

Significance of RAGE Marker in Hepatocellular Carcinoma Diagnosis

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Abstract: *Hepatocellular carcinoma (HCC) is considered as one of the most threatening cancer type. It is well known as liver cancer. Late diagnosis of HCC may lead to many complications in treatment. Molecular diagnosis is considered the switch key of safe early diagnosis of HCC. RAGE gene is located on the sixth chromosome. It is multi-ligand with multiple functions that can be used in many diseases. This study aims to find the relation between the Expression of RAGE in HCC patients. This study was conducted on 60 samples of tumor and non-tumor tissue samples and HCC serum. RNA was extracted and reverse transcribed then amplified by qRT-PCR. The expression regulated in HCC tissue and HCC serum. This marker can use it as a diagnostic tool that will help in early diagnosis of cancer and better treatment.*

Keywords: Hepatocellular carcinoma, RAGE (Receptor for advanced glycation end-products), Real time PCR, AGEs (advanced glycation end-products)

1. Introduction

Hepatocellular carcinoma (HCC) is graded as the sixth most common cancer in occurrence worldwide. It is widely spread in Asia and Africa and it affects men than women. HCC is considered as the third common cause of death related to cancers since it is mostly detected at late stages. HCC approximately affects over one million individuals annually. It includes no limit exposure to Aflatoxin B or cirrhosis that leads to loss of liver cells in irreversible way or viral infection such as hepatitis B (HBV) and C (HCV) according to [1]

HCC includes many risk factors as over consumption of tobacco and alcoholic drinks moreover the obesity. It was shown that there is correlation between diabetic person and HCC patient. These risks may increase the chance of liver chronic inflammations that is later on associated with liver cancer. About 80 % of HCC arises from background chronic liver disease person according to [2]

AGEs are considering as heterogeneous irreversible adducts that are made from non-enzymatic protein glaciation. There is endogenous AGEs that occur normally during the metabolism and exogenous AGEs that occur from tobacco and food and these are the two AGEs major sources. For example, it occurs on food when it is exposed to high temperature methods such as grilling, deep frying and boiling. The accumulation of AGEs up regulate inflammation by binding to the receptor RAGE according to [4]

RAGE gene is located on the chromosome number six and it belongs to the immunoglobulin superfamily and it is multi-ligand receptor. It is included in the class III if major histocompatibility complex. This multi ligand consists of V type domain and cytoplasmic tail more over two constants C type domains. The V domain consists of two N glycosylation sites that are responsible for the extracellular binding ligand. The cytoplasmic tail that is responsible for the intracellular ligand binding and signaling. RAGE is

highly expressed by the increase number of ligands according to [5]

RAGE is highly expressed in relation to liver cancer. Binding the RAGE to its AGEs is the key of activation the cell pathway signaling such as p44/42 MAP kinase, p38 moreover creating reactive oxygen and proinflammatory cytokines production. The liver function here is to clear the AGEs it may lead at the end to many cancers according to [6]

The aim of this study is to find alternative way rather than the several invasive techniques with their risks for diagnosis of hepatocellular carcinoma. Since early detection of cancer will help in treatment and makes it easier. RAGE marker is assumed to help in detection for hepatocellular carcinoma and its proliferation by using molecular biology techniques.

2. Material and Methods

This study was done on 60 samples from 20 HCC patient. Each patient gave a tumor, non tumor and serum sample. The collection of samples was done with collaboration of the pathology department at Theodor Bilharz Research institute, Giza, Egypt. Take into consideration the ethics in dealing with patients. Beside 10 serum control samples were collected from healthy volunteers. The inclusion criteria used was that the age between (41-59) and ALT>26.37, AST>23.56. Where the exclusion criteria were age over 60 years and any patient with HCV, HBV, HIV.

The tissue was spliced into small pieces. 1ml of the lysis buffer was added to the tissue samples and homogenization was applied. RNA was extracted from 200 μ L of the tissue mixture and 200 μ l of the serum samples using Abbot *msample* preparation system kit (Abbot Molecular, INC, DES, Plaines, IL)g according to the manufacture instructions.

