Association between Epidermal Growth Factor +61A/G (rs4444903) Gene Polymorphism with Risk of Hepatocellular Carcinoma in Cirrhotic Hepatitis C Patients

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Background and study aim: The most frequent type of primary liver cancer is hepatocellular carcinoma (HCC). Unfortunately, HCC is only diagnosed when the cancer has advanced considerably. So, comprehensive HCC screening using molecular markers is critical for early identification. However, there are few researches that have studied the link between HCC and Epidermal Growth Factor (EGF) gene polymorphism. The goal of this study was to look into the link between the EGF +61A/G (rs4444903) gene polymorphism and HCC incidence in cirrhotic hepatitis C patients.

Patients and Methods: The study involved 90 people, including 60 cirrhotic patients with HCV who were further separated into 30 HCC patients and 30 non-HCC patients, as well as 30 seemingly healthy age and sex-matched controls. A Qiagen DNA extraction kit was used to extract DNA from blood samples. The genotyping of EGF 61A > G polymorphisms was carried out using the fluorogenic 5'-nuclease test in accordance with allele-specific primers, and SNP rs444903 was detected using the TaqMan Genotyping Assay Applied Biosystems ThermoFischer kit.

Results: In comparison to the control group, HCC (p= 0.003) and non-HCC (p= 0.027) patients had a statistically significant increase in EGF G allele expression. Furthermore, those with the GG genotype were also more likely to develop HCC than those with other genotypes (AG, AA).

Conclusion: Identification of EGF gene polymorphism can be a promising screening tool for predicting HCC development in cirrhotic HCV patients. Subsequently, understanding this can potentially offer better preventive and therapeutic strategies according to the individual patient.

INTRODUCTION

Worldwide, HCC represents the fifth commonest cancer. Mostly, HCC is diagnosed in association with liver cirrhosis **[1]**. Among the Egyptian population, HCC is the fourth most frequent cancer **[2]**, furthermore, lately in Egypt, there is a twofold surge in HCC incidence among patients suffering from chronic liver disease **[3]**.

The earlier the detection of HCC, the better prognosis. Nowadays, EASL guidelines don't recommend serum AFP for HCC surveillance, owing to its poor sensitivity in detecting early lesions **[4]**. This necessitates the exploration of an alternative sensitive, reliable and non-invasive biomarker tool for early detection of HCC.

Comprehension of HCC biology has revealed that hepatocarcinogenesis is affected by accumulations of genetic mutations **[5]**.

The receptor of epidermal growth factor (EGFR) is overexpressed in many cancers and is increased in the liver. Cell survival, cell cycle progression, tumor invasion, and angiogenesis are all regulated by EGFR in carcinogenesis **[6]**.

The polymorphism of EGF + 61 A/G (rs4444903) is a frequent SNP (single nucleotide polymorphism) in the EGF gene's 5′-untranslated region that affects EGF gene transcription **[6]**.

Up to date, researches directed for understanding the relation between the polymorphism of EGF +61A/G and HCC risk are limited. Accordingly, the current work aimed to study this relationship to reveal the importance of molecular biomarkers to assess the likelihood of HCC developing in an individual patient, hence tailoring better preventive and therapeutic strategies..

PATIENTS AND METHODS

- ***Study design***

The Department of Tropical Medicine at Main University Hospital of Alexandria enlisted 90 volunteers; including 60 cirrhotic HCV infected patients and 30 seemingly healthy age and sex-matched controls.

In this case-control study; cirrhotic HCV patients were divided into 30 HCC and 30 non-HCC patients; HCV infection was confirmed in all patients by anti-HCV antibodies and HCV RNA.

Exclusion criteria included patients with: Co-infection with HBV, Malignancy other than HCC, Immunosuppression, Organ transplantation, and autoimmune diseases.

All the patients in HCC group did not receive directly acting antiviral drugs (DAAs) for HCV infection.

- ***Methods:***

Materials:

After gaining informed consent from 90 individuals, whole blood samples on EDTA were taken. Until DNA extraction, all samples were stored at -80 C.

