AMERICAN JOURNAL OF RESEARCH IN MEDICAL SCIENCES, 2019
VOL 5, NO. 2, PAGE 21–26
10.5455/ajrms.20190508095542

ORIGINAL RESEARCH

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Study of single-nucleotide polymorphism of phosphatase and tensin homolog in patients with hepatocellular carcinoma and cirrhosis associated with chronic hepatitis C virus

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ABSTRACT

Background: Phosphatase and tensin homolog (*PTEN*) is known as anti-oncogene that is located on chromosome 10q23.3. This gene has high mutations frequency in different human cancer. We investigated the prevalence of single-nucleotide polymorphisms (SNP) in the *PTEN* gene rs2299939 in patients with hepatocellular carcinoma (HCC) and cirrhosis associated with hepatitis C.

Material and Method: The study included 25 patients with HCC and 25 patients with cirrhosis. All patients had chronic viral hepatitis C genotype 4. Molecular study of SNP of PTEN rs2299939 polymorphism was carried out by restriction fragment length polymorphism and polymerase chain reaction.

Results: There was a statistically significant increase in GG genotype OR 2.2 (95% CI, 0.8–5.6) in patients with cirrhosis and in patients with HCC compared to the control group (p = 0.0001). There was a significant increase in GT genotype OR 1.9 (0.6–5.8) and TT genotype OR 0.7 (0.5–1.1) in control compared to patients with cirrhosis and with HCC associated with hepatitis C virus (HCV) (p = 0.0001). The frequency of G alleles had a statistically significant prevalence in patients compared to control (p = 0.0001) while T alleles had a statistically significant increase in control compared to the patients (p = 0.0001).

Conclusion: The GG genotype of *PTEN* gene rs2299939 showed a significant increase in patients with HCC and cirrhosis associated with HCV genotype 4. On the other hand, the TT and GT genotypes may be associated with the decreased risk of the development of HCC. Further studies are required to validate these findings.

ARTICLE HISTORY

Received May 08, 2019
Accepted September 17, 2019
Published October 13, 2019

KEYWORDS

PTEN; rs2299939 polymorphisms; hepatocellular carcinoma; hepatitis C; cirrhosis

Introduction

Hepatocellular carcinoma (HCC) is the fifth common malignancy worldwide affecting 1 million patients each year [1]. In Egypt, it is the most common malignancy of all liver malignancies and represents 4.7% of chronic liver disease [2,3]. The etiology of HCC includes chronic viral hepatitis C and B viruses, alcohol use, and aflatoxins exposure [4]. Besides these factors, human genetic predisposition plays an important role through abnormal gene expression, epigenetic changes, and abnormal chromosomal aberrations [5].

Affection of cellular signals pathway that controls cell proliferation, survival, activity, and adjustment of its functions may be involved in the development of different types of cancer. The development of HCC includes the interaction between different factors that lead to the imbalance between the proto-oncogenes and anti-oncogenes. The previous studies summarized the consequences that lead to HCC where environmental factors such as viral hepatitis B and C lead to proto-oncogene activation, anti-oncogene inactivation [6]. This imbalance leads to an overgrowth of the mutant cells

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with clonal expansion and the development of cancer which occurs with the loss of the anti-oncogene functions.

The common anti-oncogenes include *P53* and phosphatase and tensin homolog (*PTEN*). Both genes have high mutations frequency in different human cancer [5]. *PTEN* gene which has a total length of 200 kb is located on chromosome 10q23.3. The gene consists of nine exons and eight introns, and encodes a protein composed of 403 amino acid residues [7–9]. The protein encoded by the *PTEN* gene has activities of protein phosphatase and lipid phosphatase. The functions of this gene include regulation of the normal physiological cell differentiation as well as the pathological process of tumors as it is involved in the differentiation, invasion, metastasis, and prognosis of tumors [10–13]. The mutations of the *PTEN* gene have been associated with the development of different tumors. However, there are few studies about the association of mutations of the *PTEN* gene and liver cancer.