RNA concentration and purity were measured using Nanodrop (Nano drop 2000C spectrophotometer, thermo scientific, USA). Reverse transcription was applied on the extracted RNA by using Revert Aid First strand cDNA Synthesis Kit (Thermo scientific, USA) using 5 μ L of RNA template and 20 μ L of the master mix was prepared by adding 1 μ L of Revert Aid RT, 1 μ L of Ribo-lock RNAase inhibitor, 4 μ L 5X reaction buffer, 2 μ L of DNTP, 1 μ L of oligo (dt)18 primer, and 6 μ L of water free nuclease. Then samples were incubated for 60 min at 42°C. The reaction were terminated at 70°C for 5 min.

Real time PCR was applied using Maxima Syber Green QPCR Master Mix (2X) (Thermo scientific, USA). By using RAGE forward primer 5` TTG GCG AGC CAC TGG TGC TG 3` and the reverse primer was 5` GGC CTC CTC CCT GGG GAG AC 3`. 12.5 μ L of maxima Syber green qPCR master mix were added, no ROX. 0.05 μ L of ROX solution, Primers with 0.3 μ M concentration, 4.5 μ L of water, 5 μ L of DNA template was added to the mixture to have total volume 25 μ L. PCR program was progressed then the RAGE expression was detected using the relative quantification method using formula $2^{-\Delta\Delta CT}$. PCR program were applied with 40 cycles at annealing and extension temperature 62°C while denaturation at 95°C by using Real Time PCR system (AB Applied Bio systems , foster city, CA,USA)

3. Results

There are four clinical parameters those were studied in this research. In which age ($p=0.0374$), ALT ($p=0.0238$) and the AST($p=0.0286$) increased significantly in the serum of the HCC patients compared to the control. On the other hand, ALB decreased non-significantly in the serum of the HCC patients compared to the control in table (1).

RAGE marker expression was measured by using RT-PCR in tumor and non-tumor hepatocellular carcinoma tissue samples and HCC serum and also normal serum. There were some clinical parameters for the HCC patients that the age was in range of (41 to 59). The ALB was in range of (2.67 to 3.01), the ALT was in range of (44.66 to 51.66) and AST was in range of (39.46 to 46.24). Where the P values were 0.0374, 0.0001, 0.0238, 0.0286 respectively and the only non significant was ALB.

4. Discussion

Hepatocellular carcinoma (HCC) simply is liver cancer that is widely spread in the developed and non-developed nations. It is one of the highest recorded deadly diseases. About half million new cases diagnosed each year world wide especially in the developing countries. HCC is widely spread in men than women by two to four times. In the last 20 years in USA the disease has increased by 3 folds. There are many ways for HCC diagnosis such as simple physical examination or imaging tests with its side effect beside the liver biopsy that may lead to increase the vigorous of the cancer if present, according to [7]

In this study, an equivalent diagnosis type is going to be used. Checking for the significance of RAGE biomarker in

the diagnosis of HCC. RAGE is considered as important keys in many cancers type since it is mostly produced from cancerous cells. The RAGE consists of V type domain and a double C type domains followed by cytoplasmic tail and transmembrane domain. The RAGE receptor consists of three isoforms expressed secretory RAGE (esRAGE), Full length RAGE receptor and N-truncated RAGE (NtRAGE). RAGE was firstly considered as cell surface receptor. It is a heterogeneous compound of lipid and protein adducts according to [8]

This study shows the expression of RAGE marker in hepatocellular carcinoma. In the tumor tissue samples, it was found that the expression of RAGE was up regulated in comparison with the non-tumor samples with $P=0.031$. On the other hand, the HCC serum samples showed to up regulation in the expression of RAGE level in comparison with the control with $p=0.023$. This means that RAGE marker can be used in the diagnosis of HCC and it is significance in detection of HCC.

