

The Role of GLUT1 in the Diagnosis of HCC in Egyptian Patients

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Abstract: Hepatocellular carcinoma (HCC) is considered one of the most highlighted lethal cancers worldwide, its prediction and therapy are very limited and poor. This study aims to diagnose HCC by evaluation the expression of glucose transporter isoform1 (GLUT1) which is responsible for uptake of glucose across the plasma membrane through an enhancement in the glucose transporter proteins. Samples were collected from 20 patients, tumor, non-tumor tissues samples and normal and HCC serum samples. RNA extraction was done followed by a reverse transcription and a Real time PCR. Our results show a positive association between GLUT1 expression and Hepatocellular carcinoma especially in the serum level while in tissue it was non-significant. Most of the related studies that focus on the expression of GLUT1 have proved that GLUT1 can be used as a diagnostic marker for HCC.

Keyword: Hepatocellular carcinoma, Expression, GLUT1 (glucose transporter proteins), Real time PCR.

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Introduction

Hepatocellular carcinoma (HCC) is considered one of the most incurably and lethal cancers in human population worldwide and existed in very high percentage in many areas around the world [1]. Abnormal liver condition (liver cirrhosis) is the reason behind the inclining condition of HCC that are found in an increasing frequency with rate more than 80% of all cases that suffer from cirrhotic liver worldwide.[2]

In Egypt, the frequency of HCC and all liver related cancer in 1993 showed approximately 4% rate and in 2003 showed a rate of 7.3% .[3] A 4.7% of chronic

liver disease (CLD) patients interpretation was reported in HCC.[4]

However, in USA it was reported a range of 4 cases out of 100 000 population, while in Asia and Africa reported a range of 150 cases out of 100 000 population, that were manifested as the reason behind the large number of cancer death, also a 77% of death was arise from the developing country. According to studies that were done in North American countries and Western European countries, HCV was referred to the main risk factor for HCC occurrence as well as in the epidemiological studies. A rate of 70% of HCC patients have anti-HCV antibodies in their serum that was correlated to HCV associated patients [5]. Additionally large percent of HCC patients found to have liver cirrhosis and only few patients were reported with non-cirrhosis pointing out that the occurrence of the virus is due to mutagenic effect.

In order for the cell to grow and survive, there is a cellular metabolism designed to balance biosynthetic process with the supplement of energy to cancerous cells which is considered as a hallmark of cancer. One of the important sources of energy is glucose, in which it is metabolized by aerobic glycolysis with regardless to the presence of oxygen; this phenomenon is called “warbrug effect” where it is associated by an increase of glucose intake [6-7].

For the cell to uptake energy it undergoes a transportation system, this system is divided into two classes SGLT family and GLUT family. These genes are known as protein coding gene. For GLUT family it acts by transporting sugars along concertation gradient. [8] In this study we only look for one member of this family GLUT1 (Glucose transporter isoform1). Its location is on (1p34.2) based on NCBI database.

The membrane transporters are complex metabolic network and considered as an essential network for inflow of nutrition. The diffusion of glucose into the cytoplasm is inhaled by a group of protein membrane called “glucose transport” and the most distinct one

is glucose transporter 1 (GLUT1). One of the essential factors in the transport and metabolism of glucose in cancer cell is glucose transporter isoform 1 GLUT1. [3]

This study aims to observe the expression of GLUT1 in cases of hepatocellular carcinoma and to study its utility in the diagnosis of HCC.

1. Material and Methods

This study includes 20 HCC patients who undergone surgical liver resection in which tumor tissues and their corresponding non-tumor tissues were taken in addition to serum sample from each patient. The collection of the samples was done with the cooperation of the pathology department at Theodor Biliharz research institute, Giza Egypt. Also 10 serum samples were taken from healthy volunteers as control samples. Inclusion parameters were the age which was between (41-59) years, ALT > 26.37, AST > 23.56, while for the exclusive parameters, all the ages > 60 years and (HBV, HIV, HCV).

1.1 RNA Extraction

Tissue was spliced into small pieces, 200 µl lysis buffer was added before homogenizing, total RNA was extracted using Abbot msample preparation system kit, Cat.no.(02k02-96), (Abbot Molecular, Inc,Des Planies,IL) by using 200 µl lysis buffer, 100 µl RNA magnetic beads, 50 µl elution buffer, 400 µl Serum, 400 µl tissue mixture. Two microliters of each sample was measured for its concentration mean= 13.8 ng/ µl and purity were 1.8-2 by (Nano Drop 2000 Spectrophotometer, ThermoScientific, USA).

