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Circulating DiGeorge syndrome critical region (5) as a tumor suppressor gene in hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) was reported to have down-regulated DiGeorge syndrome critical region (5) (DGCR5). In HCC, it may function as a tumor-suppressive gene. To study DGCR5 relative expression level in HCC, a hundred and sixty participants were involved in this study. They were split up into two categories: Group I consisted of 70 patients with HCC identified by imaging (dynamic MRI or triphasic CT) or biopsy; Group II consisted of 90 healthy individuals acting as the control group. A complete history, a general clinical examination, the analysis of clinic pathological data for patients with HCC, and laboratory investigations were performed on all patients and controls. These procedures included the following: 1- Liver function tests, such as alpha-fetoprotein (AFP), aspartate and alanine transaminases (AST and ALT), total bilirubin and serum albumin, detection of HBsAg and HCV Ab. 2- Serum Creatinine. 3- Using real-time PCR, quantify the relative expression levels of (DGCR5). Serum creatinine, albumin, ALT, AST, and AFP levels were considerably higher in HCC patients than in controls, and DGCR5 relative expression level was down regulated. With total bilirubin, serum albumin, AST, and AFP, there was a statistically significant decline in the relative expression level of DGCR5 with TNM staging. Conclusion: DGCR5 acts as a tumor suppressor gene in HCC.

Keywords:
Alpha-fetoprotein, Alanine transaminase, Aspartate transaminase, DiGeorge Syndrome Critical Region, Hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver tumor and the third most common cause of cancer-related death in the Western area. Chronic liver infections, such as hepatitis B or hepatitis C viruses, non-alcoholic fatty liver disease, aflatoxins, and tobacco use are the known causes of HCC (Dimitroulis et al., 2017). According to the USA's Surveillance, Epidemiology and Results (SEER) database program, 65% of liver cancer cases are HCC instances (Ghouri et al., 2017). The third most frequent cause of cancer-related mortality, HCC results in over 700,000 deaths worldwide each year. The high frequency of chronic hepatitis C, immigration from regions where hepatitis B and hepatitis C are frequent, and the epidemic of nonalcoholic fatty liver disease are the only reasons why the death rate for HCC is rising in the United States and Canada (Bruix et al., 2016). Before the age of 40, HCC incidence is quite uncommon and tends to peak at around 70 years of age (Xie et al., 2017). A previous study indicated that one of the health issues Egypt's health officials are dealing with is HCC (El-Zayadi et al., 2005). Over ten years, they found that patients with chronic liver disease had an almost two-fold increase in HCC (Shaker, 2016). Over ten years, they found that patients with chronic liver disease had an almost two-fold increase in HCC (Fang and Fullwood, 2016). From brilliant

sub-nuclear foci to nearly exclusive cytoplasmic localization, lncRNAs exhibit a variety of subcellular localization patterns (Lennox and Behlke, 2016). While most are preferentially localized to the nucleus and chromatin, others are detected in both compartments (Singh and Prasanth, 2013). Evidence now available indicates that these compounds are essential for the control of particular physiological processes, as the transcriptional, post-transcriptional, and epigenetic expression of genes that code for proteins (Iyer et al., 2015). Many lncRNAs, particularly those related to cancer, have been functionally linked to human disorders. Papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3), for instance, DiGeorge Syndrome Critical Region 5 (DGCR5) is down regulated in HCC and downregulated in thyroid malignancies (Fan et al., 2013: Yu et al., 2017). The human non-coding RNA DiGeorge syndrome critical region gene 5 (DGCR5) is situated at chromosome 22q11 and has 3334 bp (Huang et al., 2016). It was discovered to be down regulated in HCC as well, and a low five-year survival rate was strongly correlated with low DGCR5 expression (Chen et al., 2017). This study's objective is to evaluate the expression levels of DGCR5 in patients with HCC.

