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Association of PTEN (*rs/1234213 rs/1234220*) Gene Polymorphisms with Liver Cancer Risk in Iraqi Patients

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ABSTRACT

Phosphatase and tensin homolog (PTEN) is a protein that doing on the cancer suppressor by dephosphorylating the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate that supervision a different of biological path way including cell proliferation, migration, and death [1]. The motive of this find out about to decide the relationships between the polymorphism of PTEN gene and threat of liver cancer by way of use single-tube method that collects the tetra-primer ARMS PCR experiment, SYBR inexperienced –with Real-time PCR, and melting point check with particular primer to locate the single nucleotide polymorphisms (SNP) of the gene. A pattern used to be performed= (65n) samples included 15 controls and 50 sufferers from Iraqi patient. It was observed that *PTEN* (***rs/1234213 rs/1234220***) gene have three genotypes .It was also noticed that The three genotype (GG, GA and AA) of *PTEN* rs1234213 and CC, TT genotype of *PTEN* rs1234220 may lead to decrease the risk of infection (OR=0.261, 0.261 and 0.00) (0.261 and 0.784) in Respectively.at the same time was discovered a statistical significance between CT and TT polymorphisms of *PTEN* rs1234220 with risk of liver cancer. The study also showed that the number of allele G of *PTEN* rs1234213 is higher than the allele A for both groups, while the genotype of *PTEN* rs1234220 was showed that the allele T is highest percentage in control and patient group from allele C.

Keywords: Meta-analysis - PTEN - liver cancer

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INTRODUCTION

Liver cancer is one of the major reason of cancer-linked death in the world; actually, this malignancy is the second most combined reason of cancer-linked death and its happening and mortality average are increasing [2-3]. And hepatocellular tumor accounts for 75% of liver tumor status [4]. Surgical intervention is a common treatment for liver cancer [5]. But at present the emphasis has been on genetic therapies and the use of advanced treatments [6]. Studies have confirmed relationship between the expression levels of the *PTEN* gene and the existence of the liver tumor [7], Phosphatase and tensin homolog (PTEN) is a protein that works on the tumor suppressor by dephosphorylating the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate that supervision a different of biological path way including cell proliferation, migration, and death[8]. *PTEN* was originally identified as abnormal growth of tissue suppressor frequently absent from a part of chromosome 10q23 in a diversity of human cancer [9-10].

This study was designed in order to determine the expression levels of *PTEN* genes in several samples from patients with liver tumor in order to find any relationships between these levels and the causes of the liver cancer in the patients.

METHODS

Registered in this study were 50 patients with liver cancer were collected during the period of January-2017 to December-2017 from the Al– Amal National Hospital for Cancer Management – Baghdad. With an average age of 48.5±18.2 years and divided on 13 stage I-II and 37 stage III-IV cases. Blood samples were collected from patients and control groups. Two and half milliliter of blood collected in EDTA anti-coagulant tubes that used for DNA extraction. Additionally, Genomic DNA was extracted from whole frozen blood using Relia™ Blood gDNAMiniprep System (Promega USA). And depending on manufacturer's instructions.

Quantification of gene expression was performed with the Real time thermo cycler type (Exicycler ™ 96). A Specific primers Tab(1)(2) were dissolved in sterile distilled water to give a final concentration of (10pmol/ µl) as recommended by provider, a working solution of 10 pmol/ µl was prepared. These primers are commercially available. Each reaction was performed in duplicate and determined by the following program: 7minutes of initial denaturation at 95°C , followed by denaturation of 35 cycle at 95 °C for 45 seconds , annealing temperatures at 60°C for 60 seconds with 40 cycles ,extension at 72°C for 60 seconds with 30 cycles and a ultimate extension at 72°C for 7 minutes , Melting curves have been fashioned by means of lowered the temperature to 65°C and last growing the heat by way of 0.2°C / s to 98 °C, and calculated the Tm values from the terrible derivation of fluorescence versus temperature (-dF/dT) of the melting curve for amplification merchandise calculated at 530 nm.

Table (1). PTEN (rs1234213) Primers and annealing temperatures.

Polymorphim	primers 5'-3'	Annealing Tempresure
PTEN Rs1234213	Inner primer Forward 5- TAACTTACTAGTTACTAACTTGGGCCAA….3- Reverse Primer: 5- ATTGAGAGTCAAAAAGACTGGGTCAC……3- Outer Primer Forward: 5- AAATCACTTATTTGAACCTGTTTCCTTT …..3- Outer primer Reveres: 5- ACTGATAAAGTTTGAGATGTTACCCAAG……3-	58°C

Table (2). Primers and annealing temperatures.

Polymorphim	primers	Annealing
	5'-3'	Tempresure
PTEN	Inner primer Forward	58°C
Rs1234220	5- GCATCCTTGCTAGTAATTTCATACCAAC….3-	
	Reverse Primer:	
	5- AGTCCTCTTTTTCCTGTTTTTCATGTAAA…..3-	
	Outer Primer Forward:	
	5- TGTTTATTTTCTGTTGTGTTGTCCTGAG…3-	
	Outer primer Reveres:	
	5- CATTGGTTTCTAACTGGTCAGACCTAAA…..3-	

Distribution case of genotypes and alleles of PTEN gene rs1234213and rs1234220, polymorphisms in the case group and the control group are shown in Table (3), (4).

The three genotype (GG, GA and AA) of *PTEN* rs1234213 and CC, TT genotype of *PTEN* rs1234220 may lead to decrease the risk of infection (OR=0.261, 0.261 and 0.00) (0.261 and 0.784)in Respectively.at the same time was discovered a statistical significance between CT and TT polymorphisms of *PTEN* rs1234220 with risk of liver cancer.