Material and Method

The study was a case control study. Twenty-five patients with HCC associated with chronic hepatitis C genotype 4 were included in the study. In addition, 25 patients with cirrhosis associated with chronic hepatitis C virus genotype 4 were included. The patients were recruited from Mansoura University Hospital from January 2018 till December 2018. The patients with HCC were recently diagnosed according to clinical, radiological, and pathological examination. They were classified according to the Barcelona classification of HCC (BCLC) [14]. The patients with cirrhosis were diagnosed by clinical and radiological investigations. The study excluded patients with HCC due to hepatitis B or other malignancies of the liver. Moreover, patients with cirrhosis due to etiology other than hepatitis C virus (HCV) were excluded, including patients with hepatitis B virus and patients with autoimmune diseases. In addition to patients, 25 healthy control subjects with normal liver function and negative for hepatitis viruses B and C were recruited as a control group. The control group was selected within the same age and sex distribution.

Ethical statement

The Institutional Review Board of Mansoura Faculty of Medicine approved this study protocol (IRB NO: R.19.04.486) and informed consent was obtained from all participants.

Laboratory investigation

Patients selection was done by performing HCV antibodies by immunoassay with the use of the Elecsys system (Roche-diagnostic). They had been genotyped previously for HCV by using the Accupower RT-PCR premix kit (Bioneer, Korea). Hepatitis B was excluded by performing HBsAg by ELISA kit (Human Biosource kit). Ten-milliliter blood sample was obtained from each subject and divided into two aliquots with ethylene-diamine-tetra acetic acid (EDTA) and one aliquot without anticoagulant for sera separation. The separated serum samples were subjected to the full biochemical study of liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin by autoanalyzer Dialab 40, and alpha fetoprotein (AFP) determination by enzyme immunoassay by (ELISA—DRG International Inc., USA.). Complete blood counts were determined by Sysmex system from blood samples with EDTA anticoagulant. DNA was extracted from blood samples with EDTA for molecular study.

Molecular study of single-nucleotide polymorphisms (SNP) of PTEN gene

DNA extraction

DNA was extracted from the whole blood by the use of Qiagen extraction mini\Kit (Qiagen-Germany). The extracted DNA was kept frozen at −20ºC for further study by polymerase chain reaction (PCR).

Genotyping with PCR-restriction fragment length polymorphism.

The primers used for the amplification of rs2299939 are listed in Table 1.

The amplification premix was supplied from Qiagen (Qiagen-Germany). The total amplification volume was 20 µl including 2.0 µl 10× PCR/ buffer liquid, 1.5 µl $MgCl_2$, 1.0 µl dNTP, 0.5 µl forward primer, 0.5 µl reverse primer, 0.5 µl Taq DNA polymerase, 2.0 µl template DNA, and the rest was filled with sterile water. PCR reaction condition included the following cycles, predenaturation at 95°C for 6 minutes; denaturation at 94°C for 50 seconds, annealing at 56°C for 50 seconds, and extension at 72°C for 50 seconds (35 cycles); and extension at 70°C for 5 minutes. The amplification products were subjected to gel electrophoresis in 2% agarose gel for 30 minutes and the gel was stained with ethidium bromide and the bands were visualized by UV light.

Restriction endonuclease Nco I enzyme was applied to digest the PCR Products (5 µl) according

Table 1. The sequences of the primers, endonuclease enzyme, and the base pair (bp) of the products.

	Sequences of the primers	Bp	Endonuclease encymes	bp
PTEN	5'-CTCAAACTCCTGACCTCGGG-3'	277	Nico I	
	5'-TCACCCGAGGTCAGGAGTTT-3'		GG	87
			GT	107, 87
			TT	107

to the manufacturer's instruction (Thermofisher-USA). Agarose gel electrophoresis (2%) was carried out at 90 V for 30 minutes, with the running buffer being 1× Tris acetic acid and EDTA [15].

Statistical analysis

The data were analyzed by SPSS 22. The qualitative data were expressed as number and percentage and the quantitative data were expressed as mean ± SD. The comparison was carried out by the use of chi-square for qualitative data and by a Nova test and Mann Whitney for non-parametric data. The *p* was significant if it was <0.05.