2. Materials and Methods

Subjects

The Medical Biochemistry and Molecular Biology Department and the Clinical Oncology and Nuclear Medicine Department of Menoufia University's Faculty of Medicine conducted this work. The study was carried out between April 2017 and May 2018. There were 160 subjects in all. They were divided into two categories: Group I consisted of 70 patients, 58 of whom were male and 12 of whom were female, with HCC diagnoses made by biopsy or imaging modalities (dynamic MRI or triphasic CT). Group II acted as the control group and consisted of 90 healthy individuals. There were 14 females and 76 males present. The study participants provided written informed consent before participating. The Menoufia University Faculty of Medicine's Ethical Committee for Medical Research accepted the plan. The clinical history, general examination, and clinicopathological data analysis for patients with HCC (presenting symptoms, cirrhosis, portal hypertension, portal vein thrombosis, tumor site, tumor number, presence of metastasis, and child score) were performed on all patients and controls. Additionally, laboratory investigations were conducted, including an evaluation of the following: 1- Test for liver function, comprising AFP, ALT, AST, serum albumin, and total bilirubin. Detection of the hepatitis C viral antibody (HCVAb) and the hepatitis B surface antigen (HBsAg). 2. Autoanalyzer serum creatinine (Cobas Integra 400-Roche, Germany, 3. Using real-time PCR, quantify the relative expression levels of (DGCR5).

Specimen collection

Ten-milliliter venous blood samples were taken by sterile venipuncture using disposable syringes. The blood samples were given out in plain test tubes with a vacutainer. Following a 20-minute clotting period, 6 ml of serum was removed, and samples were divided into two fractions using centrifugation at 4000 rpm for 10 minutes. 4ml for liver function tests, 2ml were stored at -80°C until used for determination of DGCR5 relative expression levels by RT-qPCR. Serum albumin was measured using a quantitative method of enhanced specificity of bromocresol green colorimetric by (DIAMOND diagnostic kit, Germany). Serum total bilirubin was determined using (DIAMOND diagnostics Kit, Germany), and serum aminotransferase (ALT and AST) was determined by kinetic UV optimized method IFCC (ELTEC Kit, England) (Doumous et al., 1985; Bergmeyer et al., 1986; Pinnell and Northam, 1978). Five primary processes were involved in the quantitative real-time PCR for (DGCR5) expression in serum, the first of which was the separation of RNA from the serum samples. 2. Ensuring the purity and quality of RNA. 3. First process: reverse transcription process for cDNA synthesis. Step 4: PCR (real-time PCR step): cDNA amplification, plate document setup, and PCR run initiation. 5. Data analysis using version 2.0.1 of the Applied Biosystems 7500 software. First Step - PCR: cDNA Synthesis (RT- Step) using (High-Capacity cDNA Reverse

Transcription Kits, Applied Biosystems, USA). 20 µl of total reaction volume was used for each reaction, which was conducted on ice. The reaction mixture consisted of 1 µl reverse transcriptase enzyme, 4 µl reverse transcriptase buffer, 10 µl template RNA, and 5 µl nuclease-free water. A 2720 heat cycler from Applied Biosystems (Singapore) was used for one cycle of incubation. Reverse transcriptase is inactivated for 5 minutes at 95 ^{o}C after 10 minutes at 42 ^{o}C, and then for 5 minutes at 4 ^{o}C. The generated CDNA was kept cold until the real-time PCR stage. Real-time PCR was performed using SYBR Green with low ROX for detection of (DGCR5) gene expression using (QuantiTect SYBR Green PCR Kit, Applied Biosystems, USA). Twenty microliters total were used: ten microliters of SYBR green Master Mix, one microliter of nuclease-free water, six microliters of template cDNA, and one microliter of each primer (forward and reverse). The primers from Midland, Texas were utilized; the forward primer sequence for DGCR5.

Was: 5`CACGAGTGTAGTGCCCAGTT3` and reverse primers of DGCR5: 5`GGTCAGGGACCTTTGTCTGG3`, Forward and reverse primers of GAPDH (endogenous control): 5`CCACTCCTCCACCTTTGAC3`, Reverse primer: 5`ACCCTGTTGCTGTAGCCA3`. Three stages made up the PCR conditions for DGCR5 amplification: a 30-second initial activation phase at 95 ºC, 40 cycles at 95 ºC, 60 ºC, and 72 ºC for one minute, and a 10-minute final extension phase at 72 ºC. Lastly, 7500 ABI PRISM (Applied Biosystems, USA) v.2.0.1 was used for data processing and fluorescence detection. Using the comparative ΔΔCt technique, the relative quantification (RQ) of DGCR5 gene expression was determined (Dorak, 2004). Similar to Fig. 1, the DGCR5 gene's expression is normalized about control and to the endogenous housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). To confirm the PCR products' identification and specificity, melting curve analyses were conducted.

