IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 9, Issue 6 Series. I (June 2019), PP. 08-17

Role of Hogg1 Ser326Cys Gene Polymorphism and Insulin Resistance in Chronic Hepatitis C Related Hepatocellular Carcinoma in Egyptian Patients

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Abstract:
Introduction: The *hOGG1* gene encodes a DNA glycosylase enzyme responsible for DNA repair. The Ser326Cys polymorphism in this gene may influence its repair ability and thus plays a role in carcinogenesis. Several case-control studies have been conducted on this polymorphism and its relationship with the risk of hepatocellular carcinoma (HCC) but, the results were inconsistent. Also, yet the role of IR in the development of HCC associated with chronic HCV infection has not been established**The objective of this study**to evaluate the role of hOGG1 Ser326Cys gene polymorphism and insulin resistance (IR) in The Development of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C in Egyptian patients.**Material and methods:**This study was included 130subjects, they were divided into three groups: 40 as Control group, 61 as HCCgroup(HCV with HCC) and29 as HCV group (without HCC).Their age ranged from 41- 89 years. All subjects undergo the PCR analysis;the genotyping for hOGG1 was detected by restriction fragment length polymorphism-PCR (RFLP-PCR).Insulinresistance (IR) was assessed by homeostasis model (HOMA-IR).**Results:** The frequencies for CC,CG and GG of hOGG1 Ser326Cys gene were showing non-significant differences between the three groups.and,Regarding the alleles,Showing non-significant differences in the allelic distribution between the three studied groups.**Conclusion:**There is no evidence to support that the (hogg1 ser326cys)gene polymorphism is significantly associated with HCCdevelopment in patients with chronic HCV infection in Egyptian population. Also, IRis not significantly associated with it.

Keywords: gene polymorphism, HCV, HCC, HOGG1 Ser326cys gene.

Date of Submission: 21-05-2019 Date of acceptance: 07-06-2019

I. INTRODUCTION

Hepatitis C virus (HCV) infects hundreds of millions of people persistently and causes spectrum of chronic liver diseases worldwide (1). The majority of infected individuals (60%- 80%) develop chronic hepatitis C (CHC), which is associated with progressive liver fibrosis and a 3%- 9% risk of cirrhosis after 20 years as shown in community based studies (2). CHC is also associated with significant morbidity and mortality, accounting for 50%-76% of all hepatocellular carcinoma (HCC) cases worldwide and two thirds of liver transplants in the developed world (3).HCC is the sixth most prevalent cancer and the third most frequent cause of cancer-related death worldwide (4). Insulin resistance (IR) which is a consistent finding in patients with type II DM is more prevalent in patients with HCV infection compared to healthy controls matched for age, sex and body mass index (BMI) and patients with other hepatobiliarydisorders(5).The presence of hepatitis C viral particles in the liver is regarded as the origin of the development of insulin resistance (6). Human 8-hydroxyguanine glycosylase 1 (hOGG1) is a DNA glycosylase enzyme responsible for the excision of 8-oxoguanine, a mutagenic base byproduct which occurs as a result of exposure to reactive oxygen . The *hOGG1* gene, located on chromosome 3p26.2, is composed of eight exons and seven introns. Polymorphisms in this gene may alter glycosylase function and an individual's ability to repair damaged DNA, possibly resulting in genetic instability that can foster carcinogenesis. Among many polymorphisms identified in the *hOGG1* gene, much interest has been focused on the Ser326Cys (C>G)

polymorphism. It is in exon 7 of the *hOGG1* gene, which takes the form of a single amino acid substitution, from serine to cysteine at Condon 326. Although the evidence is inconclusive that this functional polymorphic variation influences the activity of hOGG1 (7).Several studies have been conducted on this polymorphism and its relationship with the risk of hepatocellular carcinoma (HCC). But, their results were inconsistent. Also, yet the role of IR in the development of HCC associated with chronic HCV infection has not been established (8). So,the objective of this study to evaluate the role of hOGG1 Ser326Cys gene polymorphism and insulin resistance (IR) in The Development of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C in Egyptian patients.

II. MATERIAL AND METHODS

In this study (case-control) a total of 130 subjects were divided into three groups, 40 asControl group (healthy subjects), 29 as HCVgroup (without HCC) and 61 as HCC group (HCV with HCC),between July 2016 andNovember 2018. Their age ranged from 41- 89 years. All subjects have signed a written informed consent form before enrollment in the study. An approval was provided by the Institutional Review Board (IRB) of Mansoura Faculty of Medicine. Patients were excluded if they have one of the following criteria: Non –HCV related hepatocellular carcinoma, Diabetes Miletus, and Obesity (BMI >30).

