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Evaluation of PRDM1 gene polymorphism with hepatitis C virus-related hepatocellular carcinoma in a cohort of Egyptian patients

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Abstract: The third most common cancer-related cause of death worldwide is hepatocellular carcinoma (HCC). and it is the sixth most common primary malignancy overall. Egypt had the hepatitis C virus's highest prevalence (HCV). A critical topic of study is the association between HCV and HCC. PR domain zinc finger protein 1 (PRDM1) functions as a tumor suppressor in B and T cells and is critical for plasma cell development and the exhaustion of T lymphocytes caused by cancer and chronic viral infections. **Aim:** This study aimed to compare Egyptian patients with HCC to a control group to determine the allele frequencies and genotype of the rs1010273 PRDM1 gene polymorphism. **Methods:** Included 200 Egyptian patients (100 HCC and 100 control). All participants underwent laboratory evaluations, comprising hepatitis markers (HBsAg, anti-HCV-Ab), liver function tests, serum alpha-fetoprotein, and complete blood count. Using the TaqMan allelic discrimination assay method, the PRDM1 rs1010273 was genotyped. **Results:** Unlike the healthy controls, the HCC patients had a greater frequency of the GA genotype (43% vs. 20%) and the A allele (26.5% vs. 10%). A significant difference was found between the GA and A allele of PRDM1genotype of HCC patients and controls (P<.001). **Conclusions**: A statistically significant and greater frequency of the A allele and GA genotype was found in HCC patients compared to controls, indicating a relationship between the PRDM1 AA+GA gene polymorphism and the HCC Egyptian population. The A allele was assumed to be a risk factor for HCC (OR=11.212). SNP (rs1010273G/A) may be an optional valuable marker.

Keywords: PRDM1; HCC; Gene Polymorphism

1. INTRODUCTION

A form of liver cancer affecting the liver cells is hepatocellular carcinoma (HCC). Over 80% of all primary hepatic malignancies are due to HCC, the most well-known kind of liver cancer [1]. Cancer represents the third most common cause of death, per the WHO [2]. In 2020, there were an estimated 830,000 cases worldwide, with 83% of those identified in less developed countries [3]. Hepatocellular carcinoma surveillance, diagnosis, and management have come a long way in the last decade, and early detection is critical for better results [4]. The most typical surveillance tests are liver ultrasonography and serum fetoprotein. However, their accuracy for detecting hepatocellular carcinoma in its early stages is not optimal [5]. Ultrasonography has 84% sensitivity in HCC early detection. However, a recent meta-analysis found that its sensitivity for identifying early disease is 47%. (95% confidence interval 33% to 61%). The sensitivity of surveillance tests is increased by 63% by serum fetoprotein (48 % to 75 %) [6]. Chronic infection with HCV is a predisposing factor to cirrhosis and chronic liver disease (CLD), which has been described as the most important precursor to Hepatocellular carcinoma (HCC) [7].

Despite significant advances in HCC treatment, for example, nonsurgical treatment and curative surgery, HCC still has a poor prognosis; most patients pass away within 6 to 20 months [8]. The effectiveness of treatment is dictated by a stage of HCC somewhere at the time of diagnosis. If detected early enough, HCC can be treated. When patients

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consult their doctors with symptoms, most HCCs are diagnosed at an advanced stage [9]. Sex-specific age cutoffs (40 years old for men and 50 years old for women), cirrhosis, and a family history of HCC should all be taken into account when considering HCC surveillance [10].