Results

The study included three groups, normal control subjects, patients with cirrhosis associated with HCV genotype 4, and patients with HCC associated with HCV genotype 4. The basic demographic, clinical, and laboratory data of the groups were summarized in Table 2. There was statistically significant in Child-Pugh classification B among patients with HCC and Child-Pugh classification C in patients with cirrhosis ($p = 0.0001$). Barcelona classification of HCC revealed that the high frequency of the patients was in BCLC grade B followed by A. The size of the tumor was > 5 cm in 18 patients with HCC. There was the statistically significant elevation of AST, total bilirubin, direct bilirubin, albumin, AFP ($p = 0.0001$) ALT ($p = 0.03$) in patients groups compared to the control group with a significant reduction in the platelets counts ($p = 0.0001$) (Table 2).

There was a statistically significant increase in GG genotype OR 2.2 (95% CI, 0.8–5.6) in patients with chronic hepatitis C with cirrhosis and in patients with HCC associated with HCV genotype 4 compared to the control group ($p = 0.0001$). On the other hand, there was statistically significant increase in GT genotype with OR 1.9 (0.6–5.8) and TT genotype OR 0.7 (0.5–1.1) in healthy control compared to patients with chronic hepatitis C with cirrhosis and in patients with HCC associated with HCV ($p = 0.0001$) (Table 3).

There was no statistically significant prevalence of any genotypes in relation to BCLs classification in HCC patients or in relation to child classification in both HCC and cirrhosis (Tables 4 and 5).

Discussion

Egypt has the highest prevalence rate of HCV in the world. Hepatitis C causes chronic hepatitis infection in 60% up to 80% of the affected patients and cirrhosis in 10%–20% of the infected patients over the years [16].

There are studies that have shown that affection of *PTEN* gene through deletion or polymorphism leads to the low expression of its anti-oncogenic activity which is associated with the development of liver cancer. It has been proved that the *PTEN* gene plays an important role in the occurrence and development of liver cancer [17,18].

In the present study, the GT genotype and TT genotype appear to have protective effects from the development of HCC and cirrhosis as these are the mean genetics forms in the healthy control with a significant reduction in the patients with cirrhosis and HCC (Table 3). This was similar to the finding of the previous study [15 ,19]. The unique finding of the present study was the significant prevalence of GG genotype among patients with HCC (OR 2.2, 95% CI) ($p = 0.0001$) and cirrhosis compared to healthy control. This might reflect that these SNPs in the *PTEN* gene might be involved in the occurrence and development process of liver cancer. Furthermore, G haplotype has a significantly higher prevalence in patients with HCC and cirrhosis, a finding that suggests that this haplotype may be associated with the reduction of *PTEN* gene activity. The high frequency of the genotype GG and G alleles in cirrhosis patients may reflect the predisposition of this genotype with the process of cirrhosis, a finding that needs an extensive study with a large group of patients to be verified. Previous studies in

Table 2. Comparison of demographic, clinical and laboratory findings among the studied groups.

	Control group (*n* = 25)	Patients with cirrhosis (*n* = 25)	Patients with HCC (*n* = 25)	*p*
Age (years)	51.1 ± 6.0	52.2 ± 5.2	50.2 ± 5.9	*p* = 0.8
Gender: (No.-%)				
Male	18 72%	18 72%	18 72%	
Female	7 18%	7 18%	7 18%	
Child-Pugh classification				
A (No- %)		3 12%	3 12%	*p* = 0.0001
B (No- %)		5 20%	12 48%	
C (No- %)		17 68%	10 40%	
Barcelona classification				
O			3 12%	
A			5 20%	
B			13 52%	
C			2 8%	
D			2 8%	
Size of the tumor			7 28%	
< 5 cm			18 72%	
>5 cm				
HB (gm/dl)	13.1 ± 1.9	12.1 ± 0.2	11.0± 0.3	*p* = 0.1
WBCs × 10^3/µl	7.6 ± 2.1	4.4 ± 1.5	7.8± 1.5	*p* = 0.003
Platelets ×10^3/µl	221.5 ± 59.2	80.6 ± 37.3	146.8 ± 83.4	*p* = 0.0001
Total bilirubin (µmol/l)	13.6 ± 1.87	73.1 ± 25.5	39.1 ± 11.9	*p* = 0.0001
Direct bilirubin (µmol/l)	5.1 ± 1.7	39.1 ± 11.9	18.7 ± 6.8	*p* = 0.0001
AST (IU/l)	29.3 ± 4.8	66.2 ± 16.3	109.6 ± 11.7	*p* = 0.0001
ALT (IU/l)	28.9 ± 5.2	55.2 ± 18.1	85.2 ± 23.2	*p* = 0.03
Albumin (g/dl)	3.9 ± 0.5	2.9 ± 0.4	3.6 ± 0.5	*p* = 0.0001
AFP (µg/l) mean± SD	5.8 ± 2.2	12.5 ± 0.2	413 ± 110	*p* = 0.0001
Minimum				
Maximum				

mice had revealed that the loss of the *PTEN* function was associated with massive hepatomegaly and steatohepatitis with triglyceride accumulation [20].

