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Investigation Of Three BRAF V600E Mutations Related To Hairy Cell Leukemia In Al-Hillah City.

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ABSTRACT

BRAF V600E mutations are the most mutations related to Hairy cell Leukemia disorder .In the Investigation of three types of BRAF mutations there was only two mutations found in the collected samples and the third mutation was absent ,that show the verity of mutations in the HCL.

Keywords: BRAF, mutation, hairy cell leukemia.

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INTRODUCTION

Hairy cell leukemia (HCL) is B- cell lymphoproliferative disorder distinguished by proliferation of lymphocytes with abundant cytoplasm hairy projections and distinctive immunophymotype (1,2), HCL is a rare disease account for approximately 2% of leukemia disorder (3). There was no chromosomal or molecular abnormality was specifically associated with HCL , until Tiacci *etal* who found point mutations at the position 600 of codon 15 of the BRAF gene (4).The BRAF gene is oncogene when mutated the cell become cancer cell , BRAF gene responsible for RAS/MAPK pathway which regulate growth and division of cells(5) .The V600e mutation is the most common mutations of BRAF gene found in human cancers like melanoma (6,9), thyroid cancer (7,8) , colorectal cancer (9,10) , ovary cancer (11) and acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia/lymphoma, multiple myeloma and other non-Hodgkin lymphomas(11,12,16,18,19). Molecular detection of BRAF gene mutations not a routine test for diagnosis of HCL but is useful for next steps of treatment and monitoring the disease and detect relapse (12) . In this study conventional PCR had been used to detect three types of mutations in BRAF V 600 E to investigate the presence of HCL in Al-Hilla city, Iraq.

MATERIAL AND METHODS

Sample collection

60 blood samples were collected from patients diagnosed with HCL by blood and bone marrow tests in Merjan teaching Hospital . the blood sample were occupied in 5ml sterile tube with EDTA and stored in -80°c for next step of genetic study (DNA extraction and PCR)

DNA extraction

Genomic DNA were extracted from blood samples by using Genomic DNA mini kit (Geneaid) according to the manufacturing instructions, extracted DNA were stored at - 80°c.

PCR amplification

Three primers were used in this study to identify three type of mutation in BRAF V600e gene in exon 11 and 15, the first primer used amplify 256-bp fragment **(13)** ,the second primer 322-bp long and the third primer is 231-bp long **(14)** the primers are listed in table (1) . PCR conditions in table (2),the PCR product was separated on 3% agarose and visualized by UV-transllimunator.

1	First primer	Forward	5'-TAC CTA AAC TCT TCA TAA TGC TTG C-3
		Reverse	5'-GTA ACT CAG CAG CAT CTC AGG G-3
2	Second primer	Forward	TCC TGT ATC CCT CTC AGG CAT AAG GTA A
		Reverse	biotin-CGA ACA GTG AAT ATT CCT TTG AT
3	Third primer	Forward	M13-CAT AAT GCT TGC TCT GAT AGG A
		Reverse	biotin-M13-GG C CA A AA A TT T AA T CA G TG G A

	Initial denaturation							Final extension step	
	Time (min.)	Temperature (°C)	Time (Sec.)	Temperature (°C)	No. cycle	Time (Sec.)	Temperature (°C)	Time min.	Temperature (°C)
Primer 1	15	95	30	94	40	60	72	10	72
			30	59	40				
Primer 2	12	95	30	94	50	30	72	10	72
			30	55	50				
Primer 3	12	95	30	94	50	30	72	10	72
			30	55	50				

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RESULT AND DESICCATION

In this study the three primers were used to detect specific mutation on BRAF V600e gene show different pattern of result. The result of first primer (table 1) show absence of mutation in the 60 sample that studied (figure 1), Ascierto *etal* referred that mutations can be absent from specific patients diagnosed with HCL and HCL-v (6) many researchers like Tiacci etal and Arons etal found that in some cases of HCL the BRAF V600E mutations were absent (1,5), while the results of second primer and Third primer (table 1) show the present of mutations in patients , the second primer gave single band in 19 of 60 patients (figure2) in one exon that responsible for HCL (14), in the third primer used as it observed in figure 3, there is two mutations in 8 of 60 Patients these two bands mean that the mutation present in both exon 11 and 15 .Shalini, *eatl* mention the same results when they found the mutation in both exons ,many investigators referred that all cases of HCL with *IGHV4-34* had mutation in the wild type of exon 15(5,20).

The conclusion of this study was the detect of three BRAF V600 E mutation related to leukemia in Al-Hillah city there was one mutation was higher frequency than the other mutation and the third mutation was absent.