In Egypt, there is much interest in the relationship between HCV and HCC. First, there is proof that HCV and the emergence of HCC are related [11]. Second, with over 416,000 new infections each year, Egypt has a high rate of HCV transmission. Thirdly, the government's policy of screening and follow-up has led to an elevation in the cases of the two diseases [12]. According to the Global Cancer Observatory, liver cancer accounted for 19% of all recently diagnosed cases in 2018, affecting people of all ages and genders, with a 32% incidence rate and a 31% fatality rate. The severity of liver illness, cancer growth, malignant transformation, and predisposition to risk factors have all been linked to constitutional single-nucleotide polymorphisms (SNPs). Example of SNP in HCC, study indicates that functional polymorphism may contribute to the risk of hepatocellular carcinoma [13]. through case-control studies evaluating SNPs implicated in biological processes impacted during cancer development, SNPs related to inflammation (*Tumor necrosis factor* (TNFA), Interleukin 10 (*IL-10*), Interleukin 1 beta (IL1B), Transforming growth factor beta (TGFB), cell cycle, oxidative stress (*Glutathione S-transferase Mu 1* (GSTM1), Myeloperoxidase (MPO), Superoxide dismutase 2 (SOD2), iron metabolism (Human homeostatic iron regulator protein (HFE1), and DNA repair (Tumor protein (TP53), Mouse double minute 2 homolog (MDM2), X-ray repair cross complementing3 (XRCC3), Methylenetetrahydrofolate reductase (MTHFR) were discovered [14]. This indicated that some hereditary variables contribute to the development of HCC [15]. Through several repair pathways, DNA-repair genes significantly influence genomic integrity maintenance. A variety of DNA damage can be brought on by carcinogens or endogenous metabolic products. When the pace of damaged DNA outweighs the cell's ability to repair it, the cell cannot tolerate this damage, leading to cancer, apoptosis, or senescence [16]. Coordinated efforts between several centers and early diagnosis and management require a national campaign [17]. According to a recent study, tumor cells and many immune cells interact closely. T follicular helper cells, memory B cells, total B cells, and M1 macrophages heavily infiltrate HCC [18]. In addition, T cell exhaustion occurs in HCC and is associated with poor clinical outcomes [19]. The PRDM1 gene encodes a transcriptional repressor known as PR domain zinc finger protein 1 (PRDM1), often referred to as B lymphocyte-induced maturation protein 1 (BLIMP1). It influences B and T lymphocytes [20]. The promoter of PRDM1 is methylated in most studies using natural killer (NK) cell lines. As a result, it is believed that PRDM1 is a tumor suppressor gene [21]. In addition, it is essential to regulate T cell exhaustion throughout persistent viral infection and the development of various cancers, including HCC [22]. Accordingly, this study assessed the allelic discrimination of PRDM1 gene rs1010273 in developing HCC in Egyptian patients.

2. METHODS

Study population: The Ethics Committee of the Pharmacy Faculty of Al-Azhar University approved the study. Two study groups comprised a total of 200 Egyptians:

Group (A); 100 individuals with HCC-related HCV male = [68 (68%), female = 32 (32%)] aged from (36-81) years diagnosed by ultrasonography and contrast-enhanced triphasic CT; **Group (B);** As a healthy control group male = [66 (66%), female = 34 (34%)|] aged from aged from (31-77) years, 100 healthy Egyptian blood donors were enlisted. All participants were HCV-Ab positive, but HBs Ag negative. Antibodies to HCV and HBV were negative in all control subjects. The study excluded patients with positive HIV or hepatocellular injury, including any history of alcoholism, primary biliary cirrhosis, autoimmune hepatitis, and severe nonalcoholic liver diseases with metabolic syndrome. Clinical examination, laboratory testing, ultrasonography, and computed tomography were used for diagnosis.

Sample collection: Venipuncture was used to draw 10 milliliters of venous blood from each person's cubital vein in the manner described below: For complete blood count (CBC) and PRDM1 genotyping, 4 ml of blood samples were drawn into tubes containing EDTA. The remaining 6 milliliters were retained in a different vacutainer tube, and two milliliters were dispensed into a citrated tube for INR purposes (with no additives). It was centrifuged for 10 minutes at 3000 rpm after being hoisted for 15 minutes to induce clotting. The liver function, viral markers (HBsAg, anti-HCV-Ab), and AFP were then measured using aliquots of the serums. Each subject underwent the usual investigations: thorough history taking, abdominal ultrasonography, clinical examination, or computed tomography. The following laboratory examinations were carried out using an automated biochemistry analyzer: serum aspartate transaminase (AST), serum bilirubin (mol/L), CBC, serum alanine transaminase (ALT) (IU/L) [23], serum albumin (g/L), serum alkaline phosphatase (ALP), creatinine, and blood glucose. KCL equipment determined the international

normalized ratio (INR). Additionally, viral markers (HBsAg, HCV-Ab) and serum alpha-fetoprotein (AFP) were determined using ELISA as per the manufacturer's instructions [24, 25], as well as genotyping of the PRDM1 A/G SNP (rs1010273) gene polymorphism.

