GENETIC POLYMORHISM OF CASPASE 8 AND 9 IN IRAQ

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Abstract

This study examined the potential association between polymorphisms of Caspase 9 −293del (rs4645982) and Caspase8−652 6N ins / del (rs3834129) and the danger of acute and chronic myeloid leukaemia in Iraqi population. This case-control study was conducted as 128 (62 acute, and 66 chronic) myeloid leukaemia patient and 65 healthy individuals not infected with cancer. A conventional PCR method was used for detection in this study. The results showed that the Caspase9 −293del (rs4645982) in homozygous and heterozygous deletion genotypes were associated significantly with CML risk (p= 0.001 and 0.005 ,respectively) and in homozygous insertion and deletion genotypes of Caspase 8 -652 6N were also associated significantly with CML risk (p= 0.004 and p= 0.00, respectively), while there were non significant association between Caspase9 −293del (rs4645982) and Caspase8 −652 6N (rs3834129) with AML risk in all genotypes of them p > 0.05. These findings suggest that polymorphisms in the Caspase-9 and Caspase-8 genes might be useful markers for determining genetic susceptibility to CML and AML. The results of this study suggest that Caspase9-293 is associated with increased risk of CML in case of homozygous of deletion, and insertion in case of AML, while in Caspase8-652 6N insertion polymorphism are associated with increased risk of both CML and AML in a sample of Iraqi populations.

Keywords: Acute myeloid leukemia, Chronic myeloid leukemia, Apoptosis, Odd ratio

تعدد الشكل الوراثي للكاسبيز 8 و9 في العراق

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المستخلص

تم التحقيق في هذه الدراسة حول العلاقة المحتملة بين Caspase9 – 293del (rs4645982) و Caspase8 −652 6N ins / del (rs3834129)تعدد الأشكال وخطر الإصابة بسرطان الدم النخاعي الحاد والمزمن في السكان العراقيين. أجريت دراسة الحالات والشواهد على 62 مريضا بسرطان الدم النخاعي الحاد و 66 مريضا بسرطان الدم النخاعي و 65 من الأفراد الأصحاء غير مصابين بالسرطان. تم استخدام تقنية PCR تقليدية للكشف عن Caspase9−293del (rs4645982) وتم استخدام تضخيم أليل PCR ثنائي الاتجاه سريع بسيط (bi−PASA) لاكتشاف تعدد الأشكال من.(rs3834129) Caspase8 −652 6N ins / del أظهرت النتائج أن Caspase9 −293del (rs4645982) في الأنماط الجينية الحذف متجانسة الزيجوت وغير المتجانسة وارتبطت بشكل كبير مع خطر(p= 0.001 و 0.005، على التوالي) في المتجانسة الاظافة والحذف للانماط الجينية من Caspase8−652 6N ارتبطت أيضا بشكل كبير مع خطر CML (p= 0.004 و p=0.00، على التوالي)، في حين لم يكن هناك ارتباط كبير بين Caspase9 −293del (rs4645982) و(rs3834129) Caspase8 −652 6N مع خطر AML في جميع الأنماط الجينية منهم P > 0.05. تشير هذه النتائج إلى أن تعدد الأشكال في جينات. CASP−9 و CASP−8 قد تكون علامات مفيدة لتحديد القابلية الوراثية لـ CML وAML. تشير نتائج هذه الدراسة إلى أن Caspase9−293 مرتبط بزيادة خطر الإصابة بـ CML في حالة الحذف المتماثل، والحذف في حالة AML، بينما في Caspase8−652 6N، تعدد الأشكال الإظافة مرتبط بزيادة خطر كل من CML و AML في عينة من السكان العراقيين.

الكلمات المفتاحية: سرطان الدم النخاعي الحاد، سرطان الدم النخاعي المزمن، الموت الخلوي المبرمج، نسبة الاحتمالية