Lung cancer shows a unique result and opposes the results in the HCC. The level of RAGE was down regulated compared to the normal lung. There is a correlation between the down regulation of RAGE and the stage of disease according to [9]

In Prostate cancer, it was reported that RAGE was up regulated and over expressed in the prostate cancerous tissue than the normal tissue. [10] This meets with the results of expression in HCC.

On the other hand, in colon cancer there was conflict of results in the relevancy of RAGE to the cancer. according to Duke's classification it reported that there is over expression in RAGE levels while on other studies it shows no significant change in the RAGE level. [11]

Moreover, in the pancreatic cancer there were no evidence that could estimate that there are relation between RAGE expression level and the presence of cancer in the pancreas according to [12]

Finally, we can see that there are some type of cancers that RAGE appears to be significant and there are other types that shows no direct relation between the RAGE expression level in it to the presence of cancer cells. In HCC according to our results that shows a great significant in the tissue and serum samples in the expression of RAGE marker, we can use it as a diagnostic tool that will help in early diagnosis of cancer and better treatment.

5. Conclusion

Finally, we can see that there are some type of cancers that RAGE appears to be significant and there are other types that shows no direct relation between the RAGE expression level in it to the presence of cancer cells. In HCC according to our results that shows a great significant in the tissue and serum samples in the expression of RAGE marker, we can use it as a diagnostic tool that will help in early diagnosis of cancer and better treatment.

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Table 1

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

ALB; albumin, ALT; alanine aminotransferase, AST; aspartate aminotransferase, NS; non-significant, *** significant at p<0.001, * significant at p<0.05

Figures:

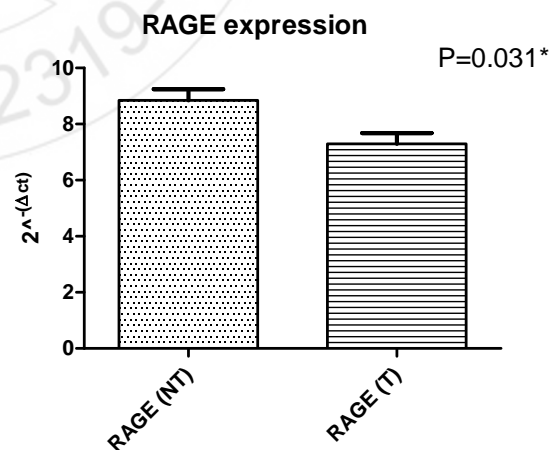


Figure 1

NT: Non tumor, T: Tumor, * significant at p<0.05

Figure (1) shows less expression of RAGE marker in the tumor tissue samples than the non-tumor.

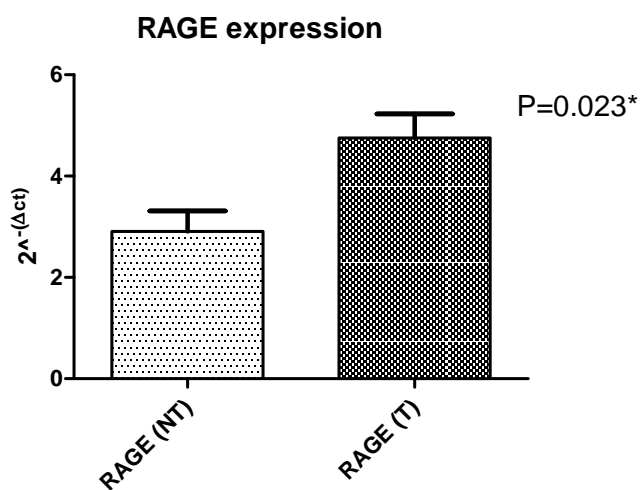


Figure 2

NT: Normal serum, T: HCC Serum, * significant at $p < 0.05$

In figure (2) The RAGE expression is up regulated in the HCC serum samples than the normal sample with P value 0.023.