1.2 Reverse transcription

Reverse transcription step was applied for 5 µl of the extracted RNA samples which was added to 20 µl of the master mix (Revert Aid first strand cDNA synthesis Kit. Cat.K1621) which contain 250 mM Tris-HCL (PH8.3), 250 mM KCl, 100 µM oligo dT, 10 mM dNTPs, 50 mM DTT, 20 µ/ml RNase inhibitor and 20 mM MgCl₂. Reaction was amplified using PCR thermal cyler BioRADT100 programmable (thermal controller, Singapore) for 60 min at 42°C and the reaction was terminated at 70°C for 5 min.

1.3 Real Time PCR reaction

5 µl of the cDNA synthesis was used as a template for Real Time PCR Reaction. Real-Time PCR master mix was 20 µl contained 12.5 µl of Syber green qPCR master mix, 0.05 µl ROX solution, 0.3 mM GLUT1 primer, GLUT1 forward primer 5'CTGCTCATCAACCGCAAC'3, GLUT1 reverse primer 5'CTTCTTCTCCCGCATCATCT'3, 4.5 µl water, and a control gene was used such as GAPDH, The reaction was subjected to 40 cycles of

denaturation (95°C, 15 s), Annealing/extension (60°C,30 s) for 40 cycle using step one Real time PCR system (AB Applied Biosystems, foster city, CA,USA).

Results

The obtained data of GLUT1 expression in HCC patients using Real Time-PCR, different clinical parameters were studied, there was a significant differences between patients and controls in terms of Age (p=0.0374*), ALT (p=0.0238*), AST (p=0.0286*). On the other hand, ALB has a non-significant decrease in the serum of patients compared to the controls. Real Time-PCR analysis revealed that GLUT1 expression was found to be down regulated in HCC tissue samples, in comparison with non-tumor tissue (Fig.1), while GLUT1 expression was significantly up regulated in HCC serum samples, in comparison with normal serum samples, p= 0.019* (Fig.2).

Discussion

Hepatocellular carcinoma (HCC) is an aggressive malignancy tumor of the liver, and it is one of the most common disorders worldwide. [9] Hepatocellular carcinoma (HCC) is considered a highlighted health problem mainly in high incidence areas. It is a tumor found with high incidence in patients with liver cirrhosis. Nowadays there is a growth in the global incidence, but with the development of the new therapies in hepatitis C virus (HCV) and hepatitis B vaccine, a gradual decrease expected in this incidence. Another significant issue is the high death rate of the patients having this tumor. In spite of the presented surveillance programs in patients with chronic liver disease, most tumors' patient are diagnosed in advanced stage, where tranquil medications can be applied and it is currently the most highlighted cause of death in this group of patients. [10]

A number of serum markers were used in HCC diagnosis as Alpha-1 fetoprotein, Lens culinaris agglutinin-reactive AFP (AFP-L3), Des-gamma carboxyprothrombin (DCP), α-L-Fucosidase, Glypican-3, Squamous cell carcinoma antigen (SCCA), Golgi protein 73 (GP73), Hepatocyte growth factor (HGF), Transforming growth factor-b1 (TGF-b1), Vascular endothelial growth factor (VEGF), Serum proteomics. [11]

GLUT1 expression is one of the markers that are used in the diagnosis of HCC. The expression in normal epithelial tissue and benign epithelial tumor is undetectable. In contrast to our results, [12] reported that the expression of GLUT1 in HCC was with high significant. As well as GLUT1 expression, tumor development, and unfavorable prognosis of

several tumors have a close relationship, which was reported by several studies. Its expression considered to be one of the suitable markers of hypoxia and glucose metabolism.

The current study was conducted to show the expression of GLUT1 in HCC patient's serum and tissue samples, the age of HCC patient was 50.71 ± 9.03 years. In serum samples the expression was significant, while in the tissue samples the expression gives non-significant results, ALB showed a non-significant decrease in the patients' serum compared to the controls.

The GLUT1 expression was compared with a related study which determines the expression of GLUT1 in intestinal type of gastric carcinoma, the result showed that the GLUT1 was not largely expressed in three normal gastric tissues but was expressed in cancerous tissues. [13] Another study for [9] showed that the expression of GLUT1 in HCC promotes tumorigenesis in tissues, the results were significantly increased in tumor cell lines and tissues compared with non-tumor tissues. This data indicate that GLUT1 expression is upregulated in tissue of HCC patients which is disagree with our study.