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INTRODUCTION

Chronic myeloid leukemia (CML) is a heamatological malignancy which is characterized by excessive proliferation of hematopoietic system myeloid progenitors. This originates from the transformation of a single hematopoietic cell carrying the BCR-ABL translocation that avoids the process of cell surveillance. The presence of BCR-ABL drives the clonal expansion of leukemic progenitor cells which depends mainly on the imbalance between proliferation rate and cell death rate. BCR-ABL oncoprotein has been found to exert its anti-apoptotic effect, both directly and indirectly, by deregulating the genes implicated in the apoptotic process via aberrant anti-apoptotic and pro-apoptotic gene expression. These results of suppression of cytochrome c release from the mitochondria, and subsequent activation of Apaf1, are found to inhibit Caspase 9 activation through precisely maintaining HSCs Properties such as self-renewal, proliferation and host-system differentiation. Initiator Caspases (Caspase-9, Caspase-8, and Caspase-10) transmit apoptotic signals and activate effector Caspases (Caspase-3, Caspase-6, and Caspase-7) that execute apoptosis (1,5,20). There are two main categorized apoptotic pathways dependent on activation by the Caspase.They are (i) the extrinsic pathway triggered by ligation of TNF family death receptors and subsequent activation of Caspase-8, and (ii) the intrinsic pathway (mitochondrial-mediated apoptosis), initiated by cell stress through cytochrome c mitochondrial release followed by activation of Caspase-9, a caspase initiator activated from mitochondria by the release of cytochrome c, triggers downstream Caspases 3 and 7. Reduced expression of Caspase-9 has also been found to be associated with the development of many types of cancers and chemotherapy resistance (21). Since Caspase-9-mediated apoptosis had been initiated in most cancer treatments. Various studies have shown that single nucleotide polymorphism. cancer treatments. Various studies have shown that single nucleotide polymorphisms (24). Therefore, variations in the Caspase-9 gene expression and functions due to SNPs or their haplotypes could also increase the risk of developing AML and CML. Caspase 9 is recognised for its regulation of cancer susceptibility. Functional SNPs in CASP9 might influence the gene expression leading to altered apoptosis which confer the risk to AML In silico analysis had revealed the deletion of 19 (-293-275 del (CGTGAGGTCAGTGCGGGGA) nucleotides was reported to alter the expression levels of Caspase-9 gene(24). Caspase-8 is important for the death pathways of extrinsic cells triggered by family members of the tumor necrosis factor (TNF). Death receptors recruit the Death-induced Signalling Complex (DISC) after binding to specific family ligands of the TNF. Then, through the adaptor protein Fas Associated via Death Domain (FADD) procaspase-8 can be recruited into this complex.Caspases are classified into two different subgroups based on their structure and function: the initiator or apical caspases (caspases 2, 8, 9, and 10) are responsible for initiating the caspase cascade upon receipt of proapoptotic stimuli. It is assumed that the effector or executioner caspases (caspases 3, 6 and 7) are responsible for typical apoptotic characteristics such as DNA fragmentation, chromatin condensation, cell shrinkage and membrane blebbing (13). Caspase 8 is an effective apoptosis initiator, a crucial anti-hyperproliferation and malignancy defence mechanism. . The Caspase-8 gene is mapped to the 2q33–34 chromosome and contains 11 highly polymorphic exons of 30 kb (30). It is well known that a six-nucleotide polymorphism deletion (−652 6N del) in the Caspase-8 promoter abolishes a transcription activator 1 (Sp1) binding location, resulting in reduced lymphocyte production of the Caspase-8 protein and lower Caspase-8 activity and activation-induced cell death of T lymphocytes (29). This deletion variant has been shown to be safe against several types of cancer (14). Other studies have found no correlation between Caspase-8 −652 6N ins / del and multiple cancer risk (3,28).

MATERIALS AND METHODS

Study design: The present case-control study was conducted on 193 individuals including 65 controls, 62 AML cases and 66 CML cases. Mean Age of control (39.23±13.46), patients AML (37.34±15.96) and, CML patients (41.98±12.86) the mean gender of control

male 36(56.30%) female 28(43.80%) ,AML patients male 40(64.50%) Female 22(35.50%) CML patients male 33(50.00%) Female 33(50.00%) matched test samples were collected from the local population for case-control analysis, without any family history of cancer; while the AML and CML cases were recruited from Iraq hospital , Hyderabad. This study was accepted by the Committee for Biomedical Research on Institutional Ethics, University of Baghdad, and the Iraq hosspital ethical committee, Hyderabad. The diagnosis of the AML and CML were confirmed based on the complete blood picture (WBC, platelets and Hb %), and molecular analysis (to detect the levels of BCR-ABL transcript) for CML patients. All the patients who participated in the study were informed for objectives, and they received written informed consent. Sex, age at diagnosis, occupation and other general information of the patient were noted in the prescribed proforma. The investigators conducted a personal interview using a standardized questionnaire consisting of demographic characteristics age gender, occupation, habits and habitats, etc.). Clinical data baseline characteristics at diagnostic age, Hb percent, platelet count, white blood cell count, were measured. Blood collection three ml of venous blood specimen was collected from each participant in ethylene diamine tetra acetic acid (EDTA) vacutainer and stored at −20 °C freezer until used.

