

Epidermal Growth Factor Gene Polymorphism in Egyptian Patients with Hepatocellular carcinoma related to Hepatitis C

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ABSTRACT

AIM: Hepatocellular carcinoma (HCC) ranks fifth among the most

prevalent cancers worldwide. In Egypt, its incidence has been doubling due to hepatitis C viral (HCV) infection. Epidermal growth factor (EGF) plays an important role in hepatocyte regeneration and had a role in malignant transformation. Single nucleotide polymorphism (SNP) G/G genotype was associated with higher risk for HCC development. This study was done to evaluate the correlation between EGF polymorphism and HCC in patients with HCV.

METHODS: Routine investigations for liver cirrhosis and HCC, also EGF genotyping were done on 2 groups; patients with HCV related cirrhosis and patients with newly diagnosed HCC on top of cirrhosis, while the control group performed EGF genotyping only.

RESULTS: EGF gene polymorphism 61*G was dominant in HCC patients. The G/G owns the highest concentration when compared with A/A and A/G genotypes, with high statistical significance between studied groups as regard number and percentage ($p < 0.0001$).

CONCLUSIONS: EGF gene polymorphism 61*G was associated with HCC risk. Moreover, the increased concentration of EGF was associated with G/G genotype.

Key words: Genetic variation; Gene polymorphism; Epidermal growth factor; Hepatitis C virus; Hepatocellular carcinoma

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INTRODUCTION

Hepatocellular carcinoma (HCC) ranks the fifth among the most prevalent malignancies worldwide and is considered the main cause for chronic liver diseases related mortality, affecting about one million patients annually worldwide with the incidence equal to the mortality rate^[1].

In Egypt, approximately 4.7% of chronic liver disease patients had HCC, also HCC represents 70.48% of liver malignancies; its

occurrence is due to cirrhosis which in turn is a complication of HCV. Egypt has very high incidence of HCV, almost 6 million persons and HCV viremia was reported as 7.3%^[2,3].

HCC has multiple etiologies such as chronic hepatitis B and C infections, alcoholism, and other many etiological factors^[4]. Abnormal gene expression, epigenetic changes and chromosomal aberrations play an important role in development of HCC, however; the molecular mechanism is not yet clear^[5]. Recently, many signaling pathways especially pathways that regulate the physiological processes such as tumor cells growth, differentiation, migration, apoptosis, and angiogenesis had been studied in the era of HCC development such as the epidermal growth factor (EGF) signal pathway^[6].

In 1962, human EGF had been known to stimulate the proliferation and differentiation of epidermal and epithelial tissues, through binding to the EGF receptor (EGFR) supporting its role in malignant process^[7-10]. In mice, overexpression of human EGF enhances HCC development^[11,12]. EGF gene polymorphism has been associated with multiple human malignancies including HCC^[13,14].

The aim of this study is to evaluate the correlation between EGF polymorphism and HCC in Egyptian patients with HCV, to the best of our knowledge; this is the first study in Egypt to correlate EGF polymorphism with HCC.

METHODS

Study population

The current prospective study enrolled 100 adult patients with HCV related cirrhosis (Without or with HCC; Group I and II, respectively) who were admitted to the Tropical Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt and Internal Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt within the period from January 2015 to October 2015. Fifty healthy subjects, age and sex matched were included as a control group (Group III) [One control subject per case from the HCC patient family who was matched to the case by sex and age (within five years)]. The study protocol was approved by the ethical scientific committee of National Hepatology and Tropical Medicine Research Institute. This study was carried out in accordance with the guidelines of the declaration of Helsinki and its subsequent amendments. Written consent was obtained from all patients before starting this study.

The patients were subdivided into two groups, Group I which included 50 patients with HCV related cirrhosis and Group II which included 50 patients with newly diagnosed HCC on top of HCV related cirrhosis.