DNA extraction and genotyping for PRDM1 rs1010273: The sample in EDTA-containing tubes was used for DNA extraction using a DNA extraction kit. Then, it was kept at -80°C in aliquots until required and was processed employing the Gene Direx®, Catalog No. SN023-0100. By identifying a single nucleic acid sequence variation, the TaqMan method was utilized to genotype the PRDM1 rs1010273 gene. It was possible to distinguish between the two likely variants at the polymorphism position in the target template sequence using two primer or probe pairs for each reaction [20]. The target sequence's length was unknown, but the unidentified samples were arranged as follows:

1. Homozygotes (with allele 1 or allele 2).
2. Heterozygotes (with both allele 1 and allele 2).

The premixed PCR master mix involved Taq DNA polymerase, MgCl2, dNTPs, and reaction buffers at optimum concentrations. Five µl of extracted DNA was added to 10 µl of master mix, 4 µl of nuclease-free water, and 1 µl of each dissolved primer. Utilizing a step-one plus real-time PCR equipment, complementary DNA (cDNA) amplification was performed (Applied Biosystems): After an initial phase of enzyme activation for 10 min at 95°C, 40 cycles of denaturation for 15 s at 95°C, annealing/collection at 60°C for 1 min, and a final extension step for 30 s at 60°C, it was held at 60°C for that time.

Statistical Methods: SPSS version 24 was utilized for data analysis (SPSS Inc., Chicago, IL). The exact test was used to identify each group's Hardy-The Weinberg equilibrium (HWE).

The number and percentage were employed to depict qualitative data, whereas the median, range or mean, and standard deviation were used to represent numerical data. The association between qualitative variables was evaluated using the chi-square (Fisher's exact) test. In order to find independent predictive factors and exclude the influence of confounding variables, a multivariate analysis of variables that were statistically significant on the univariate level was carried out utilizing a logistic regression model. Statistical significance was a p-value≤ 0.05. A two-tailed design was adopted for all testing.

3. RESULTS

Table (1) showed the demographic information for the groups under study. In addition, this table contains a comparison between the HCC group and control in demographic data. A significant difference was determined between age and body mass index (BMI) (P< 0.001).

Table 1: Comparison between the studied groups regarding to the demographic data.

Parameters	Studied groups		*p*-value
	Control 100	HCC 100	
Age (years)			
≤57 No(%)	48 (48%)[b]	20 (20%)[a]	**<0.001***
>57 No(%)	52 (52%)[b]	80 (80%)[a]	
BMI (kg/m^2)			
≤25	66 (66%)	15 (15%)	**0.662**
>25	34 (34%)	85 (85%)	

HCC: Hepatocellular carcinoma; BMI: body mass index; *P*-value *greater than 0.05* is non-significant.

Allelic and genotype frequencies of PRDM1 rs1010273 polymorphism are represented in Table (2). The most common G allele and GG genotype were considered the references to compare the HCC group a. The frequencies of the G allele and the GG phenotype were both statistically significantly higher in the control group than in the HCC (90% vs. 73.3%, p<0.001, and 80% vs. 52%, p<0.001; respectively). However, the frequencies of the A allele and both the GA and AA genotypes were statistically significantly higher in HCC patients compared to controls (26.5% and 10%, respectively, with p-value< 0.001) and (48% and 20%, respectively, with p-value< 0.001).

Table (3) showed the results of a multivariate study (logistic regression model) to determine the relationship between the prognosis for HCC and the PRDM1 gene polymorphism. There was an association between PRDM1 gene polymorphism and the diameter of the tumor or tumor size (p-value=0.0001), with OR =11.212 95% CI (4.414-28.48).