Genotyping of Caspase-9 and Caspase-8 SNPs: Blood samples collected in EDTA tube were used for genomic DNA isolation by ReliaPrep™ Blood gDNA Miniprep System kit (promega, USA) and DNA was resuspended in RNase/DNase -free distilled water after following the manufacturer's instructions. The purity and concentration of genomic DNA were determined using Nanodrop UV/VIS The DNA samples were diluted to 100 ng/µL concentration and stored at −20 °C until used. We have analysed one promoter polymorphism of Caspase9−293del (rs4645982) and one polymorphism in Caspase8−652 6N ins/del (rs3834129). PCR was performed polymorphism (CASP9 -293 del) following the PCR conditions which including initial denaturation at 95 °C for five min, followed by 28 cycles of denaturation at 94 °C for 1 min, annealing at varied temperature 64.5 °C, extension at 72 °C for one min and final extension at 72 °C for seven min using sequence specific primers (F: 5′-CGTTGGAGATGCGTCCTGCG-3′ and R: 5′-CGCCCTCAGGACGCACCTCT-3) (11,6)

The PCR master-mix was prepared in a 25-µL reaction mix that consisted of 100 ng DNA template, 2.5mM dNTP mix, pmol of forward and reverse primers, 0.5 U Taq polymerase and DNase-free water. PCR products were checked on 3% agarose gel and the sizes of the fragments were determined by using DNA ladders (100 bp). Product sizes were 222 bp for deletion allele and 241 bp for insertion allele. One polymorphism in caspase8 bi-directional PCR allele-specific amplification (bi- PASA) system for detection of 6N deletion of Caspase-8 was applied. This method is applicable—for detecting small deletions. We used two outer primers (FO:5′-AGTGAAAACTTCTCCCATGGCCT-3′; RO: 5′-GATTGATACTGGCACAGTATACTTACC-3′) and two specific primers (FI (Insertion allele): 5′-GTAATTCTTGCTCTGCCAAGCTG-3′; RI (Deletion allele): 5′-CCAAGGTCACGCAGCTAGTAAG3′). A one-tube PCR reaction can the distinction between the various genotypes. PCR was performed by using commercially available PCR premix (AccuPower PCR PreMix; BIONEER, Daejeon , Korea) according to the manufacturer's instructions. Briefly, 1 µL template DNA (~100 ng/mL), 1 µL of each primer (10 pmol/mL), and 15 µL DNase-free water were added to AccuPower PCR PreMix. Amplification was done with an initial denaturation step at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 25 s with a final extension at 72 °C for 10 min. Each reaction was verified on a 1.5% agarose gel electrophoresis.

Statistical analysis: Hardy-Weinberg genotype equilibrium was checked with a chi-square test. Using the Student's t-test, Mann-Whitney U test or chi-square test, variations in demographic and clinical characteristics and genotype distribution between patients with CHB and LC were compared. In combination with genotypes, the odds ratios (ORs) and 95%