Patients with other liver cancers, metastatic liver cancer, acute and chronic hepatitis, autoimmune liver disorders, hereditary hepatic diseases, liver disease other than HCV, and previous diagnosis of HCC were excluded.

All patients were subjected to full history taking and complete clinical assessment. HCV related cirrhosis was diagnosed according to the history, clinical assessment, laboratory investigations, and abdominal ultrasonography (US). HCC was diagnosed by abdominal US, abdominal tri-phasic computed tomography (CT) and serum alpha fetoprotein (AFP) level (HCC patients with AFP level < 200 ng/mL were excluded, even in the presence of abnormal imaging).

Laboratory investigations

Samples

Twelve hours fasting venous blood samples (10 mL) were collected on EDTA anticoagulant tubes. Whole blood samples were stored at -80°C until assayed. Laboratory investigations and Alph fetoprotein assays were performed in duplicate according to the manufacturer's instructions.

EGF genotyping

DNA was purified from whole blood sample using an Invitrogen 96 blood kit (PureLink® Genomic DNA Kits). Genotyping assay was developed for EGF at position 61 (A > G) (SNP rs4444903) polymorphism using 5' nuclease assay TaqMan Assay (TaqMan® Universal Master Mix II) according to allele specific probes designed by the manufacturer. The following primers sequences were used for Polymerase Chain Reaction (PCR) amplification of genomic DNA; forward primer sequence: GGATCCTGAGCAATCA, and reverse primer sequence: TTACCGATCTTAATAA, alleles specific probes with sequences; TTTCGCTGGCGTGAAG for reporter1 and TCGCTGGCATGAAG for reporter 2. PCR amplification using 10 ng of genomic DNA was performed in a thermal cycler (Applied Biosystem) with an initial step performed at 95°C for ten minutes followed by 40 cycles at 92°C for 15 seconds and one minute for annealing at 60°C. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results were analyzed with Sequence Detection System software.

Statistical analysis

The collected data were analyzed using SPSS version 17 software (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentage while quantitative data were expressed as mean and standard deviation. Comparison of continuous data between more than two groups was made by using one way ANOVA for parametric data and Kruskal-Wallis test for nonparametric data with post-tests (Turkey and Dunn test, respectively). Chi square test was used for comparison between Categorical data. *P*-value < 0.05 was considered significant.

RESULTS

Our study included 150 subjects, the cirrhotic group (group I) (28 males and 22 females), their ages ranged between 40 and 67 years (mean age: 56.36 ± 6.518 years), the HCC group (group II) (32 males and 18 females), their ages ranged between 45 and 74 years (mean age: 58.62 ± 6.54 years) and the control group (group III) (31 males and 19 females), their ages ranged between 44 and 69 years (mean age: 57.48 ± 6.231 years). There were insignificant differences between all the studied groups regarding age and sex (*P*-value 0.217 and 0.6955, respectively). For EGF polymorphism, there were three genotypes; A/A, A/G and G/G that were detected in the three studied groups. The genotype A/A was more dominant in the control group. The A/G genotype was more dominant in the cirrhotic group, while the G/G genotype was more dominant in the HCC group. Comparison between the studied groups in relation to number and percentage of EGF genotypes was statistically significant (*P*-value < 0.0001) (Table 1) (Figure 1).

By studying the concentration of EGF in each genotype for the three groups, it was found that there were significant differences in EGF concentration between the three genotypes in each studied group. The G/G owns the highest concentration when compared with A/A and A/G genotypes in the patients groups while in the control group, there was insignificant statistical difference between the A/G and G/G as regards to EGF level (Figure 2) (Table 2).

DISCUSSION

Epidermal Growth Factor stimulates the growth and differentiation of malignant cells, as well as normal epithelial cells. EGF has been shown to enhance the lung, breast, colon, and bladder malignancies^[15]. In mice, gene expression profiles comparing normal and malignant hepatic tissue proved the autocrine mechanism for EGF in HCC^[11].

