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RESEARCH ARTICLE
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A cross-sectional study of Iraqi patients investigating HPSE SNPs and the incidence of hepatocellular carcinoma

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ABSTRACT

In this study, 30 cases from different hospitals in Iraq were included, and they were divided into two groups (a patients group of 15 cases) (a control group of 15 cases).

Patient groups were divided according to gender, 12 males (80%) and three females (20%) in the patient's group while five females (33.3%) and 10 males (6.6%) in the control group. Information related to patients was collected within the study period of 14 months (July 1, 2019 to September 2, 2020).

The demographic data of the patients were analyzed using the statistical analysis program IBM SOFT SPSS 22, where the true value and the arithmetic mean of the results were calculated, and the value of the statistical significance to know the type of relationship was also extracted.

The results found rs 12503843 with HCV (CC for eight patients were distributed, six HCV positive and two negative patients; CT for five patients were distributed, four patients HCV positive and one negative patient; TT for two patients were distributed with two positive patients).

As for the distribution of patients according to the results of the statistical relationship between rs 12331678 with HCV (six cc patients were distributed as five positive HCV and one negative; CA for six patients distributed as two negative HCV and four positive HCV).

Finally, we concluded in this study; it was described that heparanase (HPSE) (rs 12331678 and rs 12503843) with the incidence of hepatocellular carcinoma, and a statistically significant relationship was found with a p-value < 0.01.

Keywords: *Chronic renal failure, Renin , Aspartate Amino Transferase Alanine Amino Transferase , Albumin, Globulin, Calcium , Sodium , Potassium* .

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third biggest cause of death worldwide and the most prevalent form of primary liver cancer which is the fifth most prevalent neoplasm. It accounts for around 5.4% of all malignancies.1–3 It is estimated that around 564,000 new cases are diagnosed in the world each year, and an equal number of patients die from this disease.4–6 The incidence of HCV was somewhat higher in 2015, about 12 per 100,000 inhabitants.7

The risk factor for HCC and related complications varies widely between people and is determined by both environmental and genetic factors.8,9

A thorough case-control pilot study was carried out to determine whether heparanase (HPSE) SNPs are a risk factor for HCC.10

In this paper, we examine how genes and environmental variables affect a risk factor for HCC's pathophysiological characteristics. Additional genetic variants will be found by the use of a sufficient population sample, SNP array analysis, and sequencing techniques (exome and genome as a whole). This will definitely increase the understanding of the etiology. Understanding a risk factor for HCC will enable the creation of a risk-specific method for assessing a patient's illness.11–13

In the world, HCC is one of the most prevalent tumors. In addition to chronic liver disease leading to cirrhosis, epidemiological analyzes show that liver carcinogenesis is associated with exposure to xenobiotics, in addition to substances such as aflatoxin.14,15

Glucuronosyltransferase to detoxify carcinogens, the cytochrome P450 system, and conjugate transporter proteins are candidates that can determine genetic predisposition to carcinogenesis and thus represent HCC-modulator genes.

MATERIAL AND METHOD

In this study, 30 cases from different hospitals in Iraq were included, and they were distributed into two groups (a patient group with 15 cases and a control group of 15 cases).

The patient group was distributed according to gender, 12 males (80%) and three females (20%), while 5 females (33.3%) and 10 male cases (6.6%).

The approvals required to conduct this study were obtained, and the patients were obtained for the purpose of conducting a clinical examination.

After obtaining the official approval of the protocol from the Ethics Committee located at Al-Furat Hospital in Baghdad, information related to patients was collected and the study period was of 14 months (July 1, 2019 to September 2, 2020).

Preliminary information related to the demographic characteristics of this study was collected.

Patients underwent clinical examinations in addition to lab tests and blood images. Those with liver cancer and patients younger than 60 years were excluded from the study.

