Expression of Transcription Factor -1 in Hepatocellular Carcinoma Egyptian Patients

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Abstract: Hepatocellular carcinoma (HCC) is the sixth prevalent malignancy worldwide and is a rising cause of cancer related mortality. Where majority of people are infected with HCC due to liver cirrhosis, hepatitis B and hepatitis C infection. Egypt has the highest age-standardized cirrhosis rates of mortality with 72.7 deaths per 100,000. Thyroid transcription factor -1 is a marker which is used to classify a tumor of unknown origin as lung and thyroid primary, where the recent studies have shown that the nuclear TTF-1 occurs on denocarcinoma of thyroid or non pulmonary origin that depends upon the antibody clone. This aim of this study is to check the utility of TTF-1 in the diagnosis of HCC to assess hepatic tissue the expression of thyroid transcription factor-1 in HCC in tumor and non tumor.

The diagnosis efficiency of HCC was determined using TTF-1 gene expression by RT-PCR technique for 20 patients samples serum and tissue (tumor and non-tumor) samples and the level of AST, ALB, ALT were measured.

Results
A significant result was determined as a comparison between the patients diagnosed with HCC and between healthy people in liver and in other studies the expression of TTF in Hashimoto’s disease, where the TTF-1 expression in HCC was highly expressed in tumor tissue and expressed lower in HCC serum.

Conclusion: TTF-1 gene may acts as promising marker in diagnosis of hepatocellular carcinoma.

1. Introduction
Hepatocellular carcinoma (HCC) is considered one of the most causes of liver cancer among malignancy, where the pathological diagnosis are easy to be reached although the methods to confirm these results are still in complicated cases. HCC is considered the third cancer common cause of death all over the world and one of the most common cause of cancer. [1] El Serg and Mason have mentioned an increase in the incidence of HCC with 80% over the past 20-30 years in the united states [2] All over the world liver cancer is considered the fifth known cause of cancer among men and the sixth cause of cancer death [2]. While in women it’s the seventh cause of cancer and the sixth cause of cancer death [3]. Also HCC is from 85% - 90% in histological primary liver cancer [4].

One of the most common cause of HCC is Hepatitis C Virus (HCV), Where the infection with HCC is 15 fold higher in patients with HCV that are compared with HCV negative subjects [5].

In Egypt, HCC is the major cause of death where it is proved that it’s more among patients infected with HCV [6] Liver cirrhosis is the most cause of death all over the world. Where HCC happens in the background of cirrhosis, where it is found in patients with HCC from 80% - 90% . Egypt has the highest age-standardized cirrhosis rates of mortality with 72.7 deaths per 100,000 [7]. Thyroid transcription factor -1 (TTF)-1 is an important and useful diagnostic marker which was discovered in the past years [8] Where (TTF )-1 is a 38-kDa nuclear protein. As mentioned by Gokden, Glickman and Lei that TTF -1 may work out as a marker that is used to diagnose HCC in uncertain cases [9].

(TTF) -1 is considered as DNA binding protein where it is encoded by a gene located on chromosome 14q13 [8].Where it is expressed in the thyroid and lung, also TTF -1 is expressed in C-cells & follicular cells where it works out for
activating the thyro-globulin and thyroperoxidase gene transcription [10]. TTF -1 is a marker for primary adenocarcinoma of the lung. It was shown by some studies that TTF -1 can be expressed in extra-pulmonary adenocarcinomas. Although the results in endometrial adenocarcinoma for the expression of TTF -1 are conflicting [11] TTF -1 is an epithelial marker which is a peripheral airway as it is expressed in lung adenocarcinomas but not in squamous cell carcinomas, that is used to differentiate between 2 malignancies especially those that can’t be diagnosed by common histology. It’s generally believed that TFF -1 is not expressed in squamous cell carcinomas of the lung [12]

The aim of this project is to check the utility of TTF-1 in the diagnosis of HCC and to assign clinic-pathological correlations, and also to assess hepatic tissue expression of thyroid transcription factor -1 in HCC in tumor and non tumor tissue

2. Patients & methods

From each patient diagnosed with hepatocellular carcinoma, 20 samples were tumor tissues and their corresponding non tumor tissues and 5 µl blood samples were taken and 10 µl blood from controls. Where there are two criterias; inclusion criteria the patients ages range from 41 to 59 years where the amount of ALT > 30 IU/L and the exclusion criteria includes ages above 60 years that has HBV, HCV, and HIV.