Samples collection: 2 ml venous blood samples were delivered to sterile collection tubes containing K2EDTA Stored as EDTA ant coagulated blood Samples at -70^{o}C for DNA extraction and genotyping ofhOGG1Ser326Cys gene using thespecific restriction enzyme (RFLP). Another 5 ml blood samples were delivered to Vacuum blood collection tubesand allowed to clot for 15 minutes and centrifuged at 7000 r.p.m for 10 minutes for serum separation then serum collected in other sterile tubes and stored at - 70^{o}C until used to determine serum α fetoprotein level and serum liver function tests, as well as fasting blood glucose and insulin levels for estimation of HOMA-IR.

DNA extraction: Genomic DNA was isolated from EDTA anticoagulated peripheral blood using a DNA extraction kit (Qia-ampilification extraction kit; Qiagen, New York, Frankfurt, USA).

Principle of DNA extraction:DNA extraction is the process by which DNA is separated from proteins, membranes, and other cellular material contained in the cell from which it is recovered. Samples are digested with proteinase K, which is the protease used to digest proteins from samples. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane; impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the elution buffer. The purity of template DNA is detected by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. An A260/A280 ratio of 1.7–1.9 means pure template DNA and is better suited for PCR (9). *Procedure*; 200 µl of EDTA anticoagulated blood sample was used for DNA extraction according to the manufacturer's instructions.The extracted DNA was verified by agarose gel electrophoresis (1% ethidium bromide stained agarose).The concentration and purity were tested spectrophotometrically by measuring absorbance at 260/280 nm.The extracted DNA samples were stored at −80°C until amplification by PCR.

Amplification:PCR: Amplification ofhOGG1Ser326Cys gene using the PCR: enzymatic amplification was performed by PCR using Taq polymerase enzyme and PTC-100 thermal cycler (MJ Research Inc., Watertown, Massachusetts, USA). Regarding primer sequences, we used the following oligonucleotide primers for in-vitro amplification of specific hOGG1Ser326Cys gene fragment:

The following primers were used:(forward) 5′-GGAAGGTGCTTGGGGAAT- 3′, (reversed) 3′-ACTGTCACTAGTCTCACCAG-5′.

polymorphism: The total volume of 25 ml included 30 ng genomic DNA, 0.3 mmol of each primer (Promega, Madison, Wisconsin, USA), and 1× PCR mix (Taq PCR Master Mix Kit; Qiagen GmbH, Hilden, Germany) containing 200 mmol/l of each dNTP, 5 ml 10× reaction buffer, 1.25 U Taq Gold Polymerase, and 4 mmol/l $MgCl_2$.

Cycling condition:A thermal cycler was used as follows: denaturation at 95°C for 5 min, followed by 30 cycles under the following conditions:Denaturation at 95°C for 30 sec.Annealing at 58°C for 30 s.Extension at 72°C for 1 min.Final extension cycle at 72°C for 5 min.PCR product was digested by restriction enzyme, Fnu4HI , according to kit instructions (New England Biolabs, Beverly, Massachusetts, USA) at 65°C for 16 h. Electrophoresis of 5 µl of the PCR product was separated on 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.only one 100-bp fragment was seen in subjects with the cys/cys genotype .in subjects with the ser/sergenotype two bands of 100 and 200bp were seen whereas in those subjects homozygous for the ser variant (ser/ser),only one 200-bp PCR fragment is seen.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) :In each sample, the degree of insulinresistance was estimated by the homeostasis model assessment (HOMA-IR) as described by (10). HOMA-IR wascalculated by taking into account fasting insulin and blood glucose levels according to the equation (HOMA-IR) =

fasting insulin (μU/ml) × fasting blood glucose mg/dl) / 22.5. Serum insulin level was estimated usingELISA Kit (11). The kitwas provided from Diagnostic Systems laboratories. Inc.Corporate Headquarters, 445Medical Center, Blvd. Webster, Texas 77598-4217 USA. Fasting blood glucose level was done according to method

of (12), and the kits were provided from Leitch diagnostics, Zone Industrielle- 61500, Sees France

Estimation of serum α-fetoprotein (AFP) :Serum level of α-fetoprotein (AFP) was estimated using Fetoprotein Human ELISA Kit (catalog number ab108838, Abcam, alpha Fetoprotein Human).