Statistical analysis

The clinical data was collected using a report form. The statistical software for social science, or SPSS, version 20 was used for the tabulation and analysis of this data, yielding the following results: detailed data After computing analytical and descriptive statistics for the data, the mean and standard deviation (±SD) of the quantitative data and the frequency and distribution of the qualitative data were obtained. To ascertain the significance of the variation in the statistical comparison between the several groups, one of the subsequent tests was employed: Student's t-test and Mann-Whitney test are used, respectively, to compare the parametric and non-parametric means of two sets of quantitative data.

3. Results

While there was a significant statistical difference in these categories, there was no significant difference in age, gender, or smoking among the study groups (Table 1). Serum creatinine, total bilirubin, albumin, ALT, AST, and AFP were statistically different between the two study groups (Table 2). There was a statistically significant decrease in the relative expression level of DGCR5 in Stage IV as compared to Stages II and III (Table 3). Table 4 shows that there was a significant statistical drop in the DGCR5 relative expression level in the HCC group concerning total bilirubin, serum albumin, AST, and AFP, but no significant statistical decrease concerning serum creatinine and ALT. The cutoff point for the relative expression level of DGCR5 between HCC patients and controls has a receiving operating characteristic curve (ROC) with an area under the curve of 0.807 (n=160) (Figure 2). At a cutoff point of.98, the test's accuracy is 83.1%, its specificity is 100%, its positive predictive value is 100%, its negative predictive value is 76.9%, and its sensitivity as a predictor of HCC is 61.4%.

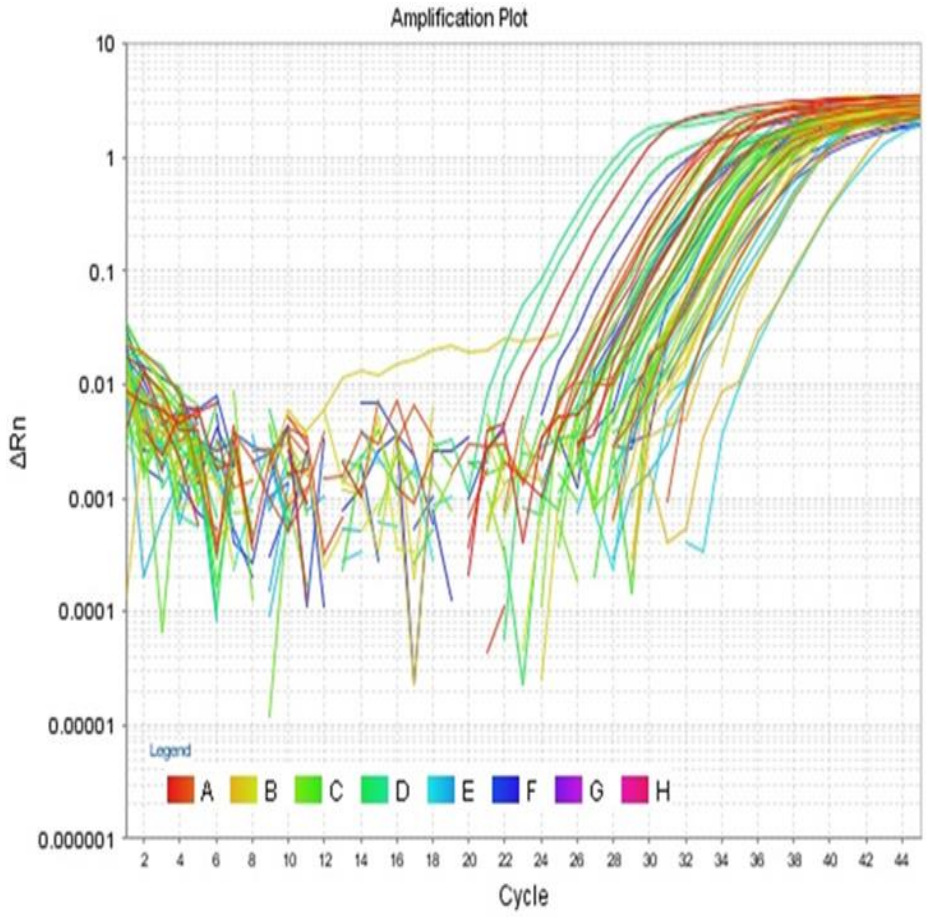

Fig. 1. Amplification plot of *DGCR5* expression (normalized fluorescence signal (Rn) plotted versus cycle number.

