

## Prognostic Impact of Telomere Maintenance Gene Polymorphisms on Hepatocellular Carcinoma Patients with Chronic Hepatitis C

HEBAT ALLAH M. SHARAF EL-DEEN, M.D.; ENAS H. MAHMOUD, M.D.; AHMED A.E. HEKEL, M.D.; ALAA A. ABDEL RAUOF, M.D.; SUZAN E. MAHROUS, M.D. and ASMAA E.A. NASR ELDIN, M.Sc.

*The Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University*

### Abstract

**Background:** Telomere length and telomerase activity are crucial for cancer initiation and the survival of tumors. Telomere length is maintained by telomerase in 90% of human cancers, while 10% of cancers use an alternative mechanism of telomere lengthening termed (ALT). Another mechanism for Cancer cells is through activating or up-regulating the normally silent human TERT gene (hTERT) that encodes telomerase.

**Aim of Study:** The aim of the present study was to assess the effect of a SNP in TERT gene rs13167280 on telomere length, furthermore, a study was conducted to investigate whether the changes of median values of Relative Telomere Length (RTL) is related to a SNP genotype.

**Patients and Methods:** The study was conducted on a total number of 100 patients, subdivided into two groups HCC and HCV groups. SNP was measuring using Taqman probe on step-one real time PCR.

**Results:** It was found that HCC patients had high frequency of heterozygous AG genotype than HCV patients 30% versus 22% and both groups had no homozygous AA genotype. Moreover, the frequency of G allele comparing to A allele in SNP rs13167280 was higher in HCV than HCC 90% versus 85% respectively.

**Conclusion:** There was no statistical significant difference observed on comparing a SNP rs13167280 in HCC and HCV groups and there was no statistically significant association was found ( $p=0.34$ ) when we investigated whether the changes of median values of RTL is related to a SNP genotype.

**Key Words:** Hepatocellular carcinoma – Relative telomeric length – Telomere maintenance gene.

### Introduction

HEPATOCELLULAR cancer is the sixth most common cancer and the third leading cause of

**Correspondence to:** Dr. Hebat Allah M. Sharaf El-Deen, The Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University

death worldwide. It has a poor prognosis and the overall survival rates are 3-5%. HCC has a mortality rate of 9.1% of global cancer [5]. In Egypt, HCC is the second most common cancer in men and the sixth most common cancer in women and it was reported that HCC accounts for about 4.7% of chronic liver disease with a remarkable increase in this proportion from 4.0% to 7.2% over the last decade [11]. This rising incidence may be due to a high prevalence of HCV and its complications. HCV infection is the main etiology of liver cancer in about 40-50% of cases [11].

Chronic infection with Hepatitis C virus is considered one of the major causes of liver cirrhosis and HCC. It affects 130-170 million people worldwide with high prevalence reported in Egypt [8]. Egypt has one of the highest rates of HCV (~15%) worldwide [6].

Telomeres consist of repetitive sequences (TTAGGG) located at the ends of eukaryotic chromosomes which protect the integrity of genomic DNA [2]. Telomere length is maintained by telomerase in 90% of human cancers, while 10% of cancers use an alternative mechanism of telomere lengthening termed (ALT) [4]. Telomere shortening is a hallmark of cirrhosis and the main risk factor for the development of liver cancer through up-regulation of hTERT gene that encodes telomerase [12].

Telomeres protect chromosome ends from fusion and from being recognized as sites of DNA damage. Telomeres dysfunction arises from a critical shortening of telomeres in normal somatic cells during progressive cell divisions elicit DDRs that trigger cellular senescence [1].

Progressive shortening of telomere length may lead to senescence, apoptosis and oncogenic transformation of somatic cells which affects health and the life span of an individual [1].

Polymorphic variations such as Single-Nucleotide Polymorphisms (SNPs) within several gene classes are considered to be important for regulation the length of telomeres and the higher order structure of the protein complex at the ends of linear chromosomes. Telomere maintenance genes are essential for maintaining genomic integrity and stability [3]. There are five telomere maintenance genes that affect tumor progression in HCC patients (TERT, TRF1, TRF2, POT1, TEP1) [7].

### Patients and Methods

#### *Study design:*

This is cross-sectional study conducted at the Tropical Medicine Department and Internal Medicine Departments, Cairo University during the period from October 2015 to December 2016. Two groups of patients were enrolled; group 1 (HCC group) included 50 patients with mean age of  $56.74 \pm 7.9$ , their diagnosis was based on their medical records and the presence of hepatic focal lesions diagnosed by abdominal ultrasound and confirmed by triphasic Computed Tomography (CT) according to American Association for the Study of Liver Diseases (AASLD) guidelines and there were also on top of HCV liver cirrhosis. Group 2 (HCV group) included 50 patients, their diagnosis was based on their medical records with mean age of  $55.38 \pm 8.9$  years.

A written informed consent obtained directly from each subject before data collection and after explanation of the study objectives and the study protocol was approved by the Institutional review board of Cairo University.

#### *Biochemical investigations:*

Blood samples were collected and investigated for serum Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), total bilirubin, albumin, creatinine, Alpha Fetoprotein (AFP), Prothrombin Time (PT) and prothrombin International Normalized Ratio (INR), as well as complete blood count.