HCV antibody was estimated by An enzyme immunoassay (ELISA) for the qualitative detection of IgG antibodies to

Hepatitis C Virus (HCV) (13, 14), in human serum using RUO kits (catalog number; 6307125& 630705respectively- LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona,

SPAIN).Diagnosis of HCV infection was confirmed by the CobasTaqMan HCV test real-time RT quantifiable PCR

for HCV.

Estimation of serum liver function :Liver functions estimation was performed colorimetrically using thecommercially available kits. Alanine and aspartate amino transferasewereestimated according to the method (15). The kits wereprovided from Leitch diagnostics, Zone Industrially 61500, Sees France.

III. STATISTICAL ANALYSIS

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 23. Polymorphisms and genotype frequencies were evaluated by gene counts. The data were tested for the goodness of fit between the observed and expected genotype frequencies (X^2 test). When the observed genotype frequencies fit to Hardy-Weinberg equilibrium, X^2 tests (2-by-2 tables) were performed to calculate significantly different genotype distributions between patients and controls and also odd's ratio(OR) and confidence interval95% were calculated to detect risk ratio.*P* value <0.05 was considered statistically significant in all analyses.

IV. RESULTS

In this study (case-control) a total of 130 subjects were enrolled (61 as HCC group, 29 as HCV group and 40 healthy controls). There was no significant difference between the three studied groups with regard to theirage, sex and smoking were not significantly different in all groups, but, HCC and HCV are more common in males than females **(table 1).** Biochemical parameters of the three studied groups are shown in **(table 2),** where**,** AFP levels which showed marked increase in HCC group which were significant when compared to HCV group and control group. Albumin level showed significant decrease in HCC group and HCV group comparing to control group.GPT and GOT levels showed significant increase comparing to control group.HOMA IR level showed significant increase when compared to control group.

There was no statistically significant difference found between the three genotypes regarding HOMA IR in all groups,while therewas statistically significant difference between different groups within each genotyping **(table 3)** and **(figure 1).** There was no significant difference found between the two alleles (C&G) within each group regarding to HOMA IR. While there wassignificant difference found between different groups within each allele, **(table 4)** and **(figure 2).**

The PCRproduct is 200 bp in length (figure 3), and is digested by the Fnu4H restriction enzyme.The three possible genotypes were defined based on the threedistinct banding patterns observed throughultraviolet spectrophotometer: only 200 bpfragments were assigned to be CC genotype, both 100 bp and 200 bp fragments wereassigned to be CG genotype, while only 100bp fragments were assigned to be GGgenotype.

The comparison of (hogg1 ser326cys)genotyping and frequencies in all groups: In H CCgroup frequencies for CC,CG and GG were 26.2%,39.3% and 34.5% respectively.In HCV group,these frequencies were 37.9%, 34.5% and 27.6% respectively. In control group, frequencies were 35.0%, 35.0% and 30.0% Respectively, showing non-significant differences**.** The comparison of (hogg1 ser326cys) allele frequencies in all groups regarding the alleles, allele C was present in 45.9% of HCCgroup,55.2 %in HCV group and 52.50% in control group.Where, allele G was present

in 54.1% of HCC group, 44.8% of HCVgroup and 47.50 %of control group,showing non-significant differences in the allelic distribution between the three studied groups.But, the frequency of allele G was higher in HCC group than in HCV group and controls **(table 5)**and **(figue4).**No significant differences in the allelic distribution between HCC and HCVgroups, HCC and Control groups nor HCV and Control groups (P>0.05)**(table 6).**