Table 2: Comparison between the studied groups regarding PRDM1 allelic discrimination

PRDM1Gene polymorphism rs1010273	Studied groups		p-value
	Control 100	HCC 100	
AA	0(0.0%)	5 (5 %)	<0.001*
GA	20 (20%)	43 (43%)	
GG	80 (80%)	52 (52%)	
Allelic frequency			
A allele N (%)	20 (10%)	53 (26.5%)	<0.001*
G allele N (%)	180 (90%)	147 (73.3%)	

Table 3: Multivariate analysis (logistic regression model) to estimate the association between the clinicopathological data of HCC and PRDM1 gene polymorphism.

Variable	Beta coefficient	Standard error	P value	Odds ratio	95% C.I.for OR	
					Lower	Upper
Maximum tumor diameter (cm)	**2.417**	**.476**	**.000**	**11.212**	**4.414**	**28.483**

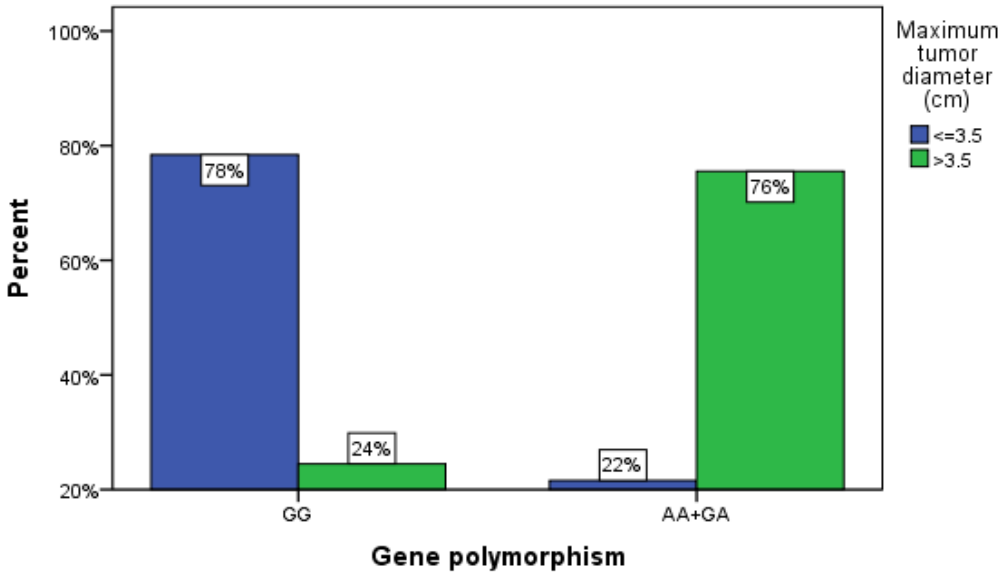

Figure 1. The diameter of the tumor or tumor size of HCC patients for measuring the correlation between prognosis of HCC and PRDM1 gene polymorphism. Data are expressed as a percentage within the group, HCC (n = 100).

4. DISCUSSION

HCC represents the fifth most frequent malignant tumor and the second-most common reason for death globally [26]. Hepatocellular carcinoma (HCC) is characterized by significant morbidity and high mortality rates worldwide [27] The relationships between hereditary and environmental factors determine cancer's propensity to spread and provide a valuable model to explain the fundamental etiologies [28]. The most prevalent type of inherited differences, genetic polymorphisms, are linked to illness susceptibility [29].

Since hepatocellular carcinoma and chronic viral infections result in T cell exhaustion, PRDM1 is crucial in regulating both processes [30]. A broad family of regulators regulating several critical biological processes in mammals includes transcription factors with zinc finger binding domains in their structural makeup. In particular, the homologous proteins Blimp1 (B lymphocyte-induced maturation protein 1) and Hobit (Blimp1 in T cells) are crucial in the control of B and T cell function [20]. The goal of the current study was to analyze the PRDM1 rs1010273 polymorphisms in a sample of Egyptian patients with hepatocellular carcinoma to identify new markers for the early diagnosis of HCC and expand the understanding of the mechanisms behind HCC.

In the current study, the demographic data of the HCC group are significantly different in age and BMI from controls. The ages of most HCC patients in this study were almost >50 years. This result

agreed with the latest World Health Report (WHO). In addition, 85% of HCC patients have BMI>25. According to Bray [3], the average age of diagnosis is typically below 60 years; in places with intermediate or low frequency, most cases manifest after 60 years of age.