According to [14], the expression of GLUT1 in the 100 paraffin embedded breast cancer tissue and normal tissue showed a high expression of GLUT1 in only 47% of breast cancerous tissue, however in the normal tissue the expression was not detected.

In a study of the prediction for prognosis of HCC in GLUT1, GLUT1 expression was significantly higher in tumor tissues than in the adjacent non-tumor tissues in the 15 HCC specimens. [15]

Conclusion

In conclusion, our results point out that the expression of GLUT1 in serum is significantly upregulated than in the tissue. This expression might provide a new biomarker for HCC diagnosis.

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References

[1] Brito, A. F., Mendes, M., Abrantes, A. M., Tralhão, J. G., & Botelho, M. F. (2014). Positron emission tomography diagnostic imaging in multidrug-resistant hepatocellular carcinoma: focus on 2-deoxy-2-(18F) fluoro-D-glucose. *Molecular diagnosis & therapy*, 18(5), 495-504.

[2] Kubicka, S., Rudolph, K. L., Hanke, M., Tietze, M. K., Tillmann, H. L., Trautwein, C., & Manns, M. (2000). Hepatocellular carcinoma in Germany: a retrospective epidemiological study from a low-endemic area. *Liver*, 20(4), 312-318.

[3] Amann, T., & Hellerbrand, C. (2009). GLUT1 as a therapeutic target in hepatocellular carcinoma. *Expert opinion on therapeutic targets*, 13(12), 1411-1427.

[4] El-Zayadi, A. R., Badran, H. M., Barakat, E. M., Attia, M. E. D., Shawky, S., Mohamed, M. K., ... & Saeid, A. (2005). Hepatocellular carcinoma in Egypt: a single center study over a decade. *World journal of gastroenterology*: WJG, 11(33), 5193.

[5] Montalto, G., Cervello, M., Giannitrapani, L., Dantona, F., Terranova, A., & Castagnetta, L. A. (2002). Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Annals of the New York Academy of Sciences*, 963(1), 13-20.

[6] Horsman, M. R. (1998). Measurement of tumor oxygenation. *International Journal of Radiation Oncology* Biology* Physics*, 42(4), 701-704.

[7] Sun, H. W., Yu, X. J., Wu, W. C., Chen, J., Shi, M., Zheng, L., & Xu, J. (2016). GLUT1 and ASCT2 as Predictors for Prognosis of Hepatocellular Carcinoma. *PloS one*, 11(12), e0168907.

[8] Szablewski, L. (2013). Expression of glucose transporters in cancers. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1835(2), 164-169.

[9] Amann, T., Maegdefrau, U., Hartmann, A., Agaimy, A., Marienhagen, J., Weiss, T. S., ... & Kreutz, M. (2009). GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *The American journal of pathology*, 174(4), 1544-1552.

[10] Pascual, S., Herrera, I., & Irurzun, J. (2016). New advances in hepatocellular carcinoma. *World journal of hepatology*, 8(9), 421.

[11] Gomaa, A. I., Khan, S. A., Leen, E. L., Waked, I., & Taylor-Robinson, S. D. (2009). Diagnosis of hepatocellular carcinoma. *World J Gastroenterol*, 15(11), 1301-1314.

[12] Airley, R., Loncaster, J., Davidson, S., Bromley, M., Roberts, S., Patterson, A., ... & West, C. (2001). Glucose transporter glut-1 expression correlates with tumor hypoxia and predicts metastasis-free survival in advanced carcinoma of the cervix. *Clinical Cancer Research*, 7(4), 928-934.

[13] Kim, W. S., Kim, Y. Y., Jang, S. J., Kimm, K., & Jung, M. H. (2000). Glucose transporter 1 (GLUT1) expression is associated with intestinal type of gastric carcinoma. *Journal of Korean medical science*, 15(4), 420-424.

[14] Kang, S. S., Chun, Y. K., Hur, M. H., Lee, H. K., Kim, Y. J., Hong, S. R., ... & Park, Y. K. (2002). Clinical significance of glucose transporter 1 (GLUT1) expression

in human breast carcinoma. Japanese Journal of Cancer Research, 93(10), 1123-1128.

[15]Shaker, M. K., Abdella, H. M., Khalifa, M. O., & Dorry, A. K. E. (2013). Epidemiological characteristics of hepatocellular carcinoma in Egypt: a retrospective analysis of 1313 cases. Liver International, 33(10), 1601-1606.