The Prevalence Of Gene NOTCH1 Mutation In Iraqi Chronic Lymphocytic Leukemia

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Abstract

Chronic lymphocytic leukemia (CLL) is a monoclonal malignancy characterized by an accumulation of small and mature looking B lymphocytes in the blood, bone marrow and other tissues. The B-lymphocytes developed in a wrong way lead to an immune disorder, and are typically characterized by expression of some of CD markers as (CD5,CD38 and ZAP-70) determined by immunophenotyping. The aim of this study was to investigate the extent to which immunological, biochemical, and molecular parameters are altered in CLL patients, and the potential of applying these alterations as biomarkers for CLL in Iraqi patients.

the present study included 64 patients of CLL and 48 healthy individuals were investigated for Notch homolog 1, translocation-associated (Drosophila) (NOTCH1) c.7544-7545delCT, The presence of NOTCH1 mutations were confirmed by genomic DNA sequencing. The NOTCH1 mutation was detected in 15% of patients and not detected in the control group.

The current study established the frequency of NOTCH1 mutations in patients with CLL from the iraqi population of the National Center of Hematology AL-Mustansiriyah University and Hematology Department of Baghdad Medical City. In summary, a high frequency of NOTCH1 and SF3B1 mutations were identified in patients with CLL compared with healthy individuals, and the NOTCH1 mutation was found in 15% of CLL patients The alignment results of the 341 bp samples revealed the presence of only one genetic variation variably distributed in some of the analyzed samples patients in comparison with the referring NOTCH1 genetic sequences , The identified variations were only found to be confined with the targeted rs763016003 frameshift variant. In a total of eleven investigated samples, S2, S3, S5, and S7 were placed as control, while the rest of the samples (S15, S19, S20, S31, S41, S49, and S51) belonged to patients. The targeted rs763016003 frameshift variant was not found in all involved control samples. Meanwhile, this rs763016003 variant was found in most of the patient samples (S15, S19, S31, S41, S49, and S51).

Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia in Europe. It is a disease that affects older people in particular, and it is often determined by the elderly , Young people rarely experience clinical symptoms that are very heterogeneous, Leukemia originates initially through changes or mutations. In the genetic material, it affects the programmed cell death of blood cells. Diagnosis: made by blood counts, blood spots, and immunoglobulin enlarged B-lymphocytes that define cloned B-cell clusters that carry CD5 antigen as well as typical B-cell markers (1) . For example the deletion of the short arm of chromosome 17 or the occurrence of mutations in the TP53 gene Demonstrating patient resistance

to chemotherapy and shorter patient time. CLL-IPI combines genetic, biological, and clinical variables to identify distinct risk groups for CLL patients (1) ,

Certain gene mutations, including Notch homolog 1, translocation-associated (Drosophila) (NOTCH1) are known biomarkers for CLL prognosis (2). NOTCH1 keep stem cells and order apoptosis in numerous tissues for the period of normal embryonic and postnatal progress.

The NOTCH signaling pathway in CLL cells play a role in survival and cell fighting to apoptosis. The most frequent mutation of NOTCH1 is C.7544-7545delCT, which accounts for ~80% of all NOTCH1 mutations ,The SF3B1, a essential component of the U2 spliceosome, serves an significant role in the excision of pre-mRNA introns and the production of mature mRNA. Mutations that inactivate the SF3B1gene result in defective splicing of various mRNAs and defective protein synthesis (3) . The presence of this mutation has been associated with an intermediate risk of CLL and transformation to high grade lymphoma (4). hole-genome sequencing revealed that NOTCH1 somatic mutations potentially affect gene function (5). The NOTCH1 gene encodes a trans membrane protein that is involved in the growth, differentiation and self-renewal of cells.

The aim of the present study was to detect the frequency ofNOTCH1 (c.7544_7545 del CT) mutations in patients with CLL from a population of Iraq compared with healthy individuals. In addition, the current study aimed to identify the association of these mutations with the CLL disease .

SUBJECT AND METHODS

Sixty four patients (46 male ,18 female) were taken at the National Center of Hematology / University of AL Mustansiriyah and Hematology Department of Baghdad Medical City were newly diagnosis and 48 healthy individual , the age range of both group between [35-84 years] , all patients group (male / female) were newly diagnosed , The total CLL patients enrolled in the present study are composed of 46 males (71.8%) and 18 females (28.2%) with higher incidence in male than in female (ratio 3:1) .

Included the study four incidence age out of 64 cases, the most common of incidence in newly CLL patients were incidence (60-69) years, and means ± SD 65.33±13.20 , Reported many studies variations in incidents for CLL patients between (60-69)years , while other incidents that including less than 60 years and over than 69 years low in ratio CLL patients compare with incidents (60-69) years.

Genotyping. Genomic DNA was extracted from EDTA-treated whole blood by using the phenol-chloroform method, as described previously (6).