Several recent mechanistic studies support relation between HCV and EGF. HCV cellular entry is facilitated by a mechanism mediated by EGFR^[16,17].

The association between EGF polymorphism and the risk of developing HCC was initially reported by Tanabe *et al* 2008 in two case-control studies and showed that; in patients with alcoholic and hepatitis C-associated cirrhosis, the EGF 61*G allele is highly associated with the increased risk of HCC compared with the A allele, where; cirrhotic patients with G/G and A/G genotype had a 4 and 2.4 fold for developing HCC, respectively when compared with A/A genotype patients^[13].

These results are in agreement with the present study which showed that the G/G genotype was the most dominant genotype in HCC patients (84% of our HCC patients) followed by A/G genotype in only 10%. A/G was the most dominant genotype in cirrhotic patients (70% of cirrhotic patients), while A/A genotype was the most prevalent in the control group (84% of controls). These results indicate that the G allele may have a key role in hepatocarcinogenesis, while A/A genotype may have a protective role.

In the direction of our results, another study carried out by Abu Dayyeh *et al* 2011, showed that the EGF genotype G/G (rs4444903) was associated with increased risk for HCC in chronic Hepatitis B virus (HBV) infected Chinese population^[18].

Also, Zhong *et al* 2012 made their meta-analysis on eight studies, involving 1,304 HCC patients and 2,613 controls and found that patients with EGF 61*G allele had significantly higher risk of HCC when compared with A/A and G/A genotypes^[19].

Suenaga *et al* 2013 stated that the ratios of A/A, A/G, and G/G

genotypes were 5.3%, 42.8%, and 51.9%, respectively, in 208 patients with HCC, whereas in 290 patients without HCC, the ratios were 8.6%, 35.9%, and 55.5%, respectively with insignificant difference. The G allele (A/G and G/G) had higher risk for developing HCC especially in HCV patients when compared with A/A patients.²⁰ But they could not explain why HCC was higher in patients with A/G genotype when compared with the G/G genotype; however, this has been observed occasionally, in other studies^[13,21,22].

Yuan *et al* 2013 showed that among non-Asians in Los Angeles, patients with G allele had higher risk of HCC when compared with the A/A genotype even after adjustment for multiple risk factors for HCC.²³ Yoshiya *et al* 2014 conducted a retrospective study on 141 patients and found that G/G was present in 69 patients (48.9%), A/G in 56 (39.7%) and A/A in 16 (11.4%)^[24].

In opposition to our results, Qi *et al* 2009 certified that there was no association of EGF rs4444903 and HCC in Chinese patients with chronic HCV infection. The researches perceive that the association between EGF rs4444903 and the risk of HCC is still controversial and ambiguous^[25]. This situation may be due to some facts such as ethnic diversity, control selecting, and small sample size selection.

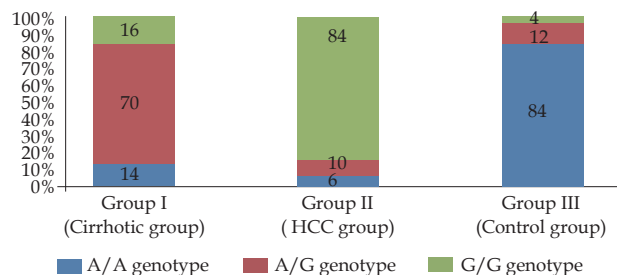


Figure 1 Epidermal growth factor genotypes distribution among the studied groups.

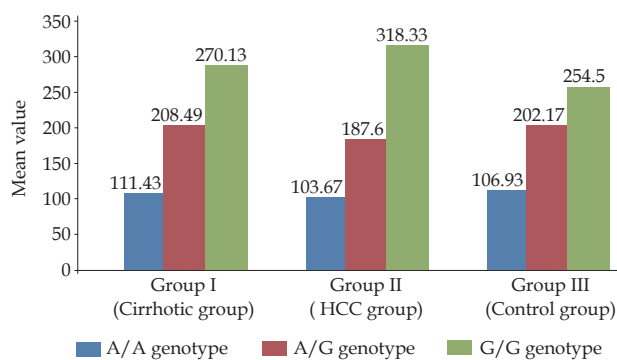


Figure 2 Epidermal growth factor serum level in different EGF genotypes of the studied groups.