On the other hand, there was a statistically significant increase in GT genotype with OR 1.9 and TT genotype OR 0.7 in healthy control compared to patients with chronic hepatitis C with cirrhosis and in patients with HCC associated with HCV ($p = 0.0001$) (Table 3). This finding was in agreement with Li et al. [15] who concluded that

the GT genotype 0f rs2299939 probably decrease the risk of hepatoma (OR = 0.483).

The development of HCC occurs due to the interaction between predisposing genetic factors and infections or other environmental risk factors [21,22]. The genetic factors include inactivation of tumor suppressor gene and activation of the proto-oncogene, epigenetic alteration, genomic instability, chromosome gain and deletion [23,24] of *PTEN* gene with BCLC in HCC or with Child-Pugh classification in patients with cirrhosis and HCC (Tables 4 and 5). Similar result was identified in a previous study that reported that *PTEN* mutation has no association with BCLC classification [25].

Table 3. Distribution of genotypes of rs2299939 polymorphism among the studied groups.

	Control group (*n* = 25)		Patients with cirrhosis (*n* = 25)		Patients with HCC (*n* = 25)		*p*
GG OR* (CI#-95%) 2.2 (0.8-5.6)	12	48%	20	80%	21	84%	*p* = 0.0001
GT OR* (CI#-95%) 1.9 (0.6-5.8)	3	12%	2	8%	1	4%	*p* = 0.0001
TT OR* (CI#-95%) 0.7 (0.5-1.1)	10	40%	3	12%	3	12%	*p* = 0.0001
G	27		42		43		*p* = 0.0001
T	23		8		7		*p* = 0.0001

*OR = Odd Ratio, #CI = Confidence Interval. The frequency of G alleles had statistically significant prevalence in patients compared to control (*p* = 0.0001) while T alleles had a statistically significant increase in control subjects compared to the patients (*p* = 0.0001)

Table 4. Distribution of genotypes of rs2299939 polymorphism among patients with HCC according to BCLC.

Genotypes	BCLC										Total	
	0		A		B		C		D			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
GG	1	4.8%	5	23.3%	11	52.4%	2	9.5%	2	9.5%	21	100%
GT	1	100%									1	100%
TT					1	33.3%	1	33.3%	1	33.3%	3	100%

p = 0.4

Table 5. Distribution of genotypes of rs2299939 polymorphism among patients with HCC and cirrhosis according to Child-Pugh classifications.

Genotype		Child-Pugh Classification						Total	
		A		B		C			
PTEN	GG	11	26.8%	12	29.3%	18	43.9%	41	100%
	GT	1	33.3%	0	0%	2	66.6%	3	100%
	TT	2	33.3%	2	33.3%	2	33.3%	6	100%
Total		14	28%	14	28%	22	44%	50	100%

p = 0.8

The study of the genes associated with susceptibility to HCC and cirrhosis can facilitate the understanding of the pathogenesis of these conditions and in the management of them. These conditions may include the interaction of various genes; therefore, there is a requirement for further studies that involve multiple genes.

The present study highlights the significant increase in the prevalence of GG genotype of *PTEN* gene rs2299939 in patients with hepatocellular carcinoma that may denote that this genotype is associated with the development of HCC associated with HCV genotype 4. Moreover, the same genotype is associated with cirrhosis. On the other hand, the TT and GT genotypes may be associated with a decrease in the risk of the development of HCC. The limits of the present study are the small number of the included subjects and more genes should be studied and linked with the environmental factors. Further studies can be deepened to validate these findings.

Conflicts of interest

There is no conflict of interest for any of the authors

Disclosure

This work was not supported by any grants. No funding was received for this work from any organization or committee.