The NOTCH1 c.7544_7545delCT mutation was found by AS-PCR in 10 (15.1%) of 64 CLL patients figure (3-11) three of cases were low band and neglected .The data compared with other sample of CLL patients , the wild type of the NOTCH1 (forward & reveres)found in all 64 sample (un mutated) , which were analyzed also by direct Sanger sequencing, the presence of a typical 2 bp deletion c.7544_7545delCT was confirmed . In all cases mutation process involved only one allele. By contrast, NOTCH1-unmutated cases by AS-PCR, and also 5 “presumably negative” cases, were negative by Sanger sequencing. So, our data are consistent with the suggestion of (7*)*. that AS-PCR method had 100% specificity in detection of NOTCH1 c.7544_7545delCT mutation .

NOTCH1 c.7544_7545delCT mutation was found in 10.1% of CLL patients, which is consistent with other reports (7 , 8, 5) . However, the results of AS-PCR in a minor number of cases (7 of 325 cases; 2.15%) were doubtful and required reinvestigation. Sanger sequencing allows to precisely identify deletion, but it is quite laborous and more expensive than conventional PCR-based methods. In this context real-time PCR being time- and cost-effective, extends the opportunities for an objective assessment of the amplification’s

specificity and thus might be used for fast screening for NOTCH1 c.7544_7545delCT mutation. Furthermore, under certain conditions it might allow a quantitative assessment of NOTCH1-mutated clone. CLL patients harboring NOTCH1 deletion showed asignificantly shorter PFS in comparison with NOTCH1- unmutated cases sustaining reported adverse impact of this alteration on outcome (7 , 9 , 8). The primer used in this research showed in table 1.

Table 1.Designed Primers Used in the Current study.

Primers used in gene expression		
Primer	Sequence (5′→3′ direction)	mutation
NOTCH 1 gene		C.7544-7545 del CT
Forward	GTGACCGCAGCCCAGTT	Chromosome 9
Reveres	AAGGCTTGGGAAAGGAAGC	chromosome 9
Mutant	TCCTCACCCCGTCCCGA	chromosome 9
Primers used in sequence		**Designed**
Primer	Sequence (5′→3′ direction)	
NOTCH 1 gene		product size
Forward	ACTGGTCAGGGGACTCAGG	200bp
Reveres	CACTATTCTGCCCCAGGAGA	200bp

The detection of the 2-bp deletion in NOTCH1 (c.7544_7545delCT) was performed using an allele specific-polymerase chain reaction (AS-PCR) method. Using two external primers as the internal PCR control, a fragment with 341-bp was amplified for both wild and mutant alleles. The PCR reaction was performed with a total volume of 20 µl containing 5 ng of genomic DNA, 12.5 master mix, 7.5 nM nuclease free water and 10 nM each of the forward, reverse and deletion detection primers (Table 2).

Table (2) Components of Quantitative AS PCR Used in NOTCH1 Genes Expression Experiment.

Components	**1 µl rxn**
qPCR master mix	12.5
Nuclease free water	7.5
Forward Primer (10 µM)	1

Reverse Primer (10 µM)	1
DNA	5

The PCR thermocycling conditions were as follows in table 3 .

Table (3) Thermal Profile NOTCH1 Expression

Step	Temperature	Duration	Cycles
Enzyme activation	95 ºC	5 minute	1
Denature	95 ºC	30 second	40
Anneal	60 ºC	30 second	
Extend	72 ºC	30 second	
Dissociation	5min /72 ºC-30 sec /55 ºC-30sec/95 ºC		

Results

The NOTCH1 c.7544_7545delCT mutation was found by AS-PCR in 10 (15.1%) of 64 CLL patients figure (3-11) three of cases were low band and neglected .The data compared with other sample of CLL patients , the wild type of the NOTCH1 (forward & reveres)found in all 64 sample (un mutated) , which were analyzed also by direct Sanger sequencing, the presence of a typical 2 bp deletion c.7544_7545delCT was confirmed . In all cases mutation process involved only one allele. By contrast, NOTCH1-unmutated cases by AS-PCR, and also 5 “presumably negative” cases, were negative by Sanger sequencing. So, our data are consistent with the suggestion of (7*)*. that AS-PCR method had 100% specificity in detection of NOTCH1 c.7544_7545delCT mutation .