Methods

First RNA extraction

The tissue was spliced into small pieces, 1 ml of lysis buffer was added to the tissue samples and then homogenization was applied. RNA was extracted from 200 µl of the tissue mixture and 200 µl of the serum samples using Abbot msample preparation system kit (Abbot, USA). Then the RNA was stored at –80 °C. Finally the RNA concentration was measured and its purity using Nano Drop 2000 spectrophotometer (Thermoscientific, USA). In which 2 µl of total RNA was measured the mean concentration was between 22 ng/µl, & the purity was between 1.8 – 2

Reverse transcription:

Where 5 µl RNA was reverse transcribed using Revert Aid first strand CDNA synthesis kit (Thermo scientific, USA). The 20 µl master mix consists of 1 µl oligo (dT)18 primer, 4 µl (5x) reaction buffer, 1 µl ribolock RNase inhibitor (20 u/µL), 2 µl (10 uM). dNTP mix, 1 µl Revert Aid M-MuLV RT (200 u/µl), and 12 µl nuclease free water. Then all samples were incubated for 60 mins at 42°C and then the reaction was terminated at 70°C for 5 mins. The Thyroid transcription factor -1 (TTF-1) real time PCR amplification ( TTF-1 gene) expression was determined using step one RT PCR system (AB applied biosystems, foster city, CA, USA) in which 5 µl cDNA were added to 20 µl master mix consists of 1 ulRoX,4.5 ulsyber green master mix (2x), 12.5 µl nuclease free water & 0.3 µM TTF-1, the forward primer 5’ AAGAACATGGCATTGGTGCC 3’, and 0.3 µM TTF-1 reverse primer,5’GGATGACCACACCCTACAG 3’ (Bio Basic Canada Inc.).The real time PCR program was 95°C for 10 mins, 95°C for 15 seconds & 60°C for 30 seconds recycled for 40 cycles. The TTF-1 expression was calculated using Relative quantification method 2^△△Ct [13].

3. Results

The clinico pathological parameters were measured for 20 patients diagnosed with (HCC), where there was a significant between patients and controls age (P= 0.0374*), ALB (P=0.001***), ALT (P= 0.0238*), & AST (P= 0.0286*) as shown in table (1).

Where the results of TTF-1 expression in tissue was significantly higher in tumor tissue than that of non tumor tissue (P=0.045*), as shown in figure (1). While the TTF-1 expression in serum was significantly up regulated in normal serum than that of HCC serum (P=0.030*), as shown in figure (2).

3.1 Figures and Tables

Patient’s Clinical parameters

Table (1) ALB; albumin, ALT; alanine aminotransferase,AST; aspartate aminotransferase, NS; non-significant, *** significant at p<0.001,* significant at p<0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCC Patients</th>
<th>Healthy Volunteers</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>(50.71±9.03)</td>
<td>(41.66±8.02)</td>
<td>0.0374*</td>
</tr>
<tr>
<td>ALB g/dl</td>
<td>(2.84±0.17)</td>
<td>(4.2±0.26)</td>
<td>0.0001***</td>
</tr>
<tr>
<td>ALT IU/L</td>
<td>(48.16±3.50)</td>
<td>(26.37±1.18)</td>
<td>0.0238*</td>
</tr>
<tr>
<td>AST IU/L</td>
<td>(42.85± 3.39)</td>
<td>(23.56± 2.14)</td>
<td>0.0286*</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>Tumor grade I/II/III</td>
<td>12/22/16</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Fibrosis score(0-2) (3-4)</td>
<td>8/42</td>
<td>---------------</td>
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</tr>
</tbody>
</table>