confidentiality. intervals (CIs) for LC were determined with unconditional logistic regression modified by age and gender. All data were analysed using version 17.0 (SPSS, Chicago, IL) of the statistical package SPSS. P values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Association of Caspase 9 Polymorphism with CML and AML risk: This study investigated the potential association between Caspase-9 polymorphism (-293del) and the danger of CML and AML. In addition, the Caspase-9 frequency distributions of polymorphism in CML and AML cases were compared with controls. The distributions of the Caspase-9 (-293del) genotype among cases (CML and AML) and controls are shown in figure 1 and tables (1a,1b and 2a,2b), respectively. The Caspase-9 (-293del) genotype was statistically significance in CML but not in AML in comparison with controls. There were no significant differences between AML patients in all genotypes of Caspase-9 (-293) (del/del(-/-) (222 bp): OR 1.15, 95%CI: 0.56-2.39 , P= 0.712, del/ ins(-/+) (222/241 bp): OR 0.84, 95%CI: 0.42-1.6 , P = 0.720 and ins/ins (+/+) (241bp) of mentioned SNP (OR 1.06 , 95%CI: 0.47-2.40 , P= 1.00) In CML patients the presence of heterozygote Caspase9 -293 del (+/−) heterozygous genotype may confer protection against CML but not AML (table 1a)as confirmed by the present case-control study (OR 0.33, 95%CI: 0.16_0.72, P= 0.005) The results obtained in the present study agreed with the study by(10) who reported protective effect exerted by heterozygous (−/+) genotype in high-risk non-muscle-invasive bladder cancer (NMIBC), while the homozygous Caspase9 -293 del (−/−) genotype conferred increased risk for the progression of CML as observed by the elevated frequency of Caspase9 -293 del (−/−) genotype in patients compared withcontrols (OR 3.35, 95%CI: 1.54-7.36, P= 0.001) (figure 1). These results therefore indicated that the presence of a single dose of a normal Caspase9-293 del (+) allele in heterozygotes might be able to compensate the risk conferred by deletion allele, where as deletion of 19 nucleotides in the promoter region, on both chromosomes (−/−) might affect the activity of Caspase-9, there by apoptosis (table 1a).The genotype distribution of the polymorphism among the controls and AML were in Hardy–Weinberg equilibrium, but genotype distribution of the Caspase-9 (-293del) polymorphism in CML was not in Hardy-Weinberg equilibrium ($p<0.05$). To achieve the equilibrium five conditions must be met: population should be very large, population must be isolated from other populations (no immigration or emigration), no alleles mutations (deletion or insertion), random mating (no inbreeding) and no natural selection (i.e. every individual has an equal chance of survival). If the five conditions are not met then evolution occurs and there is a change in allele frequency in the population and Hardy–Weinberg equilibrium is not present (7). Because the mutation occurred in Caspase-9 by deletion of Caspase-9 (-293del) by present study and there may have been inbreeding (increased recessive alleles) which led to deviation in Hardy-Weinberg equilibrium and the distribution of genotypes was not in this equilibrium. These findings indicate that polymorphism in the Caspase-9 gene may be useful markers for determining genetic susceptibility to CML in addition, the results suggest that the Caspase-9 gene may be involved in CML growth. Several studies have demonstrated that polymorphisms in the cell death pathway genes Fas and FasL contribute to the genetic susceptibility to various human cancers(19,22,23,15).The result agree with study demonstrated Presence of heterozygote CASP9 -293 del (+/−) genotype may confer protection against CML as confirmed by the present case-control study (6).

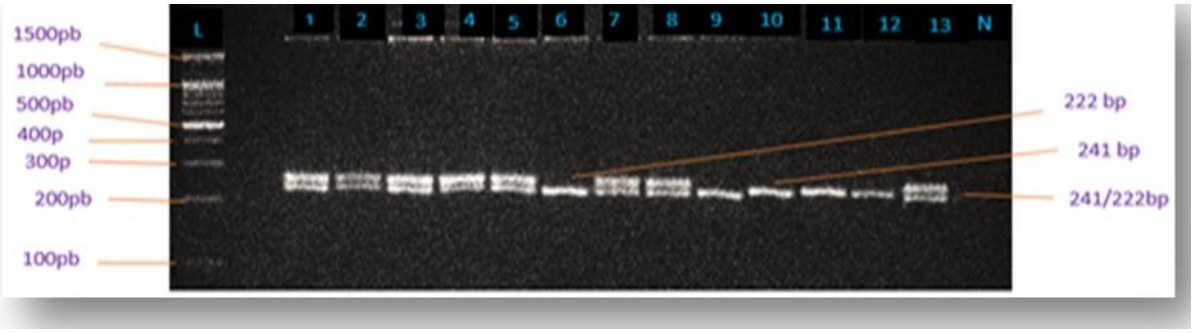

Figure 1. Detection of caspase 9 (-293) gene polymorphism on 3% agrose gel, gel viewed under Gel documentation -UV trans-illuminator after being stained with ethidium bromide stain Lane L: 100 bp DNA ladder. Heterozygous for deletion (-/+) (222/241)bp : Lane (1,2,3,4,5,7,8,13) ,Homozygous for deletion (-/-) (222bp) : Lane (6.9) , Homozygous for insertion (+/+) (241) :Lane (10,11,12) , N : negative control.