Laboratory and Radiological workup:

All patients and controls were given a complete medical history and clinical examination. Complete blood count and ESR, blood glucose level (fasting), serum level of urea and creatinine, liver enzymes and function tests including serum levels of aspartate transaminase, alanine transaminase, albumin, bilirubin, alkaline phosphatase, prothrombin time and INR, HCV Ab and HBs Ag, as well as estimation of serum AFP were all performed on all subjects. All test subjects were subjected to radiological examination, which included abdominal and pelvic ultrasonography. All patients whose ultrasound abdomen confirmed the presence of localised hepatic lesions also had a Triphasic CT abdomen done.

Liver cirrhosis was diagnosed by abdominal ultrasonography.

Quantification of HCV RNA level:

The blood HCV RNA level was measured.

The detection limit was 12 IU/mL using TaqMan® 48 automatic fluorescent quantitative polymerase chain reaction (q-PCR) kits and a Roche COBAS® AmpliPrep®/COBAS® TaqMan® 48 Analyzer (Roche Diagnostics, Mannheim, Germany).

Genotyping of EGF gene:

Collected whole blood samples from all participants, were initially utilized to extract DNA using Qiagen DNA extraction kit ® QIAmp DNA extraction Trademark Thermo Fisher Scientific Inc. Mini kit spin column. To confirm the availability of enough DNA yield, we used a nanodrop to estimate the extracted DNA. The fluorogenic 5'-nuclease assay was used to genotype the EGF 61 A > G polymorphisms using allele-specific primers and following the manufacturer's instructions. TaqMan Genotyping Assay Applied Biosystems ThermoFischer kit was utilized, which detects SNP rs444903. The following oligonucleotide primer sequences were utilized for PCR amplification, EGF forward:

CTTTCAGCCCCAATCCAAGGGTTGT [A/G] reverse:

GCTGGAACTTTCCATCAGTTCTTCC.

A total of 15 ul of reaction mixture was made up of 5.0 ul 2X TaqMan® Master Mix, 0.5 ul 20X Assay Working Stock, 4.5 ul extracted DNA, and 4 ul Nuclease free water. Stepone Applied Biosystems provided the thermal cycler, which was set to a holding temperature of 95°C for 10 minutes for polymerase to be activated, then 40 cycles of DNA denaturation at 95°C for 15 seconds each, followed by 40 cycles of 60°C annealing/extension for 1 minute each.

Allelic discrimination plots

TaqMan® Genotyper Software, Thermo Fisher Cloud Genotyping Application, and real-time PCR equipment software display allelic discrimination results as a plot of Allele 1 (VICTM dye) versus Allele 2 (FAMTM dye). Each sample is designated as a separate point on

the allelic discrimination plot as a homozygote, heterozygote, or no template control.

- ***Data Analysis (statistical):***

The current work's data was analysed by IBM SPSS software version 20.0. (Armonk, NY: IBM Corp). We utilised number and percent for qualitative data, and range (minimum and maximum), mean, median, and standard deviation for quantitative data. The significance of the findings was calculated at a 5% level of significance.

The Chi-square test was used to analyse a comparison between research groups in terms of categorical features. For quantitative variable distributions, the Kolmogorov-Smirnov test, Shapiro-Wilk test, and D'Agstino test were employed, while for the vision test, the Histogram and QQ plot were utilised. Parametric tests were used if the data were evenly distributed; non-parametric tests were used if the data were irregularly distributed.

The F-test (ANOVA) and Post Hoc test (Tukey) were employed to compare the groups if the data were evenly distributed. In the event of anomalous data distribution, the Mann-Whitney test was used to compare two independent populations, while the Kruskal Wallis test was used to compare the three groups, and the Mann-Whitney test was used to analyse pairwise comparisons. Correlations between two quantitative variables were determined using the Spearman coefficient.

RESULTS:

1- Demographic, AFP serum level (of the studied groups), triphasic CT findings, and BCLC (Barcelona Clinic Liver Cancer) for staging of HCC group: (Table I)

There was a male predominance in all the groups; 73.3%, 80%, and 66.7% in HCV with HCC, HCV without HCC, and control groups respectively without significant difference. The mean age tends to be in the fifties in all studied groups at which the difference was not significant.

