Detection of some genetic mutations in *H-Ras* gene in acute and chronic leukemia

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التحري عن بعض الطفرات الوراثية في جين الـ *H-ras* في مرض أبيضاض الدم الحاد والمزمن

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المستخلص

مرض ابيضاض الدم هو احد اكثر أنواع السرطان انتشارا في جميع أنحاء العالم في الرجال والنساء والأطفال. يتميز بارتفاع معدل الوفيات وخاصة النوع الحاد. تشكل جينات rasعائله جينات ما قبل التسرطن والتي وجدت في مجموعة متنوعة من أنواع السرطانات . تم في هذه الدراسة جمع عينات من الدم لخمسين من المرضى العراقيين وخمسة عشر من الأشخاص الأصحاء كمجموعة سيطرة بعد ذلك تمت عملية تضخيم الحمض النووي عن طريق تفاعل سلسلة البلمرة المتسلسل وقد ارسلت نتائج التفاعل إلى شركة Macrogen في الولايات المتحده الامريكية للقيام بمعرفة التتابعات.

في هذه الدراسة تم الكشف عن الطفرات في مرضى ابيضاض الدم الحاد و ظهرت النتائج كالتالي 19 طفرة 11 منهم (57,89%) كانت طفرة استبدال و 8 طفرات (42,1%) كانت من نوع الحذف . تم تحديد 8 طفرات من 19 طفرة في الشفرة 61 للجين . علاوة على ذلك ، وجد ان الأدينين شارك في 14 (73,86%) طفرة من 19 طفرة في النوع الحاد لمرض ابيضاض الدم . معظم تشوهات الأدينين تنطوي إما تغيير الموقع مع C وT (7 تغييرات للمواقع) أو حذف الادنين. وكان مجموع طفرات الجين ras في مرضى ابيضاض الدم المزمن 12 طفرة بينهم 7 من نوع الحذف (58.33٪) و 5 طفرات استبدال (41.66٪)

Abstract

Leukemia is one of the most prevalent forms of cancer worldwide in men, women and children with high rate of mortality. Fifty samples of blood were collected from Iraqi patients who were clinically diagnosed by the consultants of the National Center of Hematology and Medical City and 25 healthy as a control group. By direct sequencing in Macrogen company for PCR products ; nineteen mutations were detected in codon 61 of H-ras gene in acute leukemia patients , 11 of them (57.89%) were substitutions and 8 (42.1%) were deletions.

Moreover, the adenine found to involve in 14 (73.68%) of 19 mutations in AML detected in this study. Most of A abnormalities involve either trans version with C and T (7 transversions) or deletion of A. H-ras mutations were also detected in chronic leukemia patients, the H- ras mutations was 12 mutations including 7 (58.33%) deletions and 5 substitutions (41.66%) .

Introduction

Leukemia is a form of cancer that targets the blood. Blood contains different types of cells such as red blood cells, white blood cells (WBCs), and platelets. The normal life cycle of these cells (formation, growth, function and death) is controlled in part by the bone marrow and if the control over WBCs life cycle is disturbed, leukemia is the result [1].

In leukemia the WBCs number is higher than normal, they stop dying normally and they do not carry out their function in the body, such as fighting infections and healing wounds [2].

WBCs can be formed from different cell lineages, lymphoid or myeloid, the cell lineage affected by the cancer determines the kind of leukemia, and the affect can be sudden or "acute" or can be developing slowly or "chronic". These results are of 4 subtypes: acute lymphocytic leukemia (ALL) most common in children, acute myelogenous leukemia (AML) most common in adults, chronic lymphocytic leukemia (CLL) – most an adult disorder and chronic myelogenous leukemia (CML) – most common in adults. In general, leukemia is the most common cancer in children [3].

In Iraq statistics and epidemiological studies have shown that leukemia is the second most common malignancies in males, the third in females and the major form of pediatric cancer [4]**.**

The most common leukemic form in Iraq is ALL, followed by AML with a high rate of ALL (32%) among males and the high rate of consanguinity does not affect the incidence of leukemia

The number of patients with leukemia increased in Iraq dramatically following the first Gulf War, this might be attributed to the exposure to depleted uranium (a radioactive element used in ammunition) as well as to the exposure to other toxic environmental pollutants [5]. Genetic studies of leukemia patients have identified a small number of

genes that must be mutated in order to trigger the development of leukemia or to maintain the growth of malignant cells [6].