Table 1. Statistical comparison between the two studied groups regarding age, gender, and smoking (n=160).

	Age (years)	**Gender (No %)**		**Smoking**	
		Male	**Female**	**Yes**	**NO**
HCC group	60.57±5.24	(82.9)58	12(17.1)	46(65.7)	24(34.3)
Control group	61.49±8.82	76(84.4)	14(15.6)	6(6.7)	84(93.3)
Test	St t=0.77	x^2=0.07		x^2=62.58	
P. value	0.44	0.0.79		<0.001**	

The values represented mean ± SD; HCC: hepatocellular carcinoma, (-*X2*-value): chi-square test.

Table 2. Comparison between cases and controls regarding laboratory data (n=160).

Laboratory parameter	HCC group	Control group	Test	*P.* value
Serum creatinine (mg/dl)	0.97±0.15(0.6-1.4)	0.79±0.17(0.5-1.1)	St t=7.1	<0.001**
Total bilirubin (mg/dL)	1.73±0.95 (0-6.0)	0.57±0.24 (0.2-1)	St t=11.	<0.001**
Serum albumin (gm/dL)	3.03±0.47 (1.8-4.0)	4.22±0.55 (3.4-5)	St t=14.	<0.001**
ALT (U/L)	56.8±33.2 (18-136)	25.87±8.74 (9-43)	St t=8.4	<0.001**
AST (U/L)	63.2±38.5 (19-161)	26.20±9.6 (9-43)	St t=8.8	<0.001**
AFP (ng/ml)	3899.7±12436.7 (16.0-72776	3.67±1.41 (1-6)	St t=2.9	0.003**

The values represented means ± S.D; **: highly significant, ALT: alanine transaminase, AST: aspartate transaminase, AFP: alpha-fetoprotein, HCC: hepatocellular carcinoma.

Table 3. Comparison between DGCR5 relative expression levels among different stages of HCC (n=70).

	DGCR5 relative expression level
Stage I and II(20)	0.76±0.41 (0-1)
Stage III (20)	0.74±0.39 (0-1)
Stage IV(30)	0.50 ± 0.35^{ab} (0.1-1)
F- test	$x^2= 9.04$
P. value	0.011*

The values represented means ± S.D; a: sig&stage I; b: sig&stage II; *: significant; **: highly significant; HCC: hepatocellular carcinoma; DGCR5: DiGeorge syndrome critical region gene5.

Table 4. Correlation between DGCR5 relative expression level and laboratory data in HCC group (n=70).

relative expression of DGCR (5)		
Laboratory parameter	**r**	***P.* value**
Serum creatinine (mg/dl)	-0.142	0.24
Total bilirubin (mg/dL)	-0.285	0.017*
Serum albumin (mg/dL)	-0.249	0.038*
ALT (U/L)	-0.184	0.127
AST (U/L)	-0.551	<0.001**
AFP (ng/ml)	-0.271	0.001**

The values represented means ± S.D; *: significant, **: highly significant, ALT: alanine transaminase, AST: aspartate transaminase, AFP: alpha fetoprotein, HCC: hepatocellular carcinoma, DGCR5: digeorge syndrome critical region gene 5, r: correlation coefficient.