In this paper, genomic DNA from peripheral whole blood was purified using ABIOpure. To stabilize the DNA and aid in its removal from the solution, salts were frequently added during DNA extraction. Adding ice-cold alcohol to the sample, such as ethanol or isopropanol, is the typical procedure for DNA precipitation. This causes DNA to accumulate as fuzzy white deposits inside the fluid in the tube, which were then examined using PCL-relp hpse.

A common technique for quickly producing transcripts from a particular DNA sample is polymerase chain reaction (PCR), which enables a very tiny DNA sample to be obtained and amplified to a sufficient amount. In a series of temperature-change cycles, very little quantities of DNA sequences were transcriptionally transcribed and exponentially amplified. Today, PCR technology is widely used and frequently indispensable in clinical laboratory and medical laboratory research for a range of purposes, including medical research.

The statistical analysis program IBM SOFT SPSS 22 was used to analyze the patients' demographic data, and the arithmetic mean and true value were calculated in relation to the study's findings and the statistical significance's value to determine the nature of the association was also extracted.

To determine the variations in the statistical significance, the value of the chi-square test was retrieved together with a p-value.

RESULTS

TABLE 1. MEAN ± SD age of patient study.

Statistics			
		Patient	Control
N	Valid	15	15
	Missing	0	0
Mean		70.2000	68.1333
ST		1.58024	1.10353
Med		68.0000	68.0000
Mode		64.00a	63.00
STD1		6.12022	4.27395
Min		62.00	63.00
Max		80.00	77.00

TABLE 2. Distribution of patients according to sex.

Sex patient					
		F	%	V%	CP
V	Female	3	20.0	20.0	20.0
	Male	12	80.0	80.0	100.0
	Total	15	100.0	100.0	

TABLE 3. Distribution of the control group according to sex.

Sex control					
		F	%	V%	CP
Valid	Female	5	33.3	33.3	33.3
	Male	10	66.7	66.7	100.0
	Total	15	100.0	100.0	

TABLE 4. Characteristics of demographic results according to hematological profile.

Statistics							
		HB patient	HB control	RCB patient	RCB control	TLC patient	TLC control
N	Valid	15					
	Missing	0					
Mean		11.8000	14.8000	4.7480	4.8200	5.0180	7.1200
Std. error of mean		0.27946	0.78801	0.08826	0.11305	0.32941	0.21094
Median		12.0000	15.0000	4.8000	4.9000	4.7000	7.3000
Mode		12.00	13.00a	4.90	4.20a	4.40a	6.70
Std. deviation		1.08233	3.05193	0.34183	0.43785	1.27581	0.81696
Skewness		0.062	–0.390	–0.235	–0.361	0.397	–0.686
Error of skewness		0.580	0.580	0.580	0.580	0.580	0.580

TABLE 5. Descriptive results of focal lesion.

Descriptive					
	VAR00006			Statistic	Std. error
Patient	Absent	Mean		68.6000	1.52169
		95% CI	LB	65.1577	
			UB	72.0423	
		Frequency		Ten patients	
		Std. deviation		4.81202	
		Minimum		62.00	
		Maximum		77.00	
	Moderate	Mean		71.0000	7.00000
		95% CI	LB	–17.9434	
			UB	159.9434	
		Frequency		Three patients	
		Std. deviation		9.89949	
		Minimum		64.00	
		Maximum		78.00	
	Slight	Mean		75.0000	4.50925
		95% CI	LB	55.5983	
			UB	94.4017	
		Frequency		Two patients	
		Std. deviation		7.81025	
		Minimum		66.00	
		Maximum		80.00	

TABLE 6. Primer sequence of polymorphisms rs 12331678 and rs 12503843.

Polymorphisms	Primer sequence
rs 12503843	5’CTATAGTATTTCCTACATTATAGAGTTTGGTA-3’ (forward)’-TGGATTAGGCAATGGTCATCA-3’ (reverse)
rs 12331678	AAAGCAAAAGGATGTGAACACAAA-3’ (forward) 5’CTTACTCTAACCAATAAAAATTAATGCTATAGA-3’ (reverse)

TABLE 7. Correlation between HPSE gene polymorphism rs 12331678 with ALT, AST, and Child–Pugh score.