For the HCC, non-HCC, and control groups, the mean AFP was 102.244.28 ngml, 3.040.66 ngml, and 3.080.94 ngml, respectively. The HCC group had a statistically significant difference from the cirrhotic non-HCC group (p = 0.001), as well as a difference that is statistically significant from the controls (p = 0.001).

In the group of HCC, Triphasic CT abdomen showed that 33.3% have a single focal lesion, 50% have two focal lesions and 16.7% have three focal lesions, the mean of focal lesion size was 3.19 ± 1.18, the patients who have portal vein thrombosis were 43.3%. 43.3% of the HCC group patients have metastasis most commonly in the lung (61.5%).

In the HCC group, according to BCLC staging, stage (A) was found in 20%, stage (B) BCLC was found in 16.7%, stage (C) was detected in 13.3%, while stage (D) was presented in 50%.

2- EGF

- Distribution of EGF genotypes and alleles in the examined groups (Table II)

EGF genotypes distribution was as following: AA genotype was found in 30%,13.3% and 36.7% for HCC group, non HCC group and controls respectively. AG genotype was detected in 33.3%, 53.3%, and 60.0% in the HCC group, non HCC group and controls respectively. GG genotype was found in 36.7%, 33.3%, and 3.3% respectively. There were statistically significant differences between the HCC and control groups (p 0.004), as well as between the cirrhotic HCV and control groups (p 0.005).

EGF A allele was discovered in 46.7% of HCC patients, 40.0% of cirrhotic HCV patients, and 66.7% of control, respectively, and G allele was found in 53.3%, 60.0%, and 33.3% of HCC patients, cirrhotic HCV patients, and control, respectively. There were statistically significant differences between HCC patients and controls (p 0.027) as well as cirrhotic HCV patients and controls (p 0.00).

- Polymorphism of EGF+61 A/G Assessment as a hazard factor for HCC development in cirrhotic patients (Table III)

Patients carrying EGF GG genotype or G allele are 13.44-fold, 2.28-fold (respectively) increased risk for HCC development than those carrying AG genotype or A allele.

- Relation between Epidermal growth factor alleles and BCLC staging (Table IV)

Regarding epidermal growth factor A allele, BCLC stage D was found in 35.7% followed by stage A which present in 32.1. While in EGF G allele, BCLC stage D was detected in 62.5%

followed by stage B which present in 15.6%. Between the two alleles, there was no statistically significant difference regarding BCLC staging.

- Relation between EGF alleles and triphasic CT findings in HCC group (Table V):

Regarding focal lesions number; in patients with A allele, one focal lesion was presented in 25%, two focal lesions were presented in 53.6%, while three focal lesions were found in 21.4%. In patients with G allele, one focal lesion was found in 40.6%, two focal lesions were detected in 46.6% and three lesions were detected in 12.5%.

In patients with A allele, the mean of the focal lesion size was 3.11 ± 1.21cm, while in patients with G allele the mean lesion size was 3.26 ± 1.14.

As regard portal vein thrombosis, it was detected in 35.7% of patients with A allele, while in patients with G allele portal vein thrombosis was found in 50.0%.

In patients with A allele, tumor metastasis was found in 32.1% with the high percentage in the lung (44.4%), then in the lung and abdominal LN (33.3%) and finally in the head of the pancreas (22.2%). Regarding patients with G allele; tumor metastasis was found in 53.1% with a high percentage in the lung (70.6%) then in the bone (32.5%) and finally in the lung and abdominal LN (5.9%).

There were no significant differences between the 2 alleles and the different triphasic CT findings of HCC.