V. DISCUSSION

HCC is the seventh most common cancer and the third leading cause of cancer-related death worldwide. The incidence of HCC has been rapidly increasing in Western countries because of the increased prevalence of HCV infection (16). 8-oxoguanine is one of the most common DNA lesions resulting from reactive oxygen species (17). It has the ability to pair with adenine instead of cytosine during DNA replication, and therefore plays a role in carcinogenesis (18). In human, hOGG1 is responsible for the repair of 8-oxoguanine. The conduction of studies to examine the hOGG1 Ser326Cys polymorphism and cancer risk, is based on the notion that this polymorphism may influence the enzyme activity of hOGG1 and thus influence the process of carcinogenesis through 8-oxoguanine. The association between the hOGG1 Ser326Cys polymorphism and HCC risk was not clear so, Wang et al. (19) did a meta-analysis by pooling 8 studies with 2369 cases and 2442 controls and showed a statistically significant but very weak association between the hOGG1 Ser326Cys polymorphism and HCC risk, this finding were consistent withour results where, we found that, there were slightly high the frequency of allele G in HCC group higher than HCV group and controls, it was present as 54.1% in HCC group, 44.8% in HCV group and 47.50 % in control group.Thus, the G allele mayassociated with increased risk of HCC (OR 1.45, 95% CI 0.77–2.7), however, no significant differences in the allelic distribution between the three studied groups. Some studies suggested that the 326Cys allele confers decreased ability to repair 8-oxoguanine (20-22). Other studies, however, found no difference in activity of the *hOGG1* Ser326Cys polymorphism (23-30).Also, Tang et al. studied the urea 8-oxogudanine level in HCC patients, and did not find a relationship with the hOGG1 Ser326Cys polymorphism (31).Conflicting results may be due to the small sample size, and unable to control for other factors that may affect 8-oxogudanine levels.

Yet the role of IR in the development of HCC associated with chronicHCV infection has not been fully established. Patients with HCC have elevatedlevels of HOMA-IR compared to patients with CHC matched for age, sex andBMI. Elevated levels of IR occur regardless the presence of DM (32, 33, 34). Strong relationship between IR and HCC. These findings suggest that IR is a potential risk factor for development of HCC in patients with CHC. SeverAL hypothesis which could explain this relationship, Alexia et al**.**suggestedthat hyperinsulinemia which occur in IR can promote the synthesis andbiological activity of insulin-like growth factor 1 (IGF-1), which is a peptidehormone that regulates energy-dependent growth processes. IGF-I stimulatescell proliferation and inhibits apoptosis and has been shown to have strongmitogenic effects on a wide variety of cancer cell lines. Changes in theexpressionpattern of IGF-system components have been observed in patientswith HCC, in human HCC cell lines and in their conditioned culture medium (35). In contrast, our results showed no statistically significant difference found between the three genotypes regarding HOMA IR in all groups, but there was statistically significant difference between different groups within each genotyping**.** There was no significant difference found between the two alleles (C&G) within each group regarding to HOMA IR. While there was significant difference found between different groups within each allele, where there was no significant difference between HCC group and HCV group while there was statistical significant higher in the two groups than controls**.**In this study, HCV group, fasting blood glucose level, fasting insulin level and HOMA- IR were significantly higher than the control group This finding is consistent with (36,37,38).IR may be induced by HCV genotype-4 infection regardless of the severity of liver disease and that IR effects begin at an early phase of HCV infection and accelerate the progression of hepatic fibrosis and HCC development (39).Chenet al. proposed that hepatocyte necrosis followed by hepatic regeneration especially biliary epithelial cells may be the cause for increase serum AFP level (40). Also, Kawaguchi's study suggested that there is an association between whole-body IR and increased serum AFP level in HCV patients (41), this finding consistent with our results, there was statistically significantly higher in HCC group in regard to AFP level than HCV group (P<0.001) and this predict the risk of HCC.

VI. CONCLUSION

Our results suggest that the hOGG1Ser326Cyspolymorphism may not play a major role as an independent factorinhepatocarcinogenesis. And also, IR is not significantly associated with it. Although this case-control study of moderate size is among the largest ones that have been reported on theassociation between

HCC and genetic polymorphisms, we couldnot exclude the possibility of a weak association with the hOGG1polymorphism and its interaction with environmental factors. Further large studies are needed to address theseissues**.**

ACKNOWLEDGEMENT

The authors thank all the patients who have contributed to this study and their families by answering the questionnaire and donating biological samples and all people who help and supportus.

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Table (1): Demographic variables in three groups:

		Groups						P
		HCC		HCV		Control		
Age		58.38	±10.33	57.72	±9.17	56.85	±6.53	0.7
Sex	Male	43	70.5%	19	65.5%	22	55.0%	0.28
	Female	18	29.5%	10	34.5%	18	45.0%	
Smoker	No	35	57.4%	21	72.4%	30	75.0%	0.13
	Yes	26	42.6%	8	27.6%	10	25.0%	

Data expressed as mean±SD or s frequency(Number-percent)
SD: standard deviation P:Probability *:significance <0.05
Test used: one way ANOVA-test for data expressed as mean±SD and Chi-square for data expressed as frequency

Table (2): Biochemical parameters of the three studied groups.