The oncogenes are found in all cells and in many cancer causing viruses , approximately fifty oncogenes have been identified in malignant tumors as part of chromosomal rearrangement, or the amplification or mutations of specific genes[7] . Mutation within a coding sequence may activate oncogenes such as the activation of Ras–oncogene in acute myeloid leukemia [8].The aim of this study to determination of some mutations in codon 61 of H-ras gene.

Materials and Methods

Blood sampling

Five ml of blood has been collected by vein puncture from 50 cases (acute and chronic) who were admitted to the Center for Hematology / Yarmouk Hospital from May 2012 till September 2012. The disease has been clinically diagnosed by the consultant medical staff at the centre. Each collected blood sample has been dispensed into EDTA tubes for molecular studies. In addition, 15 apparently healthy controls have also been included .

DNA extraction

The total genomic DNA from different healthy samples, AML, and CML has been extracted using Wizard genomic DNA kit (GENAID). This kit allows efficient extraction of DNA with high yield and purity.

Agarose gel electrophoresis

adopted

to confirm the presence and integrity of the extracted DNA the reagents that used in the Gel Electrophoresis was : Agarose , 1 X TBE Buffer , Bromophenol Blue in 1 % glycerol(loading buffer) and Ethidium Bromide .

Specific primer

PCR reaction was performed using specific primers were designed for the codon 61 of H-*ras* gene, primers were designed depending on their nucleotide sequence [9]. Were used in PCR reaction at work solution concentration (10μM).The sequences of these primers were listed in(Table 1) .

Table (1): Sequences of primer**:**

Forward	Reverse	Product	Primer
5′-TCCTGCAGGATTCCTACCGG-3′	5′-GGTTCACCTGTACTGGTGGA-3′	194 bp	H-ras 61

PCR programs

DNA samples were subjected to PCR using master mix (Promega Corp., Madison, WI), and a thermal cycle (Applied Biosystem-USA). The standard cycle procedure was showed in table-2.

Table (2): PCR programs for (H-*ras* 61) gene:

Steps	Temperature (^{o}C)	Time	No. of Cycles
Initial denaturation	95	2 minute	1
First loop:			
Denaturation	95	40 second	35
Annealing	58	40 second	
Extension	72	45 second	
Final extension	72	7 minute	1

PCR products sequencing

The PCR products of the *H-ras* gene regions and primers were sending to Macrogen Company (USA) for sequencing, Macrogen is biotechnology company providing diagnostic and screening services to the healthcare community and genetic analyses and bimolecular tools. all results were directly compared with human reference mRNA-*H-ras* sequence by software program (Chromas Pro,version:1.5) that available in web site (http://www.technelysium.com.au/chromas.html).

Statistical analysis

The statistical analysis system –SAS program [10] was used to the effect of difference factors in traits in this study. Least significant difference (LSD) test was used to the significant compare between means.

Results and Discussion

Blood samples have been collected from two groups;the group of the Iraqi patients and the group of the healthy people. The total number of patients is 50, while the number of the healthy is 15 for control group detection of H-ras 61gene .In order to specify the sequence related to H-ras 61 genes, number of experiments were done which include, extraction of total genomic DNA, amplification of the sequence related to codon 61 of H-ras gene and detection if any mutations occur within sequence**,**figure(1).

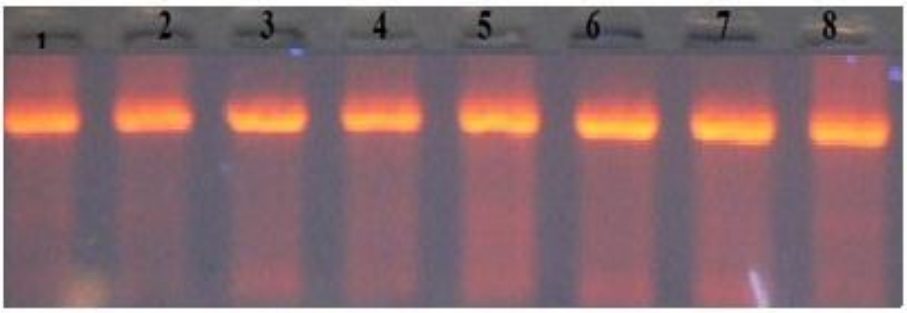

Figure (1): DNA bands extracted from blood of patients with leukemia.Bands were fractionated by electrophoresis on a 1% agarose gel at 70 voltages for (90) min. and visualized under U.V. light after staining with ethidium bromide.

