The Detection of BRAF-V600E Mutation in Hairy Cell Leukaemia by Polymerase Chain Reaction and Study of its associated Clinicohaematological Parameters

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ABSTRACT

Objective: To contemplate the occurrence of a rare mutation (BRAF V600E) in the patients having Hairy cell leukemia in our setting, which wouldserve asuseful analytic and diagnostic criteria.
Study Design: Analytical cross sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology Rawalpindi Pakistan, from Oct 2017 to Oct 2018.
Methodology:A sample of 36 patients with a suspicion of Hairy cell leukemia were included in this investigation. Complete blood counts, bone marrow examination (trephine biopsy and aspiration), cyto-chemistry and staining of the patients suspected to have Hairy cell leukemia was performed. PCR was done for detection of BRAF V600E mutation.
Results: 29 (80.5%) cases showed a positive BRAF mutational analysis and 7 (19.4%) cases showed a negative result.
Conclusion:Almost all the cases of classic Hairy cell leukemia had BRAF V600 E mutation.Isolating this mutation can be used as a regular tool in detection of disease and monitoring disease progression and relapse.

Keywords: BRAF V600E mutation, B-cell lymphoproliferative, Complete blood counts, Chronic lymphocytic leukemia, Monocytopenia, Pallor.

How to Cite This Article: *Iqbal I M, Robert M H, Khan N, Mahmood R, Khurshid A, Khan A S. The Detection of Braf-v600e Mutation in Hairy Cell Leukaemia by Polymerase Chain Reaction and Study of its associated Clinicohaematological Parameters. Pak Armed Forces Med J 2022; 72(Suppl-2): S178-181. DOI: https://10.51253/pafmj.v72iSUPPL-2.3083*

INTRODUCTION

Various Haematological malignancies include Acute Lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML) etc. Chronic lymphocytic leukaemia has many variants among which hairy cell leukaemia (HCL) is a relatively rare malignancy characterized by accumulation of abnormal B lymphocytes.[1] It was first described by distinct clinicopathologic entity by Bouroncle *et al,* in 1958.[2] Its prevalence is around 2% of all the leukaemias and 8% of all lymphoproliferative disorders. Gender predilection is more towards males as compared to females with diagnosis being made at a mean age of 50 years.[3] Thus concluding to occur more frequently in older age males.

Patients with hairy cell leukaemia usually present with splenomegaly, fatigue, pallor, weight loss and infections. Hepatomegaly may or may not be present in such cases. Lymphadenopathy is also a rare occurrence and is usually associated with aggressive disease progression.[4] Various modalities used for reaching a final diagnosis include complete blood count (CBC), peripheral blood smear, bone marrow aspiration cytology, flow cytometry etc.This unique clinical entity presents with quite contrasting blood complete picture showing pancytopenia, however if hairy cells are larger in number in blood than a higher or normal lymphocyte count could be seen. Unusual formed WBC's having hair like cytoplasmic projections which can be seen on fringe blood smear and bone marrow sample are named as hairy cells.[5]

Uncontrolled cellular proliferation and development of malignancy is associated to mutations in various genes. Various cytogenetic analyses have failed to distinguish between various chromosomal abnormalities connected with hairy cell leukaemia. Similarly, fluorescence in situ hybridization (FISH), genomic hybridization, and microarray SNP genotyping also could not make reliable distinctions. Nonetheless, exom sequencing approach in 2011 revealed BRAF V600E as the gene responsible for HCL and can be used as a molecular marker for early diagnosis. Tiacci *et al,*iscovered that 100% of the HCL patients had the oncogenic BRAF V600E mutation.[6]

Accurate diagnosis of hairy cell leukaemia is important because the natural history, prognosis, and therapeutic alternatives in this disease may be quite distinct from those in other leukaemias.[18] exons make up the BRAF proto-oncogene (7q34). Exon 15

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Received: 04 Jul 2019; revision received: 06 Jan 2020; accepted: 10 Jan 2020

undergoes transformation at position 1799, where thymine is interchanged

with adenine, that changes the amino acid valine (V) to glutamic acid (E) at codon 600 (V600E) in the BRAF protein.

Absence of this mutation in other B-cell malignancies and the existence in HCL patients show that this change can be marked as a Hallmark of classic HCL. On the contrary, Arons *et al,* and couple of other researchers have now revealed that BRAF V600E mutation may not be a distinguishing highlight of all the HCL cases.[7] Due to scarcity of literature in Pakistani population, thisstudy was conducted in our tertiary care hospital setting to determine the frequency of BRAF V600E mutation in patients with hairy cell leukaemia presenting to our department and correlate it with clinicohe-matological findings in our population.