		HCC	HCV	Control	P	P1	P2	P3
Afp	Median	188.00	45.00	18.50	<0.001*	<0.001*	<0.001*	0.004*
	IQR	76.00-648.00	19.00-65.00	9.60-28.00				
Albumin	Mean	2.76	3.05	4.50	<0.001*	0.07	<0.001*	<0.001*
	±SD	.66	.40	.53				
Gpt	Mean	88.13	72.90	26.75	<0.001*	0.036*	<0.001*	<0.001*
	±SD	36.08	21.51	5.68				
Got	Mean	94.34	76.21	27.00	<0.001*	0.01*	<0.001*	<0.001*
	±SD	36.95	22.24	5.47				
Homa	Mean	6.60	6.45	2.55	<0.001*	0.6	<0.001*	<0.001*
	±SD	.85	.68	.36				

Data expressed as mean±SD or as median(IQR)
SD: standard deviation IQR: interquartile range
P:Probability *:significance <0.05
Test used: One way ANOVA followed by post-hoc Tuckey for data expressed as mean±SD and Kruskalwallis followed by post-hoc Dunne's for data expressed as median(IQR)
P1: significance between HCC&HCV groups
P2: significance between HCC & Control groups
P3: significance between HCV & Control groups

Table(3):Association between HOMA IR and genotyping of (hOGG1 ser326 cys) gene.

HOMA IR		Genotyping			P^a
		CC	CG	GG	
HCC	Mean	6.60	6.82	6.34	0.16
	±SD	.60	1.00	.80	
HCV	Mean	6.44	6.31	6.63	0.6
	±SD	.92	.45	.56	
Control	Mean	2.68	2.65	2.50	0.2
	±SD	.34	.33	.31	
	P^b	<0.001*	<0.001*	<0.001*	

	Post-hoc	***P1=0.8*** ***P2=<0.001**** ***P3=<0.001****	***P1=0.18*** ***P2=<0.001**** ***P3=<0.001****	***P1=0.5*** ***P2=<0.001**** ***P3=<0.001****	

Data expressed as mean±SD
SD: standard deviation P:Probability *:significance <0.05
P[a]:s significance between different genotyping within each group
P[b]:s significance between different groups within each genotyping
Test used: One way ANOVA followed by post-hoc tukey
P1: significance between HCC&HCV groups
P2: significance between HCC & Control groups
P3: significance between HCV & Control groups

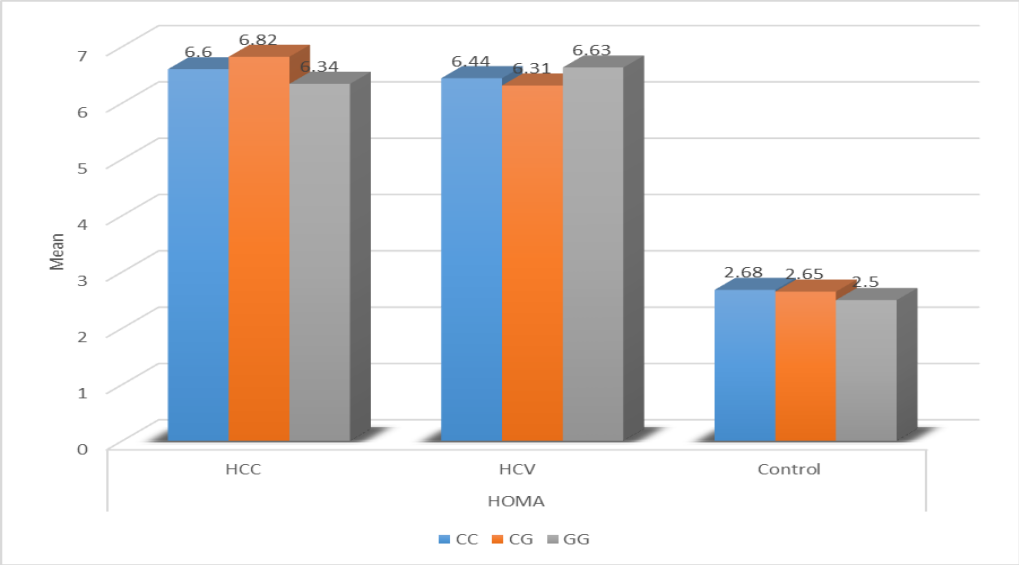

Fig 1: Association between HOMA IR and genotyping of(hOGG1 ser326 cys) gene.