Table 1a. Observed numbers and percentage frequencies of Caspase-9 (-293del) gene polymorphism in CML patients and controls

CML Genotypes		Patients (No.=66)		Controls (No.=65)		Odd Ratio	95% C.I.	p-value
		No.66	%	No.65	%			
222(-/-) (homozygous of deletion)		41	62.12	21	32.3	3.44	1.68_7.02	0.001
241/222 (+/-) (heterozygous of deletion)		14	21.21	29	44.61	0.33	0.16_0.72	0.005
241(+/+) (homozygous insertion)		11	16.66	15	23.07	0.67	0.28_1.58	0.388
Total		66	100.0	65	100.0	-	-	-
Allele frequency	*222 del*	96	72.72	71	54.6	2.22	1.33_3.70	0.003
	241 ins	36	27.27	59	45.4	0.45	0.27_0.75	0.003

Table 1b. Numbers and percentage frequencies (observed and expected) of of Caspase-9 (-293del) gene polymorphism in CML patients and their Hardy-Weinberg equilibrium (HWE) in CML patients compared with control groups

Groups			CASP-9 (-293del) genotypes polymorphism Genotypes			HWE	Alleles	
			222	241/222	241	p - value	222	241
CML patients (No. =66)	Observed	No.	41	14	11	0.0002	94	36
		%	62.12	21.21	16.66		72.31%	27.69%
	Expected	No.	33.98	26.03	4.98		Not estimated	
		%	52.28	40.05	7.67			
Controls (No. = 65)	Observed	No.	21	29	15	0.42	71	59
		%	32.3	44.61	23.07		54.62%	45.38%
	Expected	No.	19.39	32.22	13.39		Not estimated	
		%	29.83	49.57	20.6			

Table 2a. Observed numbers and percentage frequencies of in of Caspase-9 (-293del) gene polymorphism in AML patients and controls

Caspase 9 Genotypes		Patients (No.=62)		Controls (No.=65)		Odd Ratio	95% C.I.	p-value
		No. 62	%	No. 65	%			
222(-/-) (homozygous of deletion)		22	35.48	21	32.3	1.15	0.56-2.39	0.712
241/222 (+/-) (heterozygous of deletion)		25	40.32	29	44.61	0.84	0.42-1.69	0.720
241(+/+) (homozygous of insertion)		15	24.19	15	23.07	1.06	0.47-2.40	1
Total		62	100.0	65	100.0	-	-	-
Allele frequency	*222 del*	69	55.64	71	109.23	1.04	0.64-1.71	0.900
	241 ins	55	44.35	59	90.76	0.96	0.59-1.57	0.900

Table 2b. Numbers and percentage frequencies (observed and expected) of of Caspase-9 (-293del) gene polymorphism in AML patients and their Hardy-Weinberg equilibrium (HWE) in AML patients compared with control groups

Groups			CASP-9 (-293 del) genotypes polymorphism					
			Genotypes			HWE	Alleles	
			222 (-/-l)	241/222 (+/-)	241 (+/+)	p - value	222	241
AML patients (No. =62)	Observed	No.	22	25	15	0.149	69	55
		%	35.48	40.32	24.19		55.65	44.35
	Expected	No.	19.2	30.6	12.2		Not estimated	
		%	30.96	49.36	19.67			
Controls (No. = 65)	Observed	No.	21	29	15	0.42	71	59
		%	32.3	44.61	23.07		54.62%	45.38%
	Expected	No.	19.39	32.22	13.39		Not estimated	
		%	29.83	49.57	20.6			