Correlations					
		rs 12331678	ALT	AST	Child–Pugh score
Patient	R	1	–0.265	–0.190	0.566
	Sig		0.339	0.498	0.028
	Number	15	15	15	15
ALT	Pearson correlation	–0.265	1	0.541*	0.250
	Sig	0.339		0.037	0.368
	Number	15	15	15	15
AST	R	–0.190	0.541*	1	0.062
	Sig	0.498	0.037		0.827
	Number	15	15	15	15
Child–Pugh score	R	0.566*	0.250	0.062	1
	Sig	0.028	0.368	0.827	
	N	15	15	15	15

TABLE 8. Pearson correlations between rs 12503843 with ALT, AST, and Child–Pugh score.

		RS 12503843	ALT	AST	Child–Pugh Score
RS 12503843	R correlation	1	0.249	–0.605*	0.124
	SIG		0.371	0.017	0.659
	NUM	15			
ALT	R	0.249	1	–0.440	0.285
	SIG	0.371		0.100	0.304
	NUM	15			
AST	R	–0.605*	–0.440	1	0.062
	SIG	0.017	0.100		0.827
	NUM	15			
Child–Pugh score	R	0.124	0.285	0.062	1
	SIG	0.659	0.304	0.827	
	NUM	15			

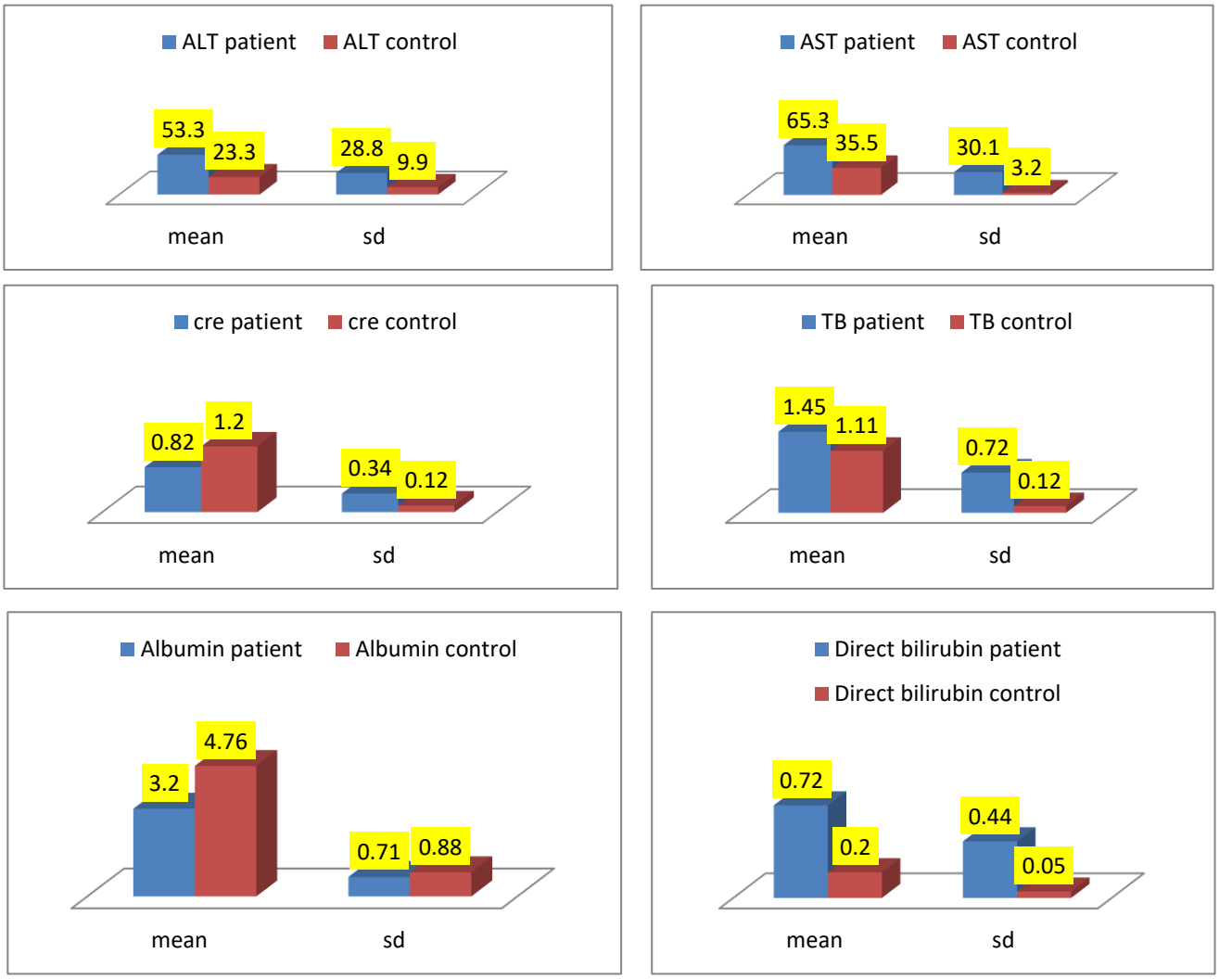

FIGURE 1. Results of patients' study according to biochemical parameters.

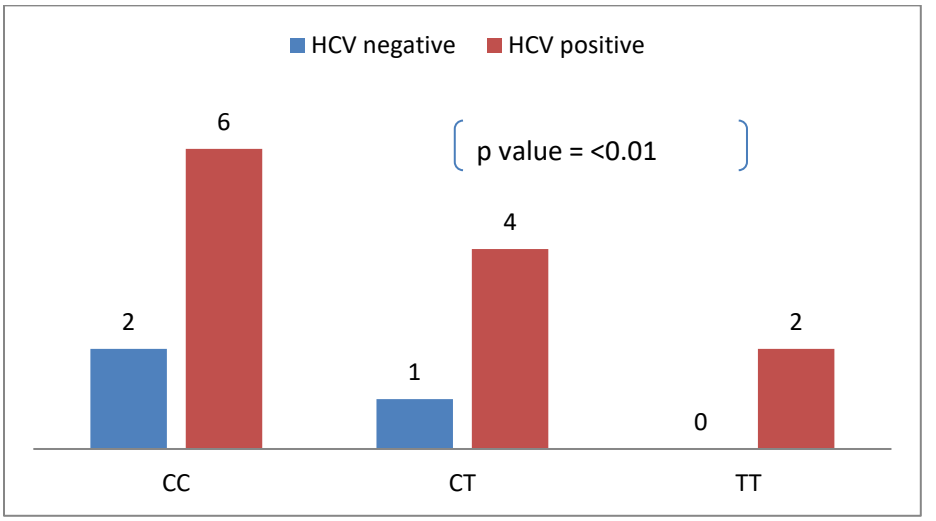

FIGURE 2. Distribution of patients according to results of the statistical relationship between rs 12503843 and HCV

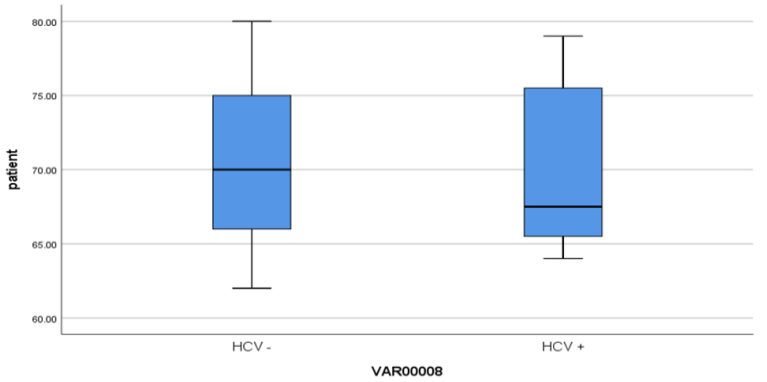

FIGURE 3. Prevalence of HCV according to age.