Table(4): Association between alleles and HOMA IR.

		Alleles				P[a]
		C		G		
HOMA		Mean	±SD	Mean	±SD	
HCC		6.70	.79	6.60	.93	0.56
HCV		6.40	.78	6.45	.52	0.8
Control		2.67	.33	2.48	.36	0.035*
	P[b]	<0.001*		<0.001*		
	Post-hoc	*P1=0.12* *P2=<0.001** *P3=<0.001**		*P1=0.78* *P2=<0.001** *P3=<0.001**		

Data expressed as mean±SD
SD: standard deviation P:Probability *:significance <0.05
P[a]:s significance between different genotyping within each group(student's t-test)
P[b]:s significance between different groups within each genotyping(One way ANOVA followed by post-hoc tukey)
P1: significance between HCC&HCV groups
P2: significance between HCC & Control groups
P3: significance between HCV & Control groups

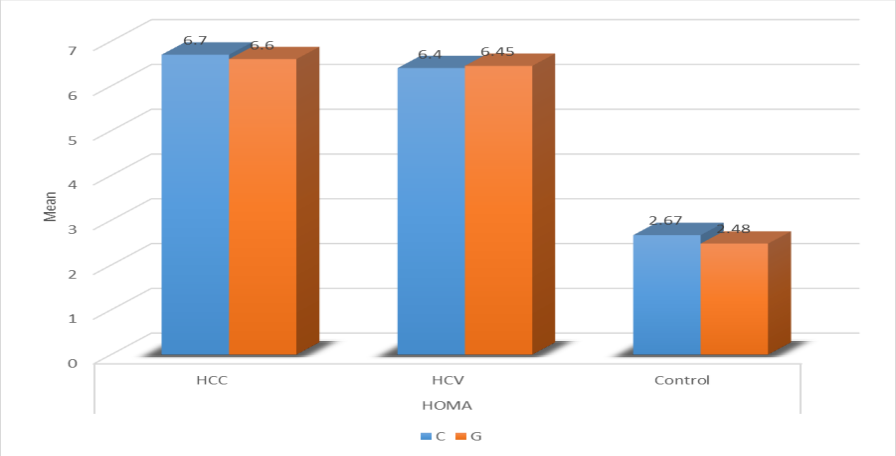

Fig 2: Association between alleles and HOMA IR.

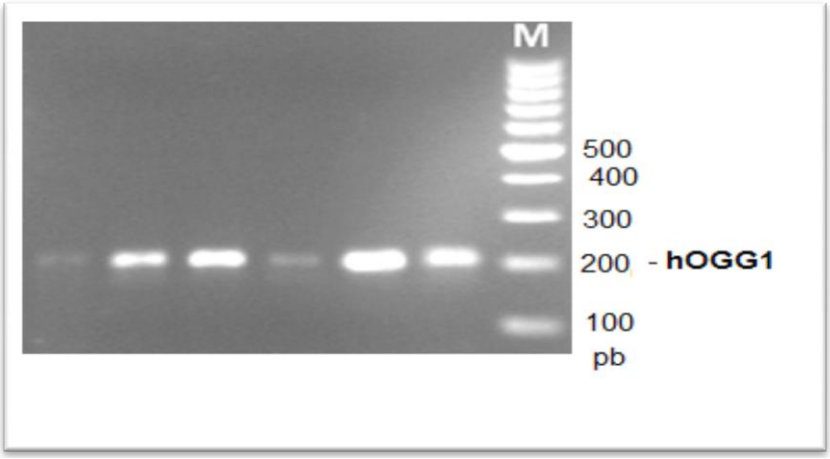

Fig 3: Agarose gel electrophoresis of the hOGG1 gene PCR amplification products. Fragments of 200 bp indicate the hOGG1 gene.