Association of CASP8 with CML and AML risk: In the current study we used a quick, reproducible, and inexpensive bi-PASA method for 6N del Caspase-8 (rs 3834129) detection (12) . In this method, a non-toxic and easy to prepare gel electrophoresis is utilized to isolate the PCR products on agarose gel electrophoresis. To date, Caspase8 −652 6N ins / del has been evaluated using a variety of methods such as Mass ARRAY system , PCR-RFLP, high-performance ml liquid chromatography and direct sequencing that involve sophisticated laboratory equipment cheap and simultaneously amplifies both wild-type and mutant alleles in a single PCR tube, together with a control fragment(2,18,20,29) No special equipment is needed except a standard PCR thermocycler. Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) is a proven single nucleotide polymorphism (SNP) detection technique (12). This technique was ustilized to detect 6N deletion of Caspase-8 by setting bi-PASA. All-specific PCR primers are prerequisites for both point mutation and deletion detection to directly discriminate between wild type and mutant allele. In the current study we investigated the effect of Caspase8 −652 6N ins / del polymorphism on CML and AML risk (figure 2). The genotypic frequencies of Caspase-8 (rs3834129) followed the HWE in both CML and AML patients (p =0.095 and 0.960), respectively and healthy individuals (P =0.715) tables (3b and 4b). Our data revealed that CML patients were significantly higher than the controls ($p < 0.05$) in homozygous forms (ins / ins) and (del / del) ($p = 0.004$ and 0.00) respectively. This finding underlines the risk role of the homozygous ins / ins as a genotype that predisposes against CML (OR 3.06 , 95%CI: 1.47-6.40) while heterozygous

ins/del and homozygous del/del have a protective role to CML in a sample of Iraqi population (OR 0.97 , 95%CI: 0.49-1.92 and OR 0.08 , 95%CI: 0.02-0.36) respectively as shown in table (3a). In addition, there was a role of Caspase 8 −652 6N genotypes and AML patients as shown in table (4a). AML patients were significantly higher than the controls ($p < 0.05$) in homozygous form (del / del) ($p = 0.084$). This finding underlines the role of the homozygous del / del and heterozygous ins/del as a genotypes have a protective role to AML in a sample of Iraqi population (OR 0.44 , 95%CI: 0.18-1.07 and OR 0.96 , 95%CI: 0.48- 1.92) respectively, while and homozygous ins/ins has risk role to AML patients (P = 0.00 , OR 1.93 , 95%CI: 0.91-4.12). This finding had came in agreement with some researchers who also stated that the Caspase8−652 6N ins / del and del/del variants were not associated with the risk of multiple cancers and have a protective effect including oesophageal squamous cell carcinoma (ESCC) (27), colorectal cancer (CRC) (26), prostate cancer (9), and cervical cancer

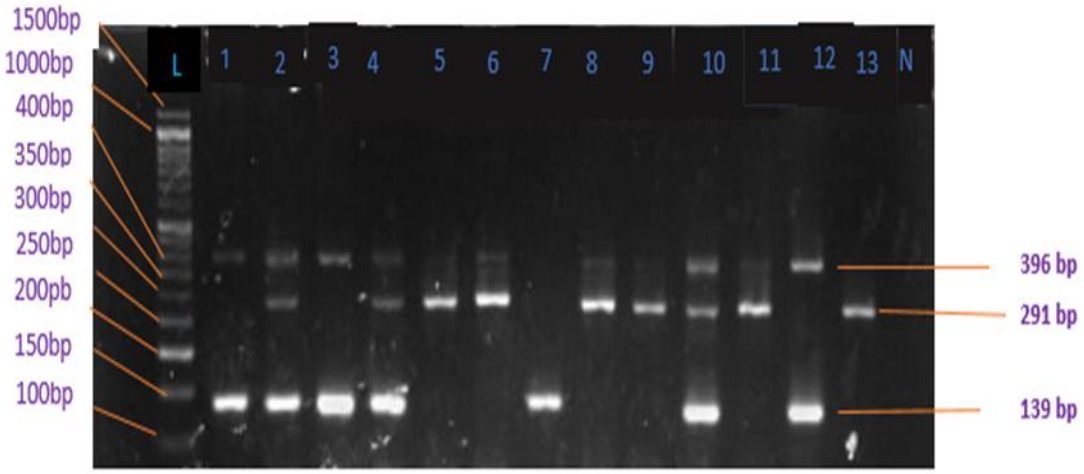

Figure 2: detection of caspase 8 (rs3834129) polymorphism by bi-PASA on 1.5% agarose gel. Gel viewed under Gel documentation - UV trans-illuminator after being stained with ethidium bromide stain. (L) 100 bp DNA ladder, (2,4,10) heterozygote genotype insertion/deletion (ins/del) (139 and 239 bp), (5,6,,8,9,11,13) homozygote genotype deletion/deletion (del/del) (239bp), (1,3,7,12) homozygote genotype insertion/insertion (ins/ins) (139 bp) and N: negative control.