Table (I): Demographic data AFP serum level (of the three studied groups) , triphasic CT findings and BCLC staging in HCC group:

	HCC group (n = 30)		Non-HCC group (n = 30)		Control (n= 30)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Gender								
Male	22	73.3	24	80	20	66.7	χ^2= 1.364	0.506
Female	8	26.7	6	20	10	33.3		
Age (years)								
Min. – Max.	40.0 – 64.0		40.0 – 63.0		40.0 – 60.0		F= 2.392	0.097
Mean ± SD.	54.03 ±5.92		53.10 ±5.42		51.10 ±4.47			
Median	53.0		53.0		51.0			
AFP (ng\ml)								
Min. – Max.	52.0 – 280.0		2.10 – 4.50		2.0 – 5.30		F= 150.313*	<0.001*
Mean ± SD.	102.2 ±44.28		3.04 ±0.66		3.08 ±0.94			
Median	90.50		3.0		2.83			
Sig.bet.Grps	p_1<0.001*, p_2<0.001*, p_3=1.000							
Triphasic CT findings								
Focal lesion number								
1	10	33.3						
2	15	50.0						
3	5	16.7						
Focal lesion size (cm)								
Min. – Max.	1.0 – 5.0							
Mean ± SD.	3.19 ± 1.18							
Median	3.70							
Portal vein thrombosis								
No	17	56.7						
Yes	13	43.3						
Metastasis								
No	17	56.7						
Yes	13	43.3						
Metastesis site (n= 13)								
Lung	8	61.5						
Bone	2	15.4						
Head of the pancreas	1	7.7						
Lung and Abdominal LN	2	15.4						
BCLC staging								
Stage A	6	20						
Stage B	5	16.7						
Stage C	4	13.3						
Stage D	15	50						

χ^2: Chi-square test F: ANOVA test U: Mann Whitney test H: Kruskal Wallis test,

p: Comparing between the different studied groups

p_1: Comparing between the **HCC** and **Non-HCC** groups

p_2: Comparing between the **HCC** and **Control** groups

p_3: Comparing between the **Non-HCC** and **Control** groups.

*: Statistically significant at $p \leq 0.05$

Table (II): Comparison of the three groups studied in terms of EGF genotypes and alleles

	HCC group (n = 30)		Non-HCC group (n = 30)		Control (n= 30)		□□	P
	No.	%	No.	%	No.	%		
Genotype								
AA	9	30.0	4	13.3	11	36.7		
AG	10	33.3	16	53.3	18	60.0	13.886*	0.008*
GG	11	36.7	10	33.3	1	3.3		
Sig.bet.Grps	p_1=0.187, p_2=0.004*, p_3= 0.005*							
Allele								
A	28	46.7	24	40.0	40	66.7	9.249*	0.010*
G	32	53.3	36	60.0	20	33.3		
Sig.bet.Grps	p_1=0.461, p_2=0.027*, p_3= 0.003*							

χ^2: **Chi-square test**
p: Comparing between the different studied groups
p_1: Comparing between the **HCV+HCC** and **HCV** groups
p_2: Comparing between the **HCV+HCC** and **Control** groups
p_3: Comparing between the **HCV** and **Control** groups
*: Statistically significant at $p \leq 0.05$

Table (III): A comparison between HCC and control groups based on EGF +61 A/G gene

	HCC group (n = 30)		Control (n= 30)		OR	p	95 % C.I	
	No.	%	No.	%			LL	UL
Genotype								
AA®	9	30.0	11	36.7				
AG	10	33.3	18	60.0	0.679	0.517	0.21	2.19
GG	11	36.7	1	3.3	13.444	0.022*	1.46	124.86
Allele								
A	28	46.7	40	66.7				
G	32	53.3	20	33.3	2.286	0.028*	1.09	4.78

®: Reference
OR: Odd`s ratio
L.L: Lower limit
U.L: Upper limit
C.I: Confidence interval
*: Statistically significant at $p \leq 0.05$

Table (IV): Relation between an allele and BCLC staging in HCC group

BCLC staging	Allele				□²	MCp
	A		G			
	No.	%	No.	%		
Stage A	9	32.1	3	9.4		
Stage B	5	17.9	5	15.6	□□□□□	0.108
Stage C	4	14.3	4	12.5		
Stage D	10	35.7	20	62.5		

χ^2: **Chi-square test** **MC: Monte Carlo**
p: Association between an allele and different parameters p-value.

