation in MPNs, MDS and in AML patients[5]. Thus, it may have an important role not only in the pathophysiology of myeloproliferative neoplasms but also in other diseases progression. In our study, the overall TET2 mutation frequency in MPNs was 16%. This frequency is higher than the frequency of 12.1% reported in the Korean population[6] and of 13% in the Western population as reported by Teffari *et al*[7].

In the Pakistani population, among different MPN entities, frequency of TET2 mutationin chronic myeloid leukemia (CML) was 28.5%, in polycythemia Vera (PV) 20%, in essential thrombocythemia (ET) 8.3% and in primary myelofibrosis (PMF) 7.1% respectively. Ha *et al*[6] in a study conducted in Korea has reported the frequency of TET2 as 22.2% in PV and 9.7% in ET. These findings are in accordance with our results. However, they have reported a much higher frequency 18.2% in PMF in comparison of our 7.1%. They have not included CML in MPNs. Teffari *et al*[7] had reported mutation frequencies as 16% in PV, 5% in ET, 17% in PMF, 14% in post-PV Myelofibrosis, 14% in post-ET Myelofibrosis and 17% in blast–phase PV/ ET/PMF.

In our population, TET2 mutation was seen in 11.11% of thirty six JAK2V617F positive cases versus 28% of JAK2V617F negative cases. These findings are in contrast to those reported by Teffari *et al*[7] who reported TET2 mutations 17% vs 7% in JAK2V617F positive vs negative cases. Another report by Martinez-Aviles *et al*[8] determined that TET2 mutation frequency was 8% in JAK2 negative MPNs and overall 14% among 62 patients cohort. In order to evaluate response to drugs targeting the myeloproliferative clone, mutational analysis of hematopoiesis should include both MPN drivers and comutated myeloid genes[9]. In PV patients, interferon α was found to target JAK2V617F-mutant clones without affecting TET2-mutant cells[10], whereas in patients with CALR-mutant ET, interferon treatment was less effective in subjects with concomitant mutations in genes like TET2, ASXL1, IDH2, and TP53[11]. A study revealed that mutations or DNA variants, other than JAK2, CALR, or MPL, are found in ~53% of patients with ET with the most frequent being TET2 (16%), ASXL1 (11%), DNMT3A (6%), and SF3B1 (5%)[12]. In recent past Venton *et al*[13] described 20%, Terra L. Lasho *et al*[14] 19%, Wong *et al*[15] 12%, Ha *et al*[16] 12.1% (22.2%) in polycythemia vera (PV), 9.7% in essential thrombocythemia (ET), 18.2% in primary myelofibrosis (PMF) and 0% in unclassified MPNs), Hidalgo López *et al*[17] 27% in blast phase of PV, Togasaki *et al*[18] and Schmidt *et al*[19] 25% and 33% respectively in CML, Song, Hussaini, Zhang, *et al*[20] 19.4%, Xie *et al*[21] 13% and Ayalew Tefferi *et al*[22] 17% (PV 16%, ET 5%, PMF 17%) revealed these mutational frequencies respectively in their populations. McPherson *et al*[23] describe TET2 mutation as Poor risk factor, seen at leukemic transformation in MPNs. No clear significance in MDS while worse prognosis in normal karyotype AML patients[23]. TET2 mutations were also seen to confer a high risk of leukemic transformation (increased by 30%) and shorter OS[24]. In addition, there is evidence that TET2 mutations appear to be an early event that may provide clonal advantage and set up a 'fertile ground' for MPN disease initiation[25]. As TET2 is a novel mutation, a lot of research is underway to determine its frequency and outcome in haematological malignancies including in MPN patients. We have conducted this study to determine the frequency of this novel mutation in our population. However, much larger studies are required to establish prognostic value of this mutation.

ACKNOWLEDGEMENT

The authors greatly acknowledge the enthusiastic efforts of Mr. Bilal khan, research officer at AFIP.

CONCLUSION

Ten-Eleven-Translocation-2 (TET2) mutations occur in both JAK2V617F positive and negative Myeloproliferative Neoplasms. TET2 gene mutation was found more in chronic myeloid leukemia as compared to PV, ET, and PMF. A slightly higher frequency of this mutation has been observed in our population in comparison of western data. However, further long term studies on a large scale are needed to further strengthen our findings, establish relation of TET2 to other gene mutations, determine response to treatment of patients harbouring this mutation and to see prognostic significance.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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