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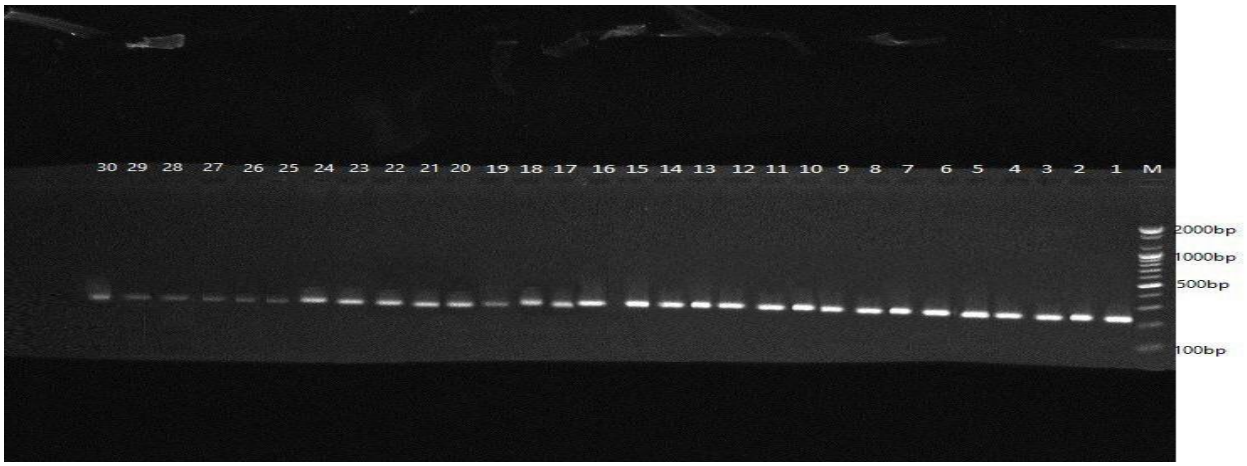
.Figure (1) mutation band of NOTCH1 gene showing band with molecular size (200bp). Electrophoresis was performed on 1.5 % agarose gel and run with a 100-.



Fig(2). The DNA chromatogram patterns of the observed frameshift mutation of the 341 bp amplicons within the targeted *NOTCH1* genomic DNA sequences. The observed frameshift mutation in the targeted rs763016003 variant was highlighted according to its positions in the PCR products

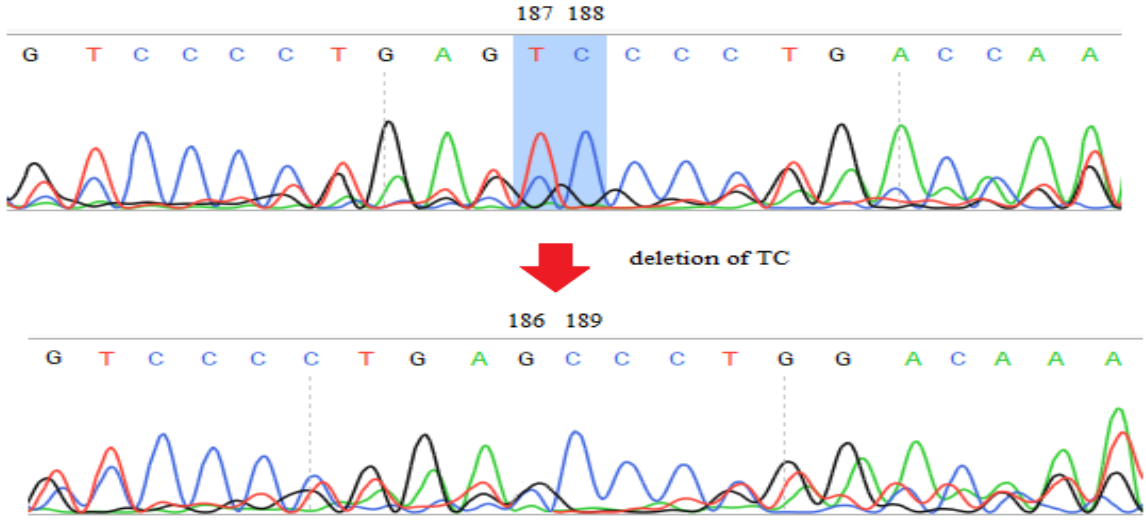


Table (4). The position and length of the 341 bp PCR amplicons that used to amplify a portion of *NOTCH1* DNA sequences. The amplified sequences were extended from 54408 to 54748 of the NCBI reference DNA sequence (GenBank acc. no. NG_007458.1).

Amplicon	Reference locus sequences (5′ - 3′)	Length

NOTCH1 DNA sequences	*CACACTATTCTGCCCCAGGAGAGCCCCGCCCTGCCCACGTCGC TGCCATCCTCGCTGGTCCCACCCGTGACCGCAGCCCAGTTCCTG ACGCCCCCCTCGCAGCACAGCTACTCCTCGCCTGTGGACAACAC CCCCAGCCACCAGCTACAGGTGCCTGAGCACCCCTTCCTCACCC CGTCCCCTGAGTCCCCTGACCAGTGGTCCAGCTCGTCCCCGCAT TCCAACGTCTCCGACTGGTCCGAGGGCGTCTCCAGCCCTCCCAC CAGCATGCAGTCCCAGATCGCCCGCATTCCGGAGGCCTTCAAGT AAACGGCGCGCCCCACGAGACCCCGGCTTCCTTT*	341 bp

CONCLUSION

In conclusion, the targeted rs763016003 frameshift variant is strongly suggested in the present work to be a competent biomarker for our investigated samples, with a remarkable association with the development of leukemia disease in Iraq.

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