Table 3a. Observed numbers and percentage frequencies of in CASP8 −652 6N ins/del gene in CML patients and controls

Caspase 8 Genotypes		Patients (No.=66)		Controls (No.=65)		Odd Ratio	95% C.I.	p-value
		No. 66	%	No. 65	%			
ins/ins (139/139)		33	50	16	24.61	3.06	1.47-6.40	0.004
ins/del (139/291)		31	46.96	31	47.69	0.97	0.49-1.92	1.00
del/del (291/291)		2	3.03	18	27.69	0.08	0.02-0.36	0.00
Total		66	100.0	65	100.0	-	-	-
Allele frequency	*ins*	97	73.48	63	48.46	2.95	1.76-4.93	0.000
	del	35	26.51	67	51.53	0.34	0.20_0.57	0.00

Table 3b. numbers and percentage frequencies (observed and expected) of Caspase-8−652 6N ins/del genotypes and their Hardy-Weinberg equilibrium (HWE) in CML patients compared with control groups

Groups			Caspase 8genotypes polymorphism					
			Genotypes			HWE	Alleles	
			ins/ins 139/139	ins/del 139/291	del/del 291/291	p - value	*ins* 139	*del* 291
CML patients (No. =66)	Observed	No.	33	31	2	0.095	97	35
		%	50	46.96	3.03		73.48%	26.52%
	Expected	No.	35.64	25.72	4.64		Not estimated	
		%	54	38.97	7.03			
Controls (No. =65)	Observed	No.	16	31	18	0.715	63	67
		%	24.61	47.69	27.69		48.46	51.54
	Expected	No.	15.27	32.47	17.27		Not estimated	
		%	23.49	49.95	26.56			

Table 4a. Observed numbers and percentage frequencies of in Caspase8 −652 6N ins/del gene in AML patients and controls

Caspase 8 Genotypes		Patients (No.=62)		Controls (No.=65)		Odd Ratio	95% C.I.	p-value
		No. 62	%	No. 65	%			
ins/ins (139/139)		24	38.7	16	24.61	1.93	0.91-4.12	0.126
ins/del (139/291)		29	46.77	31	47.69	0.96	0.48-1.92	1
del/del (291/291)		9	14.51	18	27.69	0.44	0.18-1.07	0.084
Total		62	100.0	65	100.0	-	-	-
Allele frequency	*ins*	77	62.09	63	48.46	1.74	1.03-2.96	0.032
	del	47	37.9	67	51.53	1.74	0.35-0.95	0.032

Table 4b. Numbers and percentage frequencies (observed and expected) Caspase8−652 6N ins/del genotypes and their Hardy-Weinberg equilibrium (HWE) in AML patients compared with control groups

Groups			Caspase 8 genotypes polymorphism					
			Genotypes			HWE	Alleles	
			ins/ins 139/139	ins/del 139/291	del/del 291/291	p - value	139	291
AML patients (No. =62)	Observed	No.	24	29	9		77	47
		%	38.7	46.77	14.51		%62.1	37.9%
	Expected	No.	23.91	29.19	8.91	0.960	Not estimated	
		%	38.56	47.07	14.37			
Controls (No. =65)	Observed	No.	16	31	18	0.715	63	67
		%	24.61	47.69	27.69		48.46	51.54
	Expected	No.	15.27	32.47	17.27		Not estimated	
		%	23.49	49.95	26.56			

In the FAS / FASL-mediated apoptosis pathway, Caspase 8, a cysteine peptidase can cause various cellular proteases or proteins, leading to apoptosis (16). On Caspase-8 at least 168 single nucleotide polymorphisms (SNP) were identified, mostly rare or non-functional (12). In comparison, the −652 6N del variant in the Caspase-8 promoter region has been found to cause reduced levels of Caspase-8 expression in T lymphocytes, thus influencing cancer risk through dysregulation of T lymphocyte activation-induced cell death (AICD), Which are active in immune responses to anti-tumors. T lymphocytes with the variant −652 6N del had lower Caspase-8 activity that suppresses apoptosis in T lymphocytes, leading to a decrease in the number of transformed cells that bypass the immune response to the anti-tumour, resulting in cancer defence (29). The results of this study suggest that Caspase9-293 is associated with increased risk of CML in case of homozygous of deletion, and homozygous of deletion and insertion in case of AML while in Caspase8-652 6N insertion polymorphism are associated with increased risk of both

CML and AML in a sample of Iraqi populations.

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