The current study found that men were more likely than women to have HCC, with a ratio of 2:1, consistent with Sepanlou [31]. In addition, males were more likely than females to develop and die from HCC, with an incidence ratio ranging from 2:1 to 6:1, depending on the region. According to Benkerroum [32], the higher occurrence of HCC in men may be explained by the fact that men are more susceptible to environmental carcinogens and are exposed to more environmental risk factors, such as pesticides and aflatoxin.

Different levels of exposure to risk variables may account for this. Sex hormones and other x-linked genetic variables may also have a significant role. Marrero [33], Sex hormone differences between the sexes seemed to be a significant risk factor for HCC. Contrary to estradiol, which suppresses cell-cycle regulators, hepatocyte cell-cycle regulators are positively regulated by testosterone, accelerating the liver cancer progression.

The current study found that the GA genotype were more frequently observed in HCC patients than controls for the PRDM1 rs 1010273 gene polymorphism (Table 2). Additionally, a significant difference was found in the A allele of rs1010273 between the healthy controls and HCC patients (P= < 0.001) (Table 2).

According to the genotyping results of this investigation, healthy controls had a more considerable prevalence of GG carriers than HCC (80 vs. 52%) (Table 2).

The present study examined the correlation between PRDM1 polymorphisms and HCC associated with HCV patients. The G allele and GG phenotype frequencies in the control group were significantly higher than HCC patients. However, the GA genotype and A allele frequencies were significantly more common in the HCC patients than controls. The people carrying AA or GA have risk of developing HCC than those carrying GG genotypes. This result can explain that HCC tissues expressed high levels of exhaustion markers such as TIM-3, PD-1, CTLA-4, and LAG-3 with decreasing T cell proliferation, activity, and cytokine expression[34].

Studies on the PRDM1 polymorphisms have focused on malignant lymphoma [35]. For instance, diffuse large B-cell lymphomas have been linked to PRDM1 gene mutations at exon 2-intron 2 splice sites that caused significant PRDM1 protein deletions [36]. An association between changes correlated with lower baseline expression of PRDM1 and defective induction of the PRDM1 protein following radiation exposure was identified in a study on children with Hodgkin's lymphoma who received radiation therapy [37]. HCC is a multifactorial illness that is also influenced by several ecological factors.

5. CONCLUSIONS

In conclusion, the current findings suggest that PRDM1 rs1010273 and the prevalence of HCC in Egyptians may be potential risk factors. Furthermore, the PRDM1 gene polymorphism has been implicated as a potential prognostic marker for the progression of liver diseases. The A allele of PRDM1 rs1010273 and the GA + AA genotype increase the chance of developing HCC, whereas the G allele and GG genotype have a protective effect.

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Conflicts of Interest: The authors declare that they have no competing interests.

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Ethical Statement: The whole study and experimentations have been done in compliance with the applicable regulations and guidelines. All patients and controls or their legal representatives received a formal informed consent agreement. The ethics committee of Al-Azhar University's Faculty of Pharmacy (No. 235).

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List of Abbreviations: HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; PRDM1: PR domain zinc finger protein 1 ; CLD: Chronic liver disease; NK: Natural killer; SNPs: Single-nucleotide polymorphisms; TNFA: Tumor necrosis factor A; IL-10: Interleukin 10; IL1B: Interleukin 1 beta; TGFB: Transforming growth factor beta; GSTM1: Glutathione S-transferase Mu; MPO: Myeloperoxidase; SOD2: Superoxide dismutase 2; HFE1: Human homeostatic iron regulator protein; TP53: Tumor protein, MDM2: Mouse double minute 2 homolog; XRCC3: X-ray repair cross complementing3; MTHFR: Methylenetetrahydrofolate reductase; CBC:

complete blood count; AST: aspartate transaminase; ALT: Alanine transaminase, INR: International normalized ratio; AFP: Alpha-fetoprotein; HWE: Hardy-The Weinberg equilibrium; cDNA: complementary DNA.

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