The study also showed that the number of allele G of *PTEN* rs1234213 is higher than the allele A for both groups, while the genotype of *PTEN* rs1234220 was showed that the allele T is highest percentage in control and patient group from allele C

Table 3. Distribution of sample study according to *rs/1234213* gene in patients and control

Genotype	Patients		Healthy (Control)		Chi. Square	O.R.
	No.50	%	No.15	%		
GG	42	84.00	13	86.67	0.539	0.261
GA	8	16.00	2	13.33	0.539	0.261
AA	0	0.00	0	0.00	0.00	0.00
Total	50	100%	15	100%	---	---
Chi-Square: χ^2	---	13.422 **	---	13.759 **	---	---
Allele frequency						
G	0.52		0.93		----	
A	0.48		0.07			

** (P<0.01), NS: Non-significant

Table 4. Distribution of sample study according to *rs/1234220* gene in patients and control

Genotype	Patients		Healthy (Control)		Chi. Square	O.R.
	No.50	%	No.15	%		
CC	2	4.00	1	6.67	0.539 NS	0.261
CT	12	24.00	1	6.67	6.027 **	1.052
TT	36	72.00	13	86.67	5.266 *	0.784
Total	50	100%	15	100%	---	---
Chi-Square: χ^2	---	11.961 **	---	13.644 **	---	---
Allele frequency						
C	0.16		0.10			
T	0.84		0.90		----	

* (P<0.05), ** (P<0.01), NS: Non-significant.

The second step of the melting temperature (Tm) analysis is use to analysis the variations. The nature of the PCR product has been changed by arise in temperature and as a result is emitted fluorescence emission, while in the first step can be shown dissociation curve. Which depends on the length of the amplicon can determine which temperatures.

In Fig. 1(C), G and A alleles of rs1234213 were distinguished by their Tms, 80 ± 0.2°C and 78 ± 0.2°C, respectively, Similarly, Tms of C and T alleles of rs1234220 (Fig. 2), were found to be 81.5± 0.1°C and 78 ± 0.1 °C, respectively.

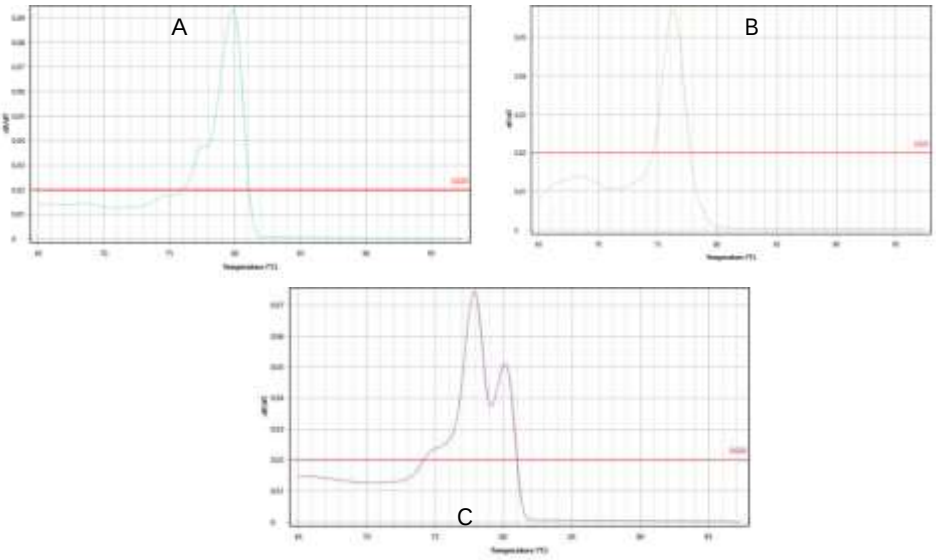

Figure (1) Real-time PCR assay for PTEN (rs1234213) genotyping. A: GG genotype, B: AA genotype, C: AG genotype

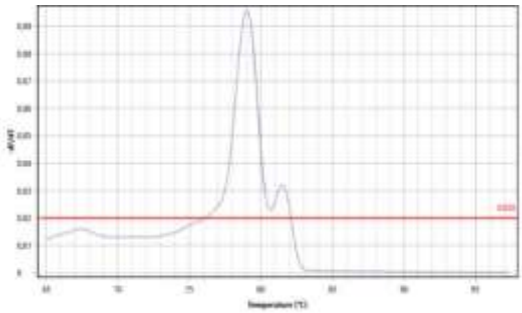

Figure (2) Real-time PCR assay for PTEN (rs1234220) genotyping. CT alleles.

In a genetically modified organism examination process , SYBR®Green qPCR put a quantity of characteristic during another fluorescence-based PCR methods: (1) SYBR®Green qPCR observe the augmentation in synoptic fluorescence during the amplification, let to evaluation the presence of non-specific amplification, (2) the test of melting temperature let post-PCR matching of the amplification not only of the foreseeable target but too

scoring the existence of carefully concerning target(s), (3) This method is economical compared to others.

Recently, many studies have shown that relationships between *PTEN* and malignant tumors, including esophageal squamous cell carcinoma, myeloid cancer,[11] ,breast tumors [12], prostate,[13] and liver cancers[6]. However, The *PTEN* gene product is effective in the regulation of cellular oxidative stresses and apoptosis [14] some studies have noticed that *PTEN* gene is possible to use as a marker for the evaluation of survival [15].

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