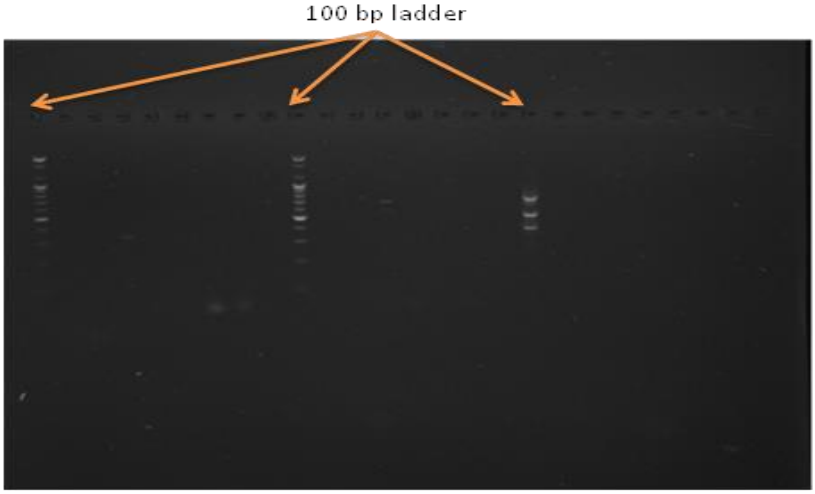

Figure (1) this figure show the result of primer 1 on agarose gel, there is no band on gel so the mutation is sbscent.

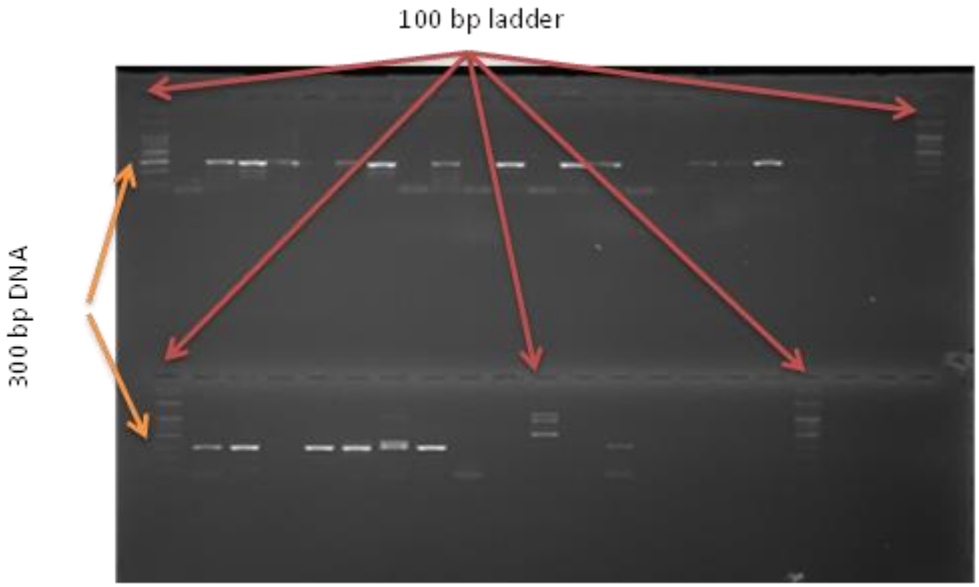

Figure (2) the result of second primer on agarose gel, there were 19 from 60 patients have this mutation

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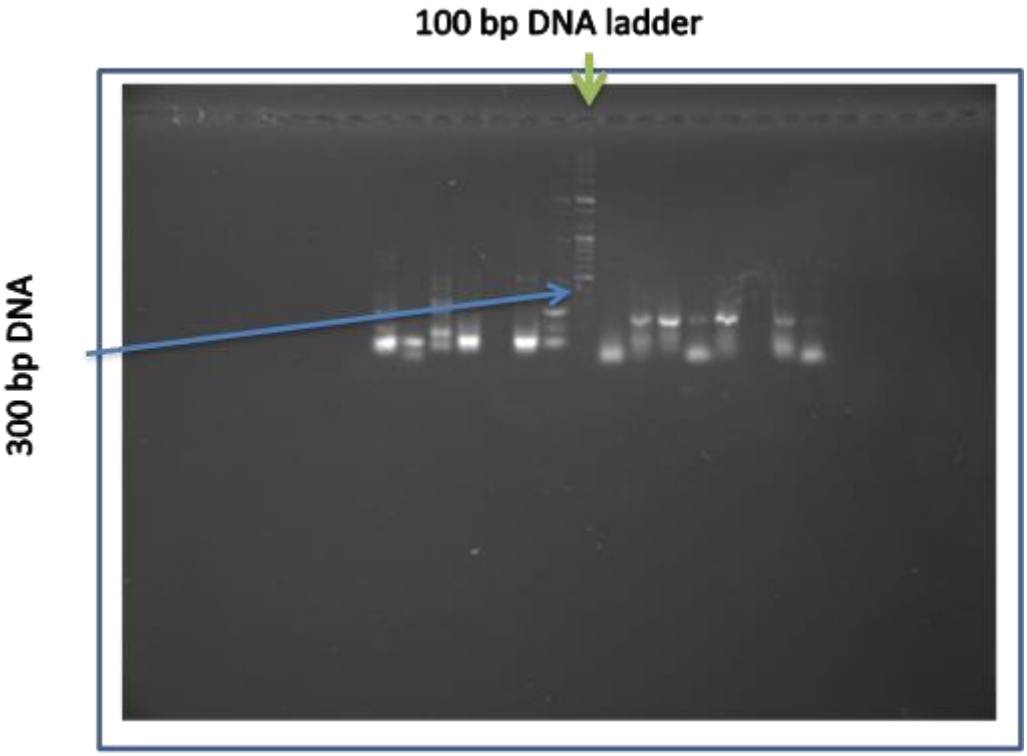

Figure (3) the result of third primer on agarose gel , there were 8 from 60 patients have this mutation

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