In the current study we found that H-ras gene have an important role in CML and AML patients. 19 mutations were detected in acute leukemia patients (Tables 3, Figure 2). 11 of them (57.89%) were substitutions and 8 (42.1%) were deletions. 8 of 19 mutations were detected in H- ras 61 codon (4 substitutions and 4 deletions).
Moreover, the adenine which is found to involve in 14 (73.68%) of 19 mutations in AML detected in this study. Most of adenine abnormalities involve either transversion with C and T (7 transversions) or deletion of Adenine.

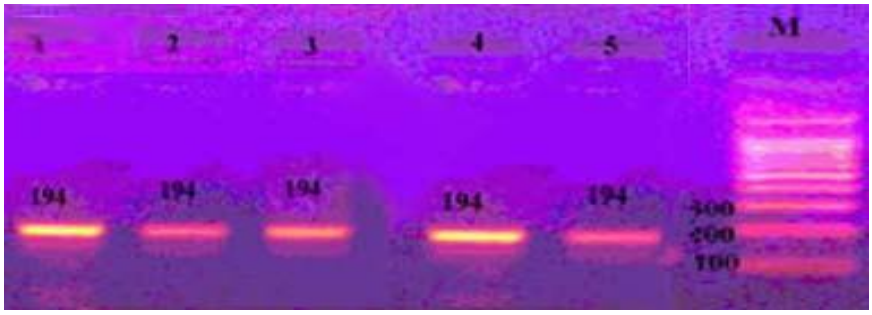

Figure (2): PCR products of H-ras 61 gene for AML on a 2 % agarose gel at 70 voltages for (90) min

Table (3): Mutations have been detected in AML patients in H (61) codon.

No. of patients sample	Mutation	Wilde type	Mutant type	Change in amino acid	Type of mutation	Effect in translation
2	T>A C.200	TAC	AAC	TYR /ASN	Substitution	Missense
3	C.6739DEL A	ACA	-CA	Deletion A 6739	Deletion	Frameshift
5	C.6739DEL A	ACA	-CA	Deletion A in site 6739	Deletion	Frameshift
8	C.6751T>A	GTT	GTA	VAL/VAL	Substitution	Silent
8	C.6739DEL A	AAA	AA-	Deletion A in site 6739	Deletion	Frameshift
8	C.6686T>G	GTA	GGA	VAL/GLY	Substitution	Missense
11	C.6739DEL A	AAA	AA-	Deletion A in 6739 Site	Deletion	Frameshift
11	C.6686T>A	GTA	GAA	VAL/GLY	Substitution	Missense

H-ras mutations were also detected in chronic leukemia patients. The total of ras mutations detected in chronic patients was 12 mutations including 7 (58.33%) deletions and 5 substitutions (41.66%) Tables (4), Figure (3).

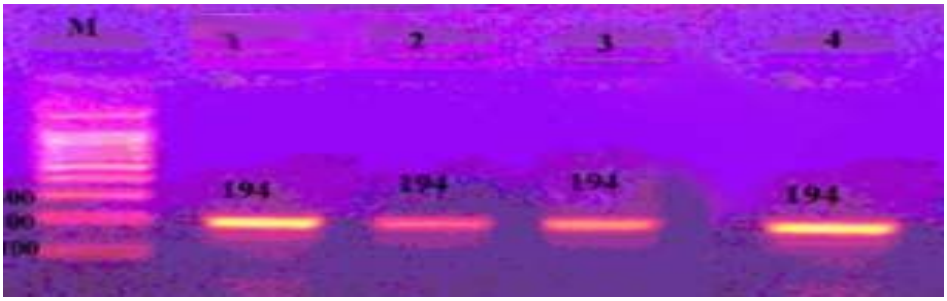

Figure (3): PCR products of H-ras 61 gene for CML on a 2 % agarose gel at 100 voltages for (35) min

Nine of 12 mutations were detected in H-ras (61) (3 substitutions and 6 deletions). The adenine role detected in acute leukemia patients was also detected in chronic leukemia patients where all the 12 mutations detected in all ras regions of chronic leukemia patients were involved A abnormalities. Most of them involve either T to A transversion (4 transversions) or deletion of A.

Table (4): Mutations have been detected in CML patients in H (61) ras gene.

