ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 12, pp. 14743-14750, December, 2016

International Journal of Recent Scientific Research

Research Article

DIFFERENTIAL GLUT1 EXPRESSION IN HEPATOCELLULAR CARCINOMA AND PERI-MALIGNANT CHRONIC VIRUS C HEPATITIS

Tarek Aboushousha[1]*., Halima Hirzi[2]., Amr Ageez[3] and Noha Helal[1]

[1]Pathology Department, Theodor Bilharz Research Institute, Cairo, Egypt
[2]Faculty of Biotechnology, Modern Sciences and Arts university, Cairo, Egypt
[3]Nucleic and protein Department at Agricultural Genetic Engineering Research Institute, Cairo, Egypt

ARTICLE INFO

Article History:

Received 17th September, 2016
Received in revised form 21th October, 2016
Accepted 28th November, 2016
Published online 28th December, 2016

Key Words:

Hepatocellular carcinoma, HCV, GLUT1, Cancer, Target Therapy

ABSTRACT

Background: Hepatitis C virus [HCV] is a major public health concern. Hepatocellular carcinoma [HCC] is one of the most fatal cancers in humans with rising incidence in many regions around the world. Glucose is the major source of energy for cells. Cancer cells are known to have increased glucose uptake and enhanced glycolytic metabolism. Glucose transporter 1 [GLUT1] is a rate-limiting transporter for glucose uptake, and its expression correlates with glycolysis. GLUT1 is over expressed in many human cancers including HCC.

Results: GLUT1 expression was detected in 85.7%, 83.3% and 50% of HCC, dysplasia and peri-malignant groups respectively. GLUT1 expression was mainly expressed as membranous staining in all studied groups; however cytoplasmic and nuclear expression were also detected. Marked intensity staining was detected only in HCC group while mild intensity predominated in peri-malignant group. Mean percentage of GLUT1 positive hepatocytes increased significantly in HCC group than in other groups and increased with rising in HCC grade. Patchy pattern of GLUT1 expression predominates in all groups.

Conclusion: GLUT1 lower expression in peri-malignant tissue and its higher expression in dysplastic lesions and sustained expression in hepatocellular carcinoma indicates that changes in GLUT1 levels represent early events during the development of hepatocellular carcinoma. So GLUT1 can be a reliable marker in the diagnosis of premalignant lesions associated with HCV infection, and usage of antagonists to GLUT1 can regulate tumor metabolism and inhibit the progression of chronic liver disease to hepatocellular carcinoma.

Copyright © Tarek Aboushousha *et al*., 2016, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Chronic liver infection by hepatitis C virus [HCV] is a major public health concern and is considered one of the leading causes of liver disease worldwide. It afflicts more than 170 million people around the world with approximately 55–85% of will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis of the liver is between 15–30% within 20 years [1]. Approximately 700 000 people die each year from hepatitis C-related liver diseases [2]. Liver cirrhosis is the main predisposing condition for developing HCC. Once HCV-related cirrhosis is established; HCC develops within 25–30 years [3]. Recently, successful eradication of the virus has been associated with development of direct antiviral agents [DAA] from 2014 onwards. These medicines are much more effective, safer and better-tolerated than the older therapies [1], with impressive responses in patients with compensated and decompensated cirrhosis [4]. However, the high costs of these drugs limit their availability worldwide [5].

Liver cancer is the fifth most common cancer in men worldwide and the seventh in women [3] and is ranked as the second most frequent cause of cancer death in men and the sixth in women [6]. Among primary liver cancers, HCC represents the major histological subtype, accounts for 85%-90% of primary liver cancers [7]. HCV is the most important risk factor for HCC, since epidemiological studies have shown up to 70% of patients with HCC have anti-HCV antibody in the serum [8].

Egypt has the highest burden of the HCV in the world with prevalence can be around 14.7% of the population [9]. In addition, Egypt has rising rates of HCC that has been associated with increased prevalence of HCV infection [10].

*Corresponding author: *Tarek Aboushousha*
Pathology Department, Theodor Bilharz Research Institute, Cairo, Egypt

HCC constitutes 70.48% of all liver tumors among Egyptians [11] [Mokhtar *et al*., 2007] and represents the main complication of cirrhosis with about 21% of cirrhotic patients at risk of developing HCC [12].

The glucose transporter [GLUT] families are composed of 14 members whereby mostly all are expressed in cell membranes of different cells. GLUT1 being the first discovered member [13].