*Selection of Candidate Genes:* A SNP (rs13167280) was selected from telomerase reverse transcriptase for testing in this study. This SNP was chosen on the basis of previous reports of their association with the risk for HCC.

*Laboratory methods:* DNA was extracted from whole blood treated with EDTA using Gene JET DNA blood Mini kit-thermo Fisher Scientific according to the manufacturer's protocol. The concentration and purity of DNA were determined by measuring the absorbance at 260 and 280 nm using NanoDrop 1 000A Spectrophotometer (NanoDrop Technologies, Waltham, MA). Genotype identification was performed with Step One real-time PCR system (Applied Biosystems). Each PCR reaction mixture included: 5  $\mu$ l of the DNA, 10  $\mu$ l TaqMan Universal PCR Master Mix (2x), No AmpErase UNG, 1  $\mu$ l 20x working stock of SNP Genotyping Assay and 4  $\mu$ l DNase-free water to a final volume of 20  $\mu$ l using the following conditions: 95°C for 10min, 95°C for 15 seconds min and 60°C for 1min. The SNP were identified by their position and fluorescent color in each well according to the position of the tagged oligonucleotides. Genotype data was generated on the basis of the relative fluorescent intensities for a SNP. The Sequence Detection System (SDS) Software used the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well.

#### *Statistical methods:*

Statistical analysis was done using SPSS for Mac, release 24 (IBM Corporation, Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median (25<sup>th</sup>-75<sup>th</sup>) as appropriate. Differences between groups are detected using Student's *t* and Mann-Whitney tests as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Correlations between variables were done using Spearman's correlation test. All tests were two-tailed. A *p*-value <0.05 was considered significant.

### Results

*Characteristics of the study population:* The baseline characteristics of the studied population in our study are shown in (Table 1).

*Genotype and Allele frequency and effects on the development of HCC:* Compared with the HCV, the patients with HCC had a higher frequency of SNP rs 13167280 GA genotypes (30% versus 22%); moreover, the frequency of G allele compared to A allele was higher in HCV than HCC patients (90% versus 85). However, the SNP showed no statistically significant difference between the two groups in the allele and genotype distribution.

Table (1): Comparison of routine laboratory parameters of the studied groups.

Variables	HCC (n=50)	HCV (n=50)	p-value
TLC X 10 <sup>3</sup> /μL	5.4 (3.8-8.1)	7.3 (5.2-10.2)	0.002
Hb (gm/dl)	11 (9.6-12)	9.65 (8.5-10.6)	<0.001
PLT X 10 <sup>9</sup> /L	129 (88.5-160)	123.5 (78.7-194)	0.926
PT (sec.)	15.7 (14.1-17.5)	17.5 (14.9-22.2)	0.025
INR	1.3 (1.2-1.5125)	1.4 (1.2-1.6)	0.297
BUN (mg/dl)	28.5 (20-38.5)	42 (31-83)	<0.001
Creat. (mg/dl)	0.9 (0.7-1.3)	1.25 (1-2)	0.002
AST (U/L)	68.5 (48.7-94.2)	63.5 (39.2-97)	0.759
ALT (U/L)	36.5 (21.7-51.2)	37.5 (19-51.7)	0.888
T. Bil (mg/dl)	1.6 (1-4.15)	2.3 (1.35-7.05)	0.043
D. Bil (mg/dl)	0.5 (0.3-1.7)	1.05 (0.4-2.95)	0.063
Albumin (g/dl)	2.8 (2.2-3.1)	2.3 (2-2.9)	0.003
ALP (U/L)	103 (72-183.2)	81.5 (59.5-102.2)	0.015
GGT (U/L)	56 (38.2-110)	35 (26.7-80)	0.009
AFP (ng/mL)	12.95 (5-99.3)	15 (4-51.2)	0.241
HCV PCR (IU/ml)	101500 (76750-164250)	90000 (75000-119000)	0.339

All data are presented as median (25th-75th).  
 p-value <0.01 is moderately significant, p-value <0.001 is highly significant.

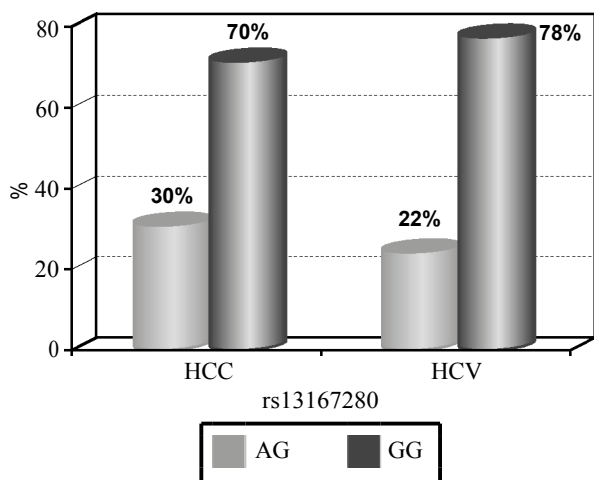


Fig. (1): Frequency distribution of rs13167280 genotypes in the two studied groups.