METHODOLOGY

This analytical cross sectional study was carried out in the department of Haematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan, from October 2017 to October 2018. Sample size of 36 was calculated using WHO sample size calculator considering level of significance as 5%, Power of test 80% and confidence interval of 95%. A total of 36 patients with a suspicion of HCL were inducted in this study. Non-probability consecutive sampling technique was used.

Consent from ethical committee board from AFIP Rawalpindi was taken before beginning this investigation (ref no. FC-HEM17-29/READ-IRB/18/946). Informed consent was also taken from the patients or their relatives. Demographic data and detailed lab and clinical parameters of every patient were endorsed in a performa for analytical purpose.

Inclusion Criteria: Patients of both gender irrespective of age, Diagnosed with HCL classic and HCL were included in this study.

Exclusion criteria: Exclusion of patients was done on the basis of presence of other haematological and lymphoproliferative issue. Patients fulfilling the inclusion criteria were deliberated about the study.

In EDTA tube 2-4ml of bone marrow aspirate or peripheral blood via venepuncture was collected.

DNA extraction and amplification:

10% Chelex method was employed forGenomic DNA extraction. Following primerswere used in a total concentration of 400 nM

- One forward primer -5'-GCTTGCTCT-GATAGGAAAATGAG-3'
- Two reverse primers -5'-ACCCACTCCATCGAGATTTCT-3’
- 5'-CTGTGGATCACACCTGCCTTA-3'
- The concentrations of 133 nM, 173 nM and 93 nM were used, respectively.25 µl. PCR mixture consisted of 1.25U HotStartTaq polymerase (Qiagen)
- 160 µM dNTPs (VWR)
- 1× Coral Load PCR Buffer (Qiagen)
- 1.5 mM MgCl2 and from 100 ng to 500 ng of genomic DNA.

PCR was carried out for 40 cycles (40 s at 94°C, 40 s at 61°C, 40 s at 72°C), with initial denaturation for 15 min at 95°C and final extension for 5 min at 72°C. Separation of PCR products was done on 1-2% agarose gels stained with ethidium bromide.

Haematological parameters:

Basic haematological parameters (Hb, WBC, Platelet count) were performed using automated analyser (Sysmex XE-5000).

Data was analyzed using SPSS version 21.0. Mean and SD was calculated for numerical variables such as age, splenomegaly, monocyte count, percentage of hairy cells, HB, WBC count and platelet count. Frequency and percentage was calculated for categorical variables like gender, BRAF mutation, presence of CD 25, 19, 11c and pallor. p value of ≤0.05 was considered statistically significant.

RESULTS

Total 36 subjects were selected for our research. Out of the 36 HCL patients, males were 27 (75%) and females were 9 (25%). Gender distribution revealed a male to female proportion of 3:1. Age range of the patients spanned between 29 to 68 years. Mean age in the HCL patients was 48.5 ± 10.1 years. Fourth and fifth decade was the most prevalent age group among HCL patients.

Presenceof BRAF V600E mutation was confirmed in 29 (80.5%) cases and was not identified in 7 (19.4%) cases. CD25, CD11c and CD19 existence was found in 30 (83.3%), 32 (88.8%) and 36 (100%) patients by stream cytometry.

Fever, pallor (84%), and abdominal distension due to splenomegaly (100%) were the most common symptoms reported. Enlargement of spleen in HCL patients was noted to be 12.93 ± 3.6cm. The highest level reported was 21cm and lowest was 4cm.

Table-I: Basic haematological parameters in BRAF positive and negative individuals.

Parameter	BRAF	n	Mean	Std. Deviation	*p*-value
WBC	Yes	29	11.5674	13.91254	0.6
	No	7	9.4600	7.76262	0.5
Splenomegaly	Yes	29	13.39	4.768	0.1
	No	7	10.80	4.756	0.1
Percentage of Hairy Cell	Yes	29	22.35	18.346	0.2
	No	7	29.90	15.808	0.2
Pallor	Yes	29	1.15	.363	0.7
	No	7	1.20	.422	0.7

CBC revealed a mean WBC count of $11.9x10^9$/L. anyway three patients had high leukocyte check. The mean Haemoglobin in the HCL group was 9.6 g/dl with a range of 5.2 g/dl to 13.3 g/dl. Pallor was reported in 36 (72%) patients. The mean platelet include in the HCL was $55x10^9$/L. Least platelet tally was $12x10^9$/L and most extreme was $150x10^9$/L. Number of hairy cell in the fringe blood film were variable. 41% of hairy lymphocytes were present on peripheral blood film. Levelof Monocytes was 0.7%. BRAF V600E mutation in classic HCL patients was statistically significant as compared to HCL variant (*p*-value <0.03).