GLUT1 is a key rate-limiting factor in the transport and metabolism of glucose in cancer cells. Its expression is primarily undetectable in normal epithelial tissues and benign epithelial tumors. However, it is overexpressed in a significant proportion of human carcinomas [14,15]. Cancer cells show increased uptake of glucose compared to normal tissue and GLUT1 is responsible for the passive transport of glucose across the cell membrane [16].

Hepatocytes play an important role in maintaining plasma glucose homeostasis by adjusting the balance between hepatic glucose production and utilization [17]. There is higher glucose uptake in HCC cell lines as compared with normal liver cells [18]. A rapid increase in glucose consumption helps in the distribution of energy that is necessary for the tumor cell´s proliferation. GLUT1 expression apparently has significant clinical function in several tumors including HCC [19].

Hypoxia has been shown to have a role in the pathogenesis of several forms of liver disease [20], moreover hypoxia results in an increased transcription of the GLUT1 gene, so it has been suggested that GLUT1 might represent an intrinsic marker of hypoxia [21].

The aim of this study was to assess hepatic tissue expression of glucose transporter [GLUT1] in HCC lesions of post-hepatitis C virus pathogenesis and the adjacent peri-malignant tissue.

MATERIAL AND METHODS

Groups

This study was conducted on 42 archival specimens for partial hepatectomy paraffin blocks taken from patients at Pathology Department, Theodor Bilharz Research Institute [TBRI], Giza, Egypt. Biopsy materials were taken from the malignant and peri-malignant liver tissue [42 biopsies each]. During histopathological examination of peri-malignant tissue, 6 cases of liver cell dysplasia were detected, and were included as separate group in the current study.

The specimens were histopathologically classified into:
Group 1: Hepatocellular carcinoma [HCC]; 42 cases.
Group 2: Dysplasia cases; 6 cases.
Group 3: Peri-malignant tissue surrounding HCC; 42 cases.
Histological Study

Five microns serial sections from paraffin blocks were stained with Hematoxylin/eosin; and Masson trichrome to be examined for routine histopathological diagnosis.
Degree of chronic hepatitis C viral disease necro-inflammation [activity] and stage of fibrosis were scored in the peri-malignant sections according to French METAVIR scoring system [22], 1994

Grade of hepatitis activity; based on amount of inflammation:

A1 = mild activity, A2 = moderate activity, A3 = severe activity.

Stage of fibrosis; representing amount of fibrosis or scarring:
F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis.

HCC grade was done according to the WHO classification of tumors of the digestive system [23].
Grade 1= [well differentiated], Grade 2= [Moderately differentiated], Grade 3= [Poorly differentiated].
Immunohistochemical technique

Formalin-fixed paraffin sections [5µm in thickness] were cut. Sections undergo deparaffinization, rehydration. Endogenous peroxidase was blocked with methanol containing 3% hydrogen peroxide. Antigen retrieval was performed by microwaving the sections in citrate buffer, pH 6.0. Sections were incubated overnight at 4^{o}C in humid chamber with the primary antibodies. GLUT1 monoclonal antibody [Abcam 41525, Cambridge, USA] at an optimal dilution of 1:100, with application of ultravision detection system HRP Polymer. The antigen was localized by the addition of DAB substrate chromogen solution [Thermo Fisher Scientific, UK]. Finally, slides were counterstained with hematoxylin, dehydrated in alcohol and mounted.

For each setting, positive and negative control slides were included within each session. As a negative control, liver core biopsy was processed in the above mentioned sequences but the primary antibody was not added and instead PBS was added in this step. Positive staining of red blood cells and perineurium of nerves were used as internal positive control.

Interpretation of Immunostaining

All immunostained slides were assessed and scored. The sections were examined by using light microscope [Scope A1, Axio, Zeiss, Germay]. Photomicrographs were taken using a microscope-camera [AxioCam, MRc5, Zeiss, Germany]. Immunopositivity was indicated by brownish cytoplasmic staining., both extent [on the basis of the percentage of positive cells evaluated in 10 microscopic fields at power X400 in each section] and intensity of immunostaining [weak, moderate or marked] were evaluated.