Figure (1): TTF-1 expression in tissue, RQ= $2^{\Delta\Delta C_{t}}$, NT: non tumor tissue, T: tumor at $P<0.045$

Figure (2): TTF-1 expression in serum, RQ= $2^{\Delta\Delta C_{t}}$, NS: normal serum, HCC serum at $P<0.030$

4. Discussion

The liver is the main source of circulating TTF-1 and it is considered as a regulatory of TTF-1 gene expression and serum levels in human diseases [1]. Where Thyroid transcription factor -1 is a marker which is used to diagnosis HCC in cases that is expressed in thyroid and lung. Also the TTF-1 is an epithelial marker that is expressed in lung adenocarcinomas but not in squamous cell carcinomas. It is a homeo-domain that contains transcription factors that is important for morphogenesis and differentiation of the thyroid as TTF-1 is produced in lung and thyroid [11]. Twenty patients were included in our study where samples of tumor and non tumor tissues, and serum samples were collected from each patient. This study shows the expression of TTF-1 gene where it is up regulated in tumor tissue than of non tumor in tissue at a significant value of $P=0.045$ and it’s expression in serum is up regulated in normal serum than HCC serum with a significant value of $P=0.030$. Compared with other studies that determined the expression of TTF-1 in Hashimoto’s disease that was tested on 10 patients and 10 control thyroid samples according to Suzuki K,1998 [14], the expression of TTF-1 was highly significant in Hashimoto’s disease more than those in the control. Where the Hashimoto’s disease is a disease that affects the thyroid gland

ALB (Albumin) is a protein that is synthesized by hepatocytes, that is formed from 580 amino acids, as well as the production and expression of ALB is reduced in different liver diseases as it’s reduction depends on the severity of the disease. According to Joh T 2014 [15], the expression of ALB in HCC ranged from (0.7 ± 0.2) with a significant $P$ value ($p < 0.001$). Our study agrees with Joh T’s results where the ALB level in patients with HCC ranges between (2.84± 0.17) and that in healthy people ranges between (4.2 ± 0.26) with very significant $P=0.0001$***

ALT ( alanine aminotransferase) is found in serum and various blood tissue but mostly secreted from liver. ALT has a clinical significant that is used to make a diagnose the evaluation of hepatocellular injury to determine liver health. In our study, the ALT level was determined in 20 patients with HCC that ranges between (48.16± 3.50 IU/L) but in healthy people it ranges between (26.37± 1.18 IU/L) with $P$ value (0.0238*) that is significant, While according to Tarao K,1997 [16], He determined the ALT expression among 26 people who were diagnosed with HCC, and was divided them into 2 groups. Where the average level of expression of ALT in Group A (48.8 +/- 26.0 IU/L) was significantly smaller than that in Group B (101.1 +/- 47.3 IU/L) ($P < 0.005$).

AST ( aspartate aminotransferase) is an enzyme which is used to test its amount in the blood where it is secreted from liver. The level of AST was elevated when the liver is diseased or damaged, so it is a test used to diagnosis liver damage and identify the liver diseases such as hepatitis. So the AST level in our study was determined between the tumor and non tumor HCC patients that ranges between (42.85± 3.39) and among healthy people that ranges between (23.56± 2.14) with a significant $P=0.0286*$. But in other studies according to Yang Z,2015 [17] he determined the AST among 189 patients that were diagnosed with HCC where 64 were not survived and 125 were survived, where the level of AST in non survived patients were higher than survived ones with $P$ value<0.05
5. Acknowledgements

This research was supported by Dr. Samah Mamdouh, the lecture at Biochemistry and molecular biology department at Theodore bilharz research institute who helped and supported me a lot with her great experience. Also I’m grateful to Fatma Khorsheed the specialist at Biochemistry and molecular biology department at Theodor Bilharz Research Institute for her assistant, and who moderated this paper and that line improved the manuscript significantly, as well as a special thanking to Dr Amr Aggez for his supervision and supporting to me at Modern university for science and arts(MSA).

And finally many thanks to Theodor Bilharz Research Institute for their welcoming and endless help.

5. References


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