Table (V): HCC group; EGF alleles and triphasic CT findings relation:

	Allele				Test of Sig.	p
	A		G			
	No.	%	No.	%		
Focal lesion number						
1	7	25.0	13	40.6	□□□	0.379
2	15	53.6	15	46.9	□□□□□	
3	6	21.4	4	12.5		
Focal lesion size (cm)						
Min. – Max.	1.0 – 5.0		1.0 – 5.0		**U**=424.0	0.720
Mean ± SD.	3.11 ± 1.21		3.26 ± 1.14			
Median	3.70		3.80			
Portal vein thrombosis						
No	18	64.3	16	50.0	□□=1.241	0.265
Yes	10	35.7	16	50.0		
Metastasis						
No	19	67.9	15	46.9	□²□	0.102
Yes	9	32.1	17	53.1	□□□□□	

χ^2: Chi-square test U: Mann Whitney test MC: Monte Carlo
p: Association between an allele and different parameters p-value.

DISCUSSION

Treatment of CHC with directly-acting antiviral Understanding liver carcinogenesis has revealed that accumulations of mutations (epigenetic and genetic) can play an important role in its development. Information from investigations carried on candidate-gene and genome-wide associations studies have emphasized the genetic variants contribution to determine HCC susceptibility **[5]**.

Hepatocarcinogenesis induced by HCV is a multifactorial process and is triggered by many elements such as HCV viral proteins and immunologic reply to cytokines.

EGFR stimulation is essential for cellular internalization of several viruses, including HCV. It belongs to the ErbB family of receptor tyrosine kinases, which are found on liver cells. It also plays a role in a variety of cancers. It regulates cell survival, cell cycle progression, tumour invasion, and angiogenesis, all of which are important in carcinogenesis. Epidermal growth factor ligands bind to EGFR stimulating signal transduction pathways, eventually causing differentiation and proliferation of epithelial and epidermal tissue. Thus the EGFR signaling pathway was postulated to contribute to the occurrence of inflammation and HCC **[6]**.

The +61A/G polymorphism (rs4444903), which is an SNP found in the EGF gene's 5′-untranslated region (5′-UTR), affects EGF serum levels. People who have EGF genotype G/G will have higher EGF level, in addition to having an increased risk of different malignancies such as gastric cancer, lung cancer, and malignant melanoma **[6]**. The current work aimed to study the EGF gene polymorphism impact in patients with HCV induced cirrhosis complicated by HCC or not.

Genotypic analysis of studied groups showed that G/G was the predominating genotype in HCC patients followed by A/G genotype. A/G was the predominant genotype in cirrhotic patients. Furthermore, statistically significant differences were discovered between the HCC control groups, and between cirrhotic HCV group and control group. These results indicate that the G allele may have a key role in hepatocarcinogenesis. These results were in the same line as El Bendary et al where they also concluded a predominance of G allele in their HCC studied patients **[7]**.

The current study revealed that EGF A allele was found in 46.7%, 40.0%, and 66.7% in HCC, cirrhotic HCV, and control groups respectively, G allele was detected in 53.3%,60.0%, and 33.3% in HCC, cirrhotic HCV, and control groups respectively. These results suggest a protective role of the A allele in cirrhosis on top of HCV.

Tanabe et al. **[8]** found a substantial link between the EGF +61A/G SNP G allele and HCC, with an odd ratio of roughly 4.0, which matches the findings of the current study. Furthermore, Abu Dayyeh et al **[9]** detected a link between G/G genotype of EGF and the development of HCC in both Caucasians and Afro-Americans. In addition, Jian-Min Yuan et al.**[10]** found that an EGF genetic variant causes a rise in EGF serum levels, which may raise the risk of HCC in low-risk patients.

On the contrary, Qi et al.**[11]** conducted a study on hepatitis B virus chronically infected Chinese patients, they discovered no link between the EGF +61A/G genotype and the risk of HCC. This may be linked to a fact that other loci may be associated with HCC due to HBV infection.

In this study, EGF genotypes and alleles were not associated with HCC progression (tumor number, tumor size, portal vein thrombosis, and tumor metastasis). In addition, no correlation was found between the different alleles, BCLC staging and Child score which were agreed with Samy Kohla et al.**[12]** and Sergany et al.**[13]** Unfortunately, diagnosis of HCC is usually done after considerable progression of the disease, besides, current HCC therapeutic options are ineffective for most patients. So, early detection of high-risk populations is of great importance through effective screening program. Ordinarily, measurement of serum AFP and liver scan by ultrasound are considered the two main pillars for high-risk people screening although the low sensitivity and specificity of both tools. Accordingly, molecular markers identification associated with elevated HCC risk will improve definition of high-risk populations. This also assists in prevention and treatment strategies, through refining the selection of patients and tailoring specific preventative measures or acquiring adapted screening policies.