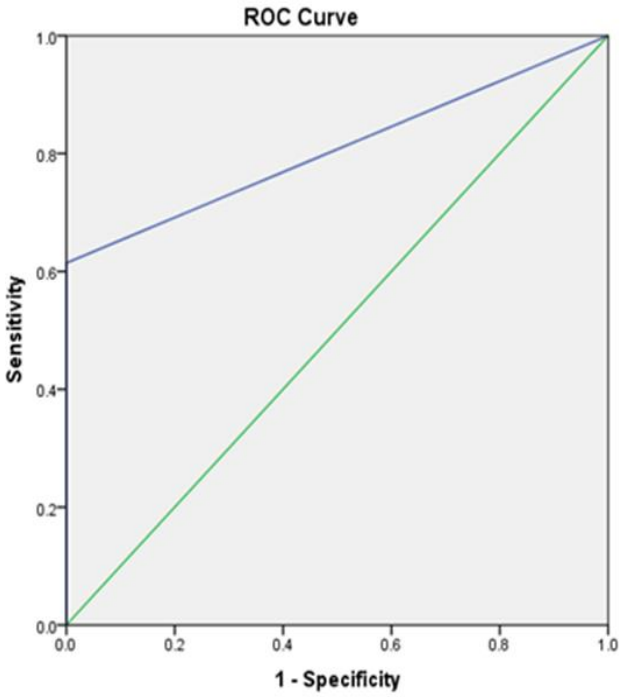

Fig. 2. Receiving operating characteristic curve (ROC) DGCR5 between HCC patients and controls (n=160).

Discussion:

As on the World Health Organization's estimations (Marrero et al., 2018) HCC is presently the fifth most frequent cancer and the third cause of cancer-related mortality globally. The development of HCC is frequently caused by chronic liver dysfunction. Viral hepatitis, which can result from an HBV or HCV infection, is thought to be the most frequent cause of HCC (Wang et al., 2017). Lately, a growing number of lncRNAs have been discovered to be important regulators in a number of cancers, including HCC. Consequently, it's critical to pinpoint specific lncRNA targets linked to the growth of HCC (Wang et al., 2018). LncRNAs are noncoding transcripts with more than 200 nucleotides (Wang et al., 2018). DiGeorge syndrome critical area gene 5 has been linked to HCC and is thought to be a potential biomarker for the condition (Wang et al., 2018). This investigation compares the expression levels of DGCR5 in HCC patients. The current study's findings showed that the age and gender distributions of the patient and control groups were comparable. The current study's mean age of HCC patients was 60.57±5.24) years, which is comparable to findings from Yapali and Tozun (Yapali and Tozun, 2018), who found that the median age at which HCC cases are diagnosed in Egypt is 66 years. This study shows that there is a male majority among HCC patients, with men accounting for 82.9% of malignant cases (Abd-Elsalam et al., 2018). We found a statistically significant difference in smoking between the two groups that were the subject of our study. According to Petrick et al. (2018), smoking is a significant risk factor for the development of HCC. These results support their findings. According to the investigation's liver biochemical profile, the HCC group's serum albumin levels were significantly lower, and their AST and ALT levels were significantly greater than those of the control group. Abou Ammo et al. (2018) and Yakut et al. (2018) hypothesized that this could be connected to the liver's reduced ability to synthesis albumin and vitamin K, which is a co-factor in the extrinsic coagulation pathway. Their findings supported their theories. Serum AFP levels in this investigation showed a statistically significant rise between the patients and the control group. The outcomes matched those of Abou Ammo et al. (2018). They talked about how the immune system's ability to fight liver cancer was compromised by the malignant hepatocytes' more selective transcriptional activation of the AFP gene, which raised AFP synthesis throughout the course of HCC development. We found a highly significant statistical decline in the relative expression level of DGCR5 between the HCC group and the control group. This is in line with the findings of Huang et al. (2016). Wang et al. (2018) reported that during their examination, they discovered that HCC cells and tissues have downregulated DGCR5. Overexpression of DGCR5 was able to inhibit the migration, invasion, and proliferation of HCC cells. According to our analysis, the DGCR5 relative expression level in stage IV of TNM staging for patients with HCC was statistically substantially lower than in stages I and II and III. This is consistent with Wang et al. (2018), which found evidence associating a reduction in DGCR5 with an unfavorable outcome for HCC. Regarding total bilirubin, serum albumin, AST, and AFP, the HCC group's relative expression level of DGCR5 was statistically substantially lower in our analysis. We found that there was no statistically significant drop in the DGCR5 relative

expression level in the HCC group concerning ALT. This result was in line with Huang et al. (2016). Study Restrictions: comparatively limited sample size and ethnicity that is concentrated in one area.

Conclusion

Serum DGCR5 expression could be considered a noninvasive marker for the diagnosis of HCC. DGCR5 expression may aid in tumor staging which could help in clinical evaluation of HCC patients. Serum DGCR5 relative expression levels could detect the outcome of HCC patients so, they also could be considered as prognostic biomarkers of HCC.

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Conflict of interest: All authors declare no conflicts of interest.

the patients

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