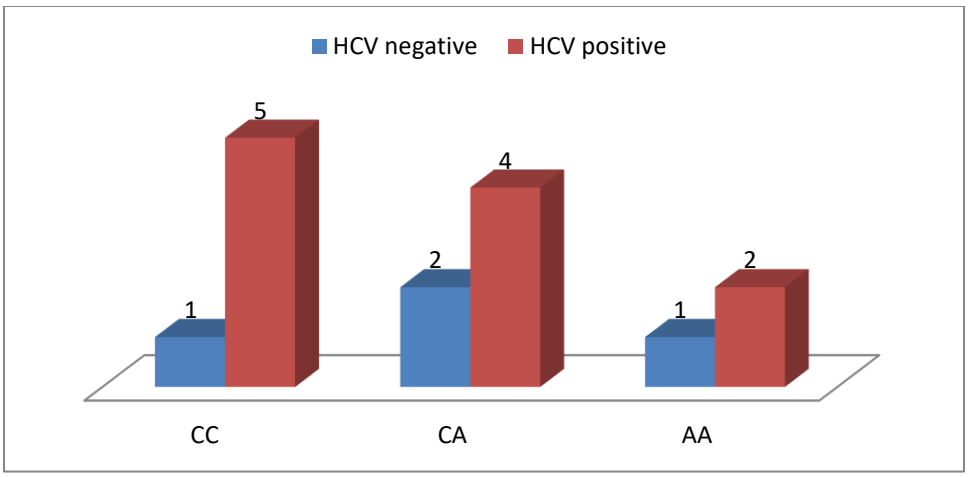

FIGURE 4. Distribution of patients according to results of the statistical relationship between rs 12331678 and HCV.

DISCUSSION

In this study, 30 patients participated and they were distributed into two groups (15 patient's group with a mean SD age of 70.2 ± 6.1 and 15 cases control group with mean SD age of 68.13 ± 4.2), and the data were analyzed, and demographic information about patients was obtained with the help of IBM SOFT SPSS PROGRAM and Microsoft Excel 2013

In this study, 15 patients with HCC and 15 control groups were genotyped for two genetic polymorphisms.

HPSE (rs 12331678 and rs 12503843) and through Figure 2 in this study, which shows the distribution of patients according to results of the statistical relationship between rs 12503843 with HCV (CC of eight patients were distributed, six HCV positive and two negative patients; CT for five patients distributed, four HCV positive and one negative patient; TT for two patients distributed with two positive patients), a statistically significant relationship was found with a p value of 0.01.

As for the distribution of patients according to the results of the statistical relationship between rs 12331678 with HCV (six cc patients were distributed as five positive HCV and one negative, CA for six patients distributed as two negative HCV, and four positive HCV as shown in Figure 4).

Through the statistical relationship found in this study, it is concluded that there is a significant association with 0.01 between HPSE (rs 12331678 and rs 12503843) and the incidence of HCC.

And in another study of Lao Tu Sin in 2014, in Chinese individuals, where 200 patients were enrolled in this study; they were distributed into 130 male patients and 70 females, and a significant positive correlation was found with the incidence of HCC.

Despite the small number of patients collected in our study, it agreed with the study Lao Tu Sin in 2014.

Tables 7 and 8 show a correlation between HPSE gene polymorphism rs 12331678 and rs 12503843 with ALT, AST, and Child–Pugh scores. A positive correlation was found in the clinical features, and this explains the negative effect on the likelihood of incidence of HCC.

In the previous studies, it was found that a study did not agree with our study. It was by John Wright in 2009, where 10 patients were collected, distributed as eight males and two females. In this study, no statistical relationship was found, and this was due to several explanations, including the lack of a number of samples collected in this study.

CONCLUSION

In this study, it was described HPSE (rs 12331678 and rs 12503843) with the incidence of HCC in Iraqi patients whose ages ranged between 60 and 80 years, and a statistically significant relationship was found.