**Discussion**

Hepatocellular cancer is the sixth most common cancer and the third leading cause of death worldwide. It has a poor prognosis and the overall survival rates are 3-5%. HCC has a mortality rate of 9.1% of global cancer [6]. The Telomerase Reverse Transcriptase (TERT) gene encodes the catalytic subunit of telomerase, which mediates pleiotropic effects, including the regulation of senescence and proliferation and plays an important role in carcinogenesis [3]. This study attempts to clarify the genetic predisposition to HCC, focusing on the rs13167280 polymorphism. Because of its close association with telomere length, SNPs in TERT have been suggested as potential target genes for cancer therapy [9,10].

In the current study, compared with the HCV, the patients with HCC had a higher frequency of

SNP rs13167280 GA genotypes (30% versus 22%, p=); moreover, the frequency of G allele compared to A allele was higher in HCV than HCC patients (90% versus 85%, p=), but the difference did not reach the level of statistical significance (p-value >0.05).

In a study by Jung et al., on TERT gene other SNPs like TEP 1, POT 1, TERF 1 genes on tumor progression and their overall survival in patients with HCC on top of HBV, they reported that the TERT rs13167280 SNP (TC/TT genotype) was strongly associated with a lower likelihood of major vessel invasion and distant metastasis. Accordingly, the TERT rs13167280 SNP was associated with significantly improved survival, and this suggests that the T allele of TERT 13167280 acts as a preventive factor for the progression of HCC (p=0.015) [7]. They also measured RTL on patients subjected to surgical resection for HCC and found that the median level of RTL was 0.68 [7].

*Limitations:* Some limitations still exist and thus cautions are needed before interpreting the results obtained from the current study. First, ethnicity and small sample size. Second, the variation in HCC etiology might be discount as significant between-study heterogeneity was observed in some comparisons. Third the lack of other important information like environmental factors, age, drinking status, and gene-environment interactions.

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## التأثير النذير لصيانة التيلومير تعدد الأشكال الجينية على مرضى سرطان الكبد الوبائي المزمن المصابين بإلتهاب الكبد الوبائي المزمن

يعتبر سرطان الكبد هو سادس السرطان الأكثر شيوعاً والسبب الثالث الرئيسي للوفاة في جميع أنحاء العالم. يعتبر التيلومير تسلسلات متكررة (TTAGGG) الموجودة في نهايات الكروموسومات في داخل النواة التي تحمي سلامة الحمض النووي الجيني. يتم الحفاظ عليها من قبل التيلوميراز الذي هو النسخ العكسي ترانسكربتاس ويمد التيلوميراز ينتج إلى تقصير التيلومير مع دورات النسخ المتماثل.

العامل الجيني مثل تعدد أشكال النوكليوتيد التي يمكن أن تنظم تعبير التيلوميراز قد تؤثر على قابلية الإصابة بالسرطان وتم تحديد خطر زيادة كبيرة في تطوير سرطان الخلايا الكبدية للبروتين المرتبط بالتيلوميراز (TEP1 rs1713449) في كل من مرحلتى الإكتشاف والتكرار وبالإضافة إلى ذلك، فإن TEP1 rs872072، TEP1 rs1713449، وحمية التيلوميراز ١ هومولوغ rs7784168، تيلوميراز النسخ العكسي rs13167280، وتيلوميريك تكرار عامل ملزمة rs2306494. تعدد الأشكال كان لها تأثير كبير على البقاء على قيد الحياة بشكل عام.

في هذه الدراسة تم إختيار خمسين مريضاً تم تشخيصهم على أنهم مرضى سرطان الكبد وخمسين مريضاً بإلتهاب الكبد المزمن. تم قياس طول تيلومير النسبي في كلتا المجموعتين باستخدام سلسلة الوقت الحقيقي تفاعل البوليميراز. بالإضافة إلى طول التيلومير النسبي، درست أيضاً العلاقة بين جين التيلوميراز ترانسكربتاس العكسي (TERT) وخطرها لتطوير سرطان الكبد. تم إختيار سنسب للإختبار rs2306494. تم إجراء تحديد النمط الجيني باستخدام تكمان سنسب فحص الأنماط الجينية.

كشفت الدراسة الحالية أن مرضى سرطان الخلايا الكبدية لديهم تواتر أعلى في النمط الوراثة AG المتخالف وفقاً ل rs13167280 مقارنة بمرضى فيروس الإلتهاب الكبدى ٣٢٪ مقابل ٢٢٪ على التوالي وكان تواتر G أليل مقارنة ب A أليل أعلى في فيروس الإلتهاب الكبدى من سرطان الخلايا الكبدية ٩٠٪ مقابل ٨٥٪ على التوالي. ولكن الفرق لم يصل إلى مستوى دلالة إحصائية ( $p < 0.05$ ).

أجريت الدراسة الحالية للتحقق فيما إذا كانت التغيرات في القيم المتوسطة على طول التيلومير النسبي ترتبط بكل من النمط الجيني ولكن مع عدم وجود ارتباط ذو دلالة إحصائية.