Table 1 Comparison between the studied groups regarding number and percentage of epidermal growth factor genotypes.

EGF genotypes		Group I (Cirrhotic group) (N = 50)	Group II (HCC group) (N = 50)	Group III (Control group) (N = 50)	P*
A/A genotype	No	7	3	42	<0.0001*
	%	14%	6%	84%	
A/G genotype	No	35	5	6	
	%	70%	10%	12%	
G/G genotype	No	8	42	2	
	%	16%	84%	4%	

P*: P-value.

Table 2 Comparison between the studied groups regarding epidermal growth factor level in different EGF genotypes.

EGF level	A/A genotype;		A/G genotype;		G/G genotype;		P- value	Post-test	
	Mean ± SD (Range) (Median)		Mean ± SD (Range) (Median)		Mean ± SD (Range) (Median)				
Group I (Cirrhotic group) (No=50)	111.43 ± 11.802 (94-128) (114)		208.49 ± 32.866 (139-258) (219)		270.13 ± 39.223 (190-303) (285.5)	> 0.0001 *	P1	> 0.001 *	
							P2	> 0.001 *	
							P3	> 0.001 *	
Group II (Hepatocellular carcinoma group) (No=50)	103.67 ± 9.609 (95-114) (102)		187.6 ± 23.891 (152-211) (199)		318.33 ± 43.559 (237-404) (325)	> 0.0001 *	P1	> 0.05	
							P2	> 0.001 *	
							P3	> 0.001 *	
Group III (Control group) (No=50)	106.93 ± 8.019 (85-130) (106)		202.17 ± 9.704 (189-214) (202)		254.5 ± 14.849 (244-265) (254)	> 0.0001 *	P1	> 0.001 *	
							P2	> 0.05 *	
							P3	> 0.05	

P1: group A/A vs. A/G; P2: group A/A vs. G/G ; P3: group A/G vs. G/G* Significant.

In our study, we found that there were significant differences in EGF concentration between the three genotypes in each studied group. The G/G owns the highest concentration when compared with A/A and A/G genotypes in all groups except in the control group there was insignificant difference between the A/G and G/G as regards to EGF level.

In accordance with our results, some researchers have shown (in a subset of subjects selected randomly from their larger cohorts) significantly higher serum and hepatic EGF levels in those with genotype G/G compared with those with genotype A/A^[13,21]. This may be due to that EGF gene polymorphism affects serum EGF concentration^[19]. Also, Tanabe *et al.*^[13]. 2008 found that epidermal growth factor levels were significantly higher in G/G patients than A/A patients. Abu Dayyeh *et al* 2011 showed that serum EGF protein levels varied among the different EGF genotypes and between subgroups with and without HCC. An association between the EGF genotype and higher serum EGF protein levels was seen among subjects with at least one G allele^[18].

Furthermore, although falling short of statistical significance, subjects with HCC had a median serum EGF protein level that was 27% higher than that of subjects without HCC, an increase that was more pronounced among those with genotype G/G (median level for subjects with HCC was 63% higher than that for subjects without HCC) but was less pronounced among those with genotype A/A (median level for subjects with HCC was 20% higher than that for subjects without HCC). This was noticed also by Yoshiya *et al* 2014 who found that the A/A group had a significantly lower serum EGF concentration than the AG/GG group^[24].

To conclude, in the present study, EGF gene polymorphism 61*G was associated with HCC risk. Add to that, the increased concentration of EGF was associated with G/G genotype.

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