Our current study also presented the clinical significance and HPSE genetic polymorphism as one of the causes of HCC.

REFERENCES

1. Liu YB, Gao SL, Chen XP, et al. Expression and significance of heparanase and nm23-H1 in hepatocellular carcinoma. World J Gastroenterol. 2005; 11: 1378–1381. https://doi.org/10.3748/wjg.v11.i9.1378
2. Ostrovsky O, Shimoni A, Baryakh P, et al. Modification of heparanase gene expression in response to conditioning and LPS treatment: strong correlation to rs4693608 SNP. J Leukoc Biol. 2014; 95: 677–688. https://doi.org/10.1189/jlb.0313147
3. Zhang H, Zhai Y, Hu Z, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet. 2010; 42: 755–758. https://doi.org/10.1038/ng.638

4. Zhai Y, Qiu W, Dong XJ, et al. Functional polymorphisms in the promoters of MMP-1, MMP-2, MMP-3, MMP-9, MMP-12, and MMP-13 are not associated with hepatocellular carcinoma risk. Gut. 2007; 56: 445–447. https://doi.org/10.1136/gut.2006.112706
5. Yang JM, Wang HJ, Du L, et al. Screening and identification of novel B cell epitopes in human heparanase and their anti-invasion property for hepatocellular carcinoma. Cancer Immunol Immunother. 2009; 58: 1387–1396. https://doi.org/10.1007/s00262-008-0651-x
6. Ha NB, Ha NB, Ahmed A, et al. Risk factors for hepatocellular carcinoma in patients with chronic liver disease: a case-control study. Cancer Causes Contr. 2012; 23: 455–462. https://doi.org/10.1007/s10552-012-9895-z
7. Zhang Y, Li L, Wang Y, et al. Downregulating the expression of heparanase inhibits the invasion, angiogenesis, and metastasis of human hepatocellular carcinoma. Biochem Biophys Res Commun. 2007; 358: 124–129. https://doi.org/10.1016/j.bbrc.2007.04.068
8. Xiao Y, Kleeff J, Shi X, et al. Heparanase expression in hepatocellular carcinoma and the cirrhotic liver. Hepatol Res. 2003; 26: 192–198. https://doi.org/10.1016/S1386-6346(03)00107-4
9. Weng CJ, Hsieh YH, Tsai CM, et al. Relationship of insulin-like growth factors system gene polymorphisms with the susceptibility and pathological development of hepatocellular carcinoma. Ann Surg Oncol. 2010; 17(7): 1808–1815. https://doi.org/10.1245/s10434-009-0904-8
10. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127: 2893–2917. https://doi.org/10.1002/ijc.25516
11. Goldshmidt O, Yeikilis R, Mawasi N, et al. Heparanase expression during normal liver development and following partial hepatectomy. J Pathol. 2004; 203: 594–602. https://doi.org/10.1002/path.1554
12. Yano Y, Yamashita F, Kuwaki K, et al. Clinical features of hepatitis C virus-related hepatocellular carcinoma and their association with 훼-fetoprotein and protein induced by vitamin K absence or antagonist-II. Liver Int. 2006; 26: 789–795. https://doi.org/10.1111/j.1478-3231.2006.01310.x
13. Chen G, Dang YW, Luo DZ, et al. Expression of heparanase in hepatocellular carcinoma has prognostic significance: a tissue microarray study. Oncol Res. 2008; 17: 183–189. https://doi.org/10.3727/096504008785114138
14. Ralph S, Brenchley PE, Summers A, et al. Heparanase gene haplotype (CGC) is associated with the stage of disease in patients with ovarian carcinoma. Cancer Sci. 2007; 98: 844–849. https://doi.org/10.1111/j.1349-7006.2007.00461.x
15. Sasisekharan R, Shriver Z, Venkataraman G, et al. Roles of heparan-sulfate glycosaminoglycans in cancer. Nat Rev Cancer. 2002; 2: 521–528. https://doi.org/10.1038/nrc842