However, the small sample size of this study was a limitation; hence a larger study group is recommended.

CONCLUSION

This study found that identifying such features as the EGF +61 A/G polymorphism is of clinical value, as it can influence liver carcinogenesis and could be exploited to enhance preventative and curative approaches in managing HCC.

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Conflict of interest: None.

Ethical consideration :
The Research Review Committee of the Alexandria Faculty of Medicine approved this work (16#8#2018, EC serial protocol number: 0105630) that was confirmed to the 1975 Declaration of Helsinki. Each participant in the research gave his or her informed consent.

Highlights:

1- Patients carry EGF GG genotype are at higher risk for hepatocellular carcinoma development in patients with HCV cirrhosis.
2- Statistically significant increased expression of EGF G allele in cirrhotic patients with and without hepatocellular carcinoma.
3- Genotypic analysis of EGF gene and identification of 61* A/G polymorphism, might be a promising screening tool to predict disease outcome and hepatocellular carcinoma development in cirrhotic HCV patients.
4- Molecular markers should be considered for tailoring HCV disease management and prevention of hepatocellular carcinoma development.

REFERENCES

1. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018; 391: 1301–1314.
2. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level. *JAMA Oncol*. 2017; 3:1683–1691.
3. El Zayadi A-R, Badran HM, EMF B, Attia Mel-D, Shawky S, Mohamed MK, et al. Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J. Gastroenterol*. 2005; 11: 5193–5198.
4. Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J-L, et al. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol.* 2018; 69(1): 182-236.
5. Yu MC, Yuan J-M, Lu SC. Alcohol, cofactors and the genetics of hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2008; 23 Suppl 1(Suppl 1):S92-S7.

6. Stoscheck CM, King LEJCr. Role of epidermal growth factor in carcinogenesis. *Cancer Res*. 1986; 46(3): 1030-1037.
7. Mahmoud El-Bendary, Mustafa. A. Neamatallah, Mohamed Abd El-Maksoud, Ahmed Elgendy, Maha Amin, Adel El-Wehedy, et al. Epidermal growth factor genetic polymorphism and its circulating serum level predict the risk of hepatocellular carcinoma in Egyptian patients with HCV (genotype-4)-related cirrhosis. *Intern Jour of Adv Res*. 2015; 3:697-705.
8. Tanabe, K. K., Lemoine, A., Finkelstein, D. M Kawasaki, H., Fujii, T., Chung, R et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. *JAMA*. 2008; 299: 53-60.
9. Abu Dayyeh BK, Yang M, Fuchs BC, Karl DL, Yamada S, Sninsky JJ et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. *Gastroenterol*. 2011; 141(1): 141- 149.
10. Jian-Min Y, Yunhua F, Simona O, Renwei Wang, David Van Den Berg, Sugantha Govindarajan, et al. Genetic polymorphisms of epidermal growth factor in relation to risk of hepatocellular carcinoma: two case-control studies. *BMC Gastroenterol.* 2013; 13:32.
11. Qi P, Wang H, Chen YM, Sun XJ, Liu Y, Gao CF. No association of EGF 5'UTR variant A61G and hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *Pathology*. 2009; 41: 555-560.
12. Samy Kohla M, Al-Haddad O, Al-Warraky M, Amer M, Ezzat S, Gabal A, et al. Association of serum levels of epidermal growth factor with disease severity in patients with unresectable hepatocellular carcinoma. *Hepatoma Res*. 2016; 2:18-25.
13. Sergany H, Mohamed AM, Madkour NK, Elsebeaey MA, Mohamed A, Elshaer SS, et al. Epidermal Growth Factor Gene Polymorphism in Egyptian Patients with Hepatocellular carcinoma related to Hepatitis C. *J Gastroenterol Hepatol Res*. 2017; 6(6): 2481-2485.