No. of patients sample	Mutation	Wilde type	Mutant type	Change in amino acid	Type of mutation	Effect in translation
14	C.6751T>A C.6739delA	TGT ACA	AGT _CA	CYS/SER Deletion A IN 6739 site	Substitution Deletion	Missense Frameshift
16	C.6751T>A C.6739delA	TGT ACA	AGT _CA	CYS/SER Deletion A6739 site	Deletion Deletion	Frameshift Frameshift
19	C.6751T>A C.6739delA	TGT ACA	AGT _CA	CYS/SER Deletion A IN 6739 site	Substitution Deletion	Missense Frameshift
22	C.6751T>A C.6739delA	TGT ACA	AGT _CA	CYS/ SER Deletion A IN 6739 site	Substitution Deletion	Missense Frameshift
25	C.6739delA	ACA	_CA	Deletion A IN 6739 site	Deletion	Frameshift

H-ras mutations show their own specific pattern, with highest percentage of mutations detected in codon 12 (about a 54%), followed by codon 61 (34.5%) and codon 13 (9%). Ras mutation rates vary widely in hematopoietic cancers, with values ranging in leukemias from as low as 5% in chronic myeloid leukemia (CML) to 27% in chronic myelomonocytic leukemia (CMML) [11]**.** Some studies have also reported exceedingly higher percentages (70%) in CMML and plasma cell myeloma [12]**.**

In general, mutations are almost inexistent in H-ras, rare events for K-ras (with the exception of CMML), and are much more frequent for N-ras, reaching rates of up to 20% in juvenile myelomonocytic myeloid leukemia (JMML) or plasma cell myeloma. Despite sharing this genetic modification with melanomas or thyroid carcinomas, the pattern of N-ras mutations in hematopoietic tumors is very different [13].

The Ras gene coding for protien P21 that located on the inner surface of the plasma membrane that has GTPase activity and may participatate in single transduction [14]. The ras oncogenes are activated by piont mutation that alter amino acid sequence of P21 ,piont mutation in the ras gene specially those effecting amino acid at codon 12 and codon 61 resulting in exaggerated response to growth factor and excessive cell proliferation [15].

References

1-**Al-Hadad, S.A., Al-Jadiry, M.F., Al-Darraji, A.M., Al-Saeed, R.M., Al-Badr, S.F. and Ghali, H.H. (2011)**. Reality of Pediatric Cancer in Iraq, *J. Pediatr. Hematol. Oncol*.vol.(33):S154–S156

2- **Rice, K.L., Buzzai, M., Altman, J. and Licht, J.D.(2011)**. The molecular basis of adult acute myeloid leukemia (in Education program for the 16th Congress of the European Hematology Association London, UK June 9-12, 2011)p: 27-35.

3- **Salim E, Moore MA, and Al-Kayed S. (2009).** Cancer Epidemiology in The Arab Region-Past Present And Future. *Asian Pac J Cancer Prev*, 10, 27-44.

4-**WHO (World Health Organization) (2008)**. Cancer Incidence and Mortality Worldwide, Cancer Mondial, International Agency for Research on Cancer, Lyon, France.

5-**Essa, S.S., Habib, O.S., Al-Diab, J., Al-Imara, K., Ajeel, N.A.H. (2007).** *Cancer Mortality In Basrah, MJBU,* Vol. 25, No.1.

6- **Mittleman F. and Heim S. (2007).**Quantitative acute leukemia cytogeneticsGenes. Chromosomes and Cancer, 5, 57-66.

7-**Jeffrey, A., McClenaghan, R.(1999).** Oncogenes the discovery of oncogenes, properties of oncogenes. *Encylopedia of Genetic* ; II: 438-440.

8- **Bos, J; Takosz, D and Marshall, J. (1985).** Amino acid substitutions at codon 13 of the N-ras oncogene in human myeloid leukemia. *Nature*; vol(52)315:725

9-**Mitchell, K. and Warshawsky,D. (1998).** MutationalAnalysisUsingEnrichedPCR andCycleSequencing, BioTechniques24:1028-1031.

10- **SAS. (2004).** SAS / STAT Users Guide for Personal Computers. Release 7.0. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).

11- **Forbes SA, Bindal N, Bamford S, (2011).** Mining Complete Cancer Genomes in the Catalogueof Somatic Mutations in Cancer. *Nucleic Acids Res*.; VOL. (39):945-50.

12- **Reuter CW, Morgan MA. and Bergmann L.(2000).** Targeting The Ras Signaling Pathway: A Rational, Mechanism-Based Treatment for Hematologic Malignancies. *Blood*. vol. (5):1655-69.

13- **Fernández-Medarde, A. and Santos, E.(2011).** Ras in Cancer and Developmental Diseases **,** *Genes and Cancer*vol. (3) 344–358

14-**Rom,W.N.;Hay,J.G.;Lee,T.C.;Jiang,Y.; and Wong,K.(2000).**Molecular and genetic aspects of lung cancer.Am.J.Respir.Crit.Med,161(4):1355-1367.

15-**Richared ,M.T.(1997).** Advanced molecular biology.(1ist) Bios Scientific publishers, Pp,273-279.