References

[1] Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009; 27(9):1485–91.

[2] Holah NS, El-Azab DS, Aiad HA, Sweed DM. Hepatocellular carcinoma in Egypt: epidemiological and histopathological properties. Menoufia Med J 2015; 28:718–24.

[3] Doss W, Shiha G, Hassany M, Soliman R, Fouad R, Khairy M, et al. Sofosbuvir plus ribavirin for treating Egyptian patients with hepatitis C genotype 4. J Hepatol 2015; 63:581–5.

[4] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003; 362(9399):1907–17.

[5] Zender L, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Cancer gene discovery in hepatocellular carcinoma. J Hepatol 2010; 52(6):921–9.

[6] Theodoropoulos G, Papaconstantinou I, Felekouras E, Nikiteas N, Karakitsos P, Panoussopoulos D, et al. Relation between common polymorphisms in genes related to inflammatory response and colorectal cancer. World J Gastroenterol 2006; 12:5037–43.

[7] Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997; 275:1943–7.

[8] Li DM, Sun H. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res 1997; 57:2124–9.

[9] Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997; 15:356–62.

[10] Hopkin K. A surprising function for the PTEN tumor suppressor. Science 1998; 282:1027, 1029–30.

[11] Hu TH, Wang CC, Huang CC, Chen CL, Hung CH, Chen CH, et al. Down-regulation of tumor suppressor gene PTEN, overexpression of p53, plus high proliferating cell nuclear antigen index predict poor patient outcome of hepatocellular carcinoma after resection. Oncol Rep 2007; 18:1417–26.

[12] Kong G, Zhang J, Zhang S, Shan C, Ye L, Zhang X. Upregulated microRNA-29a by hepatitis B virus X protein enhances hepatoma cell migration by targeting PTEN in cell culture model. PLoS One 2011; 6:e19518.

[13] Vinciguerra M, Foti M. PTEN at the crossroad of metabolic diseases and cancer in the liver. Ann Hepatol 2008; 7:192–9.

[14] EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma European Association for the Study of the Liver. J Hepatol 2018; 69:182–236.

[15] Li HG, Liu FF, Zhu HQ, Zhou X, Lu J, Chang H, Hu JH. Association of *PTEN* gene polymorphisms with liver cancer risk. Int J Clin Exp Pathol 2015; 8(11):15198–203. eCollection 2015.

[16] Westbrook RH, Dusheiko G. Natural history of hepatitis C. J Hepatol 2014; 61(1):S58–68.

[17] Valiente M, Andres-Pons A, Gomar B, Torres J, Gil A, Tapparel C, et al. Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. J Biol Chem 2005; 280:28936–43.

[18] Tang Y, Eng C. p53 down-regulates phosphatase and tensin homologue deleted on chromosome 10 protein stability partially through caspase-mediated degradation in cells with proteasome dysfunction. Cancer Res 2006; 66:6139–48.

[19] Du Y, Zhang YW, Pu R, Xue Han X, Jian-Ping H, Zhang H-W, et al. Phosphatase and tensin homologue genetic polymorphisms and their interactions with viral mutations on the risk of hepatocellular carcinoma. Chin Med J 2015; 128(8):1005–13.

[20] Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, et al. Hepatocyte-specific PTEN deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest 2004; 113(12):1774–83.

[21] Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1994; 3:3–10.

[22] Edenberg HJ, Xuei X, Wetherill LF, Bierut L, Bucholz K, Dick DM, et al. Association of NFKB1, which encodes a subunit of the transcription factor NF-kappaB, with alcohol dependence. Hum Mol Genet 2008; 17:963–70.

[23] Kirk GD, Bah E, Montesano R. Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from The Gambia, West Africa. Carcinogenesis 2006; 27:2070–82.

[24] Adjei AA, Hidalgo M. Intracellular signal transduction pathway proteins as targets for cancer therapy. J Clin Oncol 2005; 23:5386–403.

[25] Hou W, Liu J, Chen P, Wang H, Ye B C, Qiang F. Mutation analysis of key genes in RAS/RAF and PI3K/PTEN pathways in Chinese patients with hepatocellular carcinoma. Oncol Lett 2014; 8(3):1249–54.