Table (5): Comparison of (hogg1 ser326cys)genotyping and allele frequencies in all groups:

		HCC(n=61) No (%)	HCV (n=29) No (%)	Control (n=40) No (%)	P value
Genotypes	CC	16 (26.2%)	11(37.9%)	14 (35.0%)	0.81
	CG	24(39.3%)	10(34.5%)	14 (35.0%)	
	GG	21 (34.5%)	8 (27.6%)	12(30.0%)	
	CG+GG	45(73.8%)	18(62.1%)	26 (65.0%)	
Alleles	C	56(45.9%)	32(55.2%)	42 (52.50%)	0.43
	G	66 (54.1%)	26(44.8%)	38 (47.50%)	

Data expressed as frequency(Number-percent)
P:Probability *:significance <0.05
Test used: Monte-Carlo

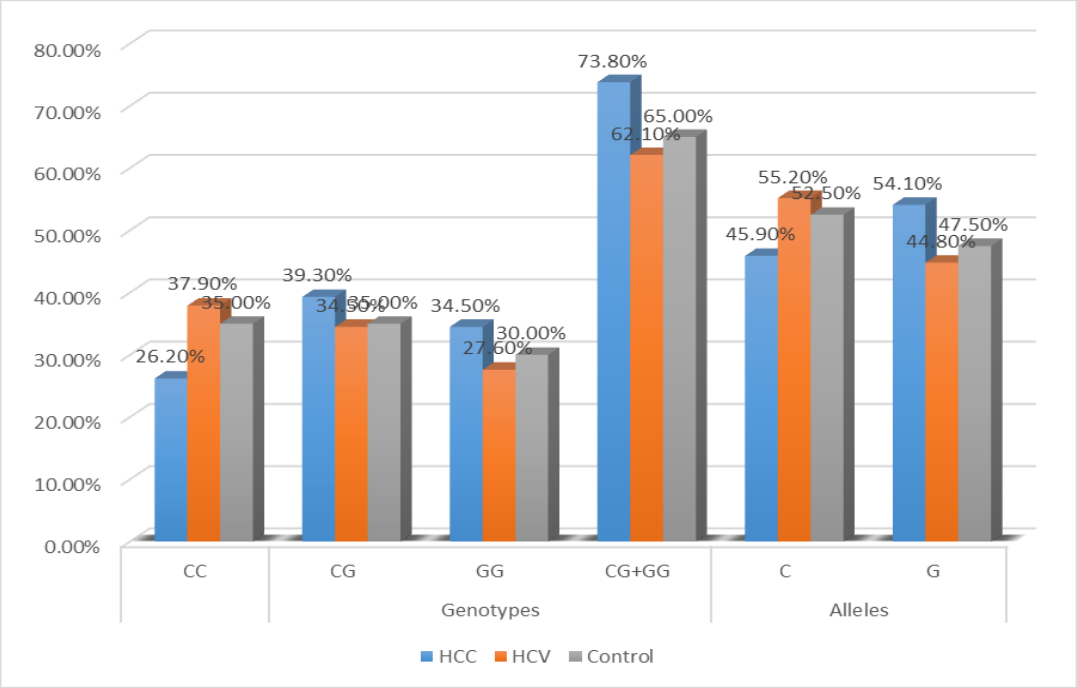

Fig 4: Comparison of (hogg1 ser326cys)genotyping and allele frequencies in all groups:

Table (6): Comparison of (hogg1 ser326cys)genotyping and allele frequencies between each two groups and risk ratio.

		P 1	OR1 (CI 95%)	P 2	OR2 (CI 95%)	P3	OR3 (CI 95%)
Geno types	CC	-	1(Ref)	-	1(Ref)	-	1(Ref)
	CG	0.35	1.65(0.57-4.78)	0.4	1.5(056-3.97)	0.86	0.9(0.3-2.8)
	GG	0.29	1.8(0.58-5.5)	0.4	1.53(0.55-4.2)	0.78	0.84(0.25-2.8)
	CG+GG	0.25	1.7(0.67-4.4)	0.34	1.5(0.6-3.58)	0.8	0.88(0.32-2.37)
Allels	C	-	1(Ref)	-	1(Ref)	-	1(Ref)
	G	0.24	1.45(0.77-2.7)	0.35	1.3(0.74-2.3)	0.75	0.89(0.45-1.77)

P1: significance between HCC and HCV groups
P2: significance between HCC and Control groups
P3: significance between HCV and Control groups
OR: odd's ratio CI: confidence interval
Test used: Pearson's Chi-square for data expressed as frequency

Mohamed A. M. Hegazi.“ Role of Hogg1 Ser326Cys Gene Polymorphism and Insulin Resistance in Chronic Hepatitis C Related Hepatocellular Carcinoma in Egyptian Patients.”IOSR Journal of Pharmacy (IOSRPHR), vol. 9, no. 6, 2019, pp. 08-17.