DISCUSSION

Hairy cell leukaemia is an infrequent lymphoproliferative disorder of the B-cell lineage, which presents with distinctive clinical symptoms and pathological characteristics distinguished by enlarged spleen, unpredictable disease progression, decreased mature blood cell production usually affecting two or three cell pedigrees and complete mono-cytopenia. Moderate to severe pancytopenia, neutropenia and monocytopenia are present on blood complete picture.[8]

B-RAF mutation is detected in almost 80-90% of HCL patients. Other clinical entities presenting with this mutation are cutaneous melanoma, lung, ovarian, bladder, thyroid, prostatic cancers and cholangiocarcinoma. BRAF changes with V600E, the most widely recognized tumors occurring in humans, naturally triggers the MAPK–ERK (Mitogen activated protein kinase-extracellular signal regulated kinase) pathway, prompting cell multiplication at a higher rate, increased survival resulting ultimately in neoplastic changes.[9] Research facility examinations on Peripheral blood smear depicts hairy cells with trade-mark morphological highlights which incorporate an increased sized lymphoid cells with a diameter of 10-15µm, lesser Nuclear to cytoplasmic ratio, oval or round indented nuclei with centric or eccentric position, reticular chromatin, with undifferentiated or absent nucleoli. Fine hair-like projections or ruffled borders are present on cytoplasm, showing positive expressions of CD19, CD20, CD22 and CD200 and negative for CD5, CD23, CD10.[10]

Bone marrow aspiration film shows the presence of a small number of hairy cells. A more informative and accurate diagnostic modality is bone marrow trephine biopsy which uncovers 3 trademark example of invasion with cells seeming isolated on account of their bounteous clear cytoplasm. Reticulin stains in practically all patients of HCL indicates discernible increment in reticulin filaments. Tartrate resistant acid phosphatase (TRAP) staining gave a positive results forhairy cells.[11]

Scarcity of literature on molecular analysis of HCL mutations in Pakistani population prompted us to carry out this study in our setup. This study was designed to analyse the frequency of BRAF v600E mutation in HCL patients which would be a highly effective diagnostic modality in such cases and during follow ups, for monitoring disease progression and relapse. Our study showed that 80.5% of the HCL cases were positive for BRAF mutation where as 19.4% of the cases showed a negative result. Similarly, Tiacci *et al*, upon sanger sequencing, observed that 100% of the cases of classic HCL has BRAF V600E mutation, but not the other B cell leukaemias.[6] The presence of BRAF V600E transformations in all patients with HCL was affirmed by Blombery *et al*, who related it to immunophenotypic variations.[12]

Our study confirmed a male predominance of 75% whereas females accounted for only 25% of the total sample size. Results of a study conducted by Arcaini *et al,* has shown that out of total 62 patients, 16% (n=10) were females and 84% (n=52) were males.

Male to female ratio was 5.2:1.[13] Similarly, Tiacci *et al,* in his study validated the previous study by reporting a male dominance in total 47 patients, 79% (n=37) were males and 21% (n=10) were females.[14] Out of 56 patients only 10 patients were screened negative for BRAF mutation. Out of this 2 patients had a raised WBC count of 63×10^9/L and 61×10^9/L with massive splenomegaly.

In our study, Out of total 7(19.4%) patients screened negative for BRAF mutation, 4 patients showed negative response to CD25 on immunophenotyping, thus were labelled as HCL variant cases lacking mutation. This was in accordance to the study performed by Xi *et al,* which also shows that all HCL variant cases were screened negative for BRAF mutation.[15] HCL variant is a separate clinical entity because of differing clinicopathological findings as compared to classic HCL. HCL variant lacks the expression of CD25 and CD123.[16]

BRAF V600E mutation in classic HCL patients was statistically significant as compared to HCL variant (p-value <0.03). Classic HCL responds to purine nucleoside analogues (cladribine and pentostatin) whereas HCL variant cases are resistant and more destructive than the former entity.[17] Thus, their distinction is a mandatory.

The alteration of BRAF V600E gene will prove to be a helpful diagnostic tool in patients with HCL who do not have a reaction (or have an insignificant reaction) to starter treatment with purine analogs, just as in patients with repeated deterioration of symptoms or unwanted toxic impacts.[18] The presence of BRAFV600E mutation overlays way for targeted therapy and monitoring of residual disease in patients of HCL.

CONCLUSION

Almost all the cases of classic HCL had BRAF v600 E mutation. Isolating this mutation can be used as a regular tool in detection of disease and monitoring disease progression and relapse.

Conflict of Interest: None.

Autor's Contribution

MII: Direct contribution to conception, design, analysis, interpretation, HMR: Data analysis, NK:, SAK: Intellectual contribution to analysis and interpretation, RM: Manuscript preparation, AK: Data collection.

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