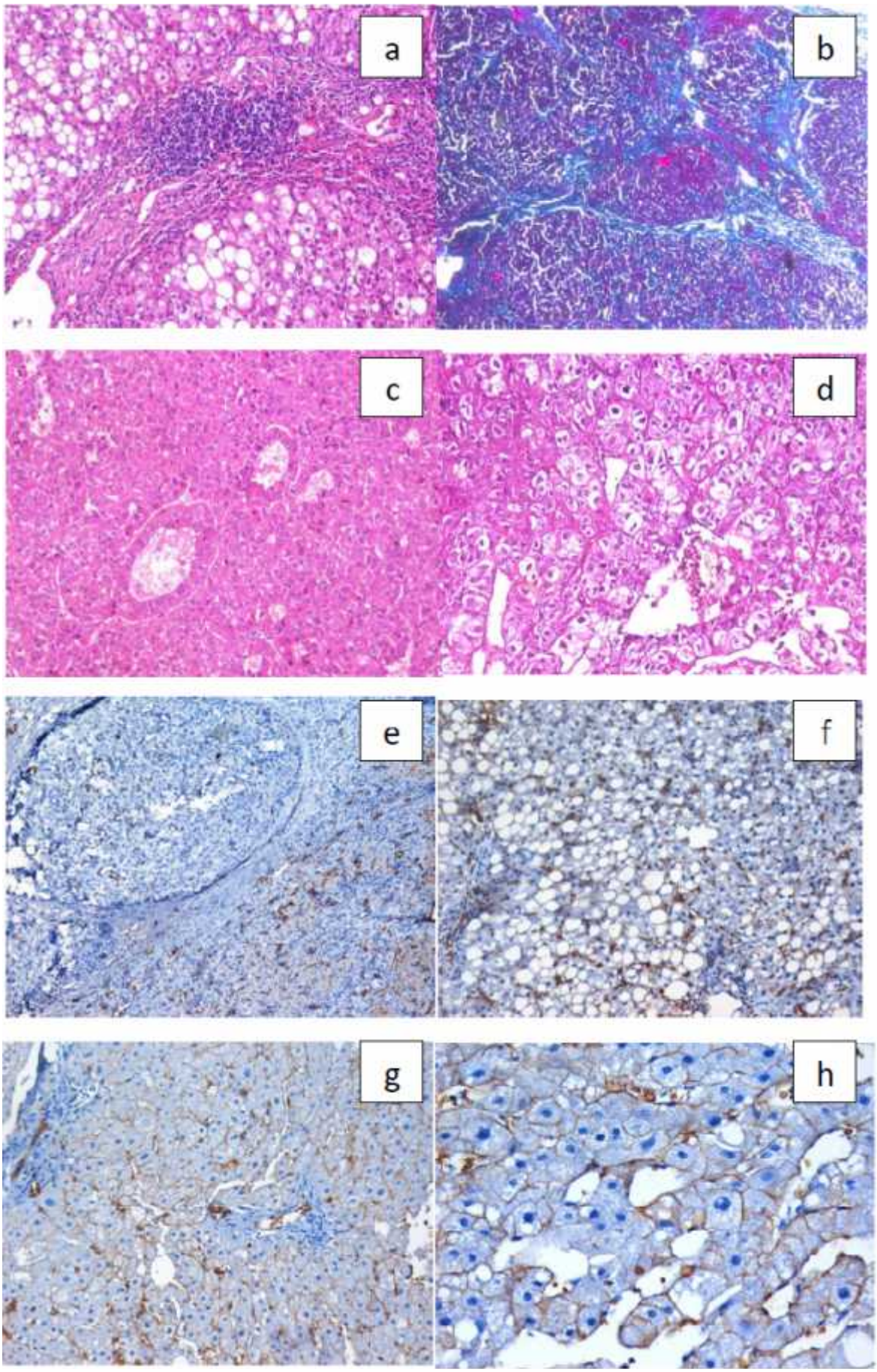
Statistical analysis

Data were summarized as percentage and means±standard deviation. They were analyzed using SPSS version 20 [IBM Corporation, Armonk, New York, USA]. T-test and ANOVA [analysis of variance] tests were used for quantitative variables and Pearson's Chi square test for percentage differences with P-value <0.05 was considered significant.

RESULTS

Our study included 84 biopsy materials from cases of partial hepatectomy done for patients suffering of hepatocellular carcinoma. 25 of these patients were males and 17 were females. The mean age of studied cases of male sex [54.79±7.59 years] was significantly higher than that of female patients [48.57±9.08 years] [p<0.01].

Studied lesions were categorized into 3 main groups according to histopathological examination; peri-malignant [chronic hepatitis C] [Fig. 1-a,b],


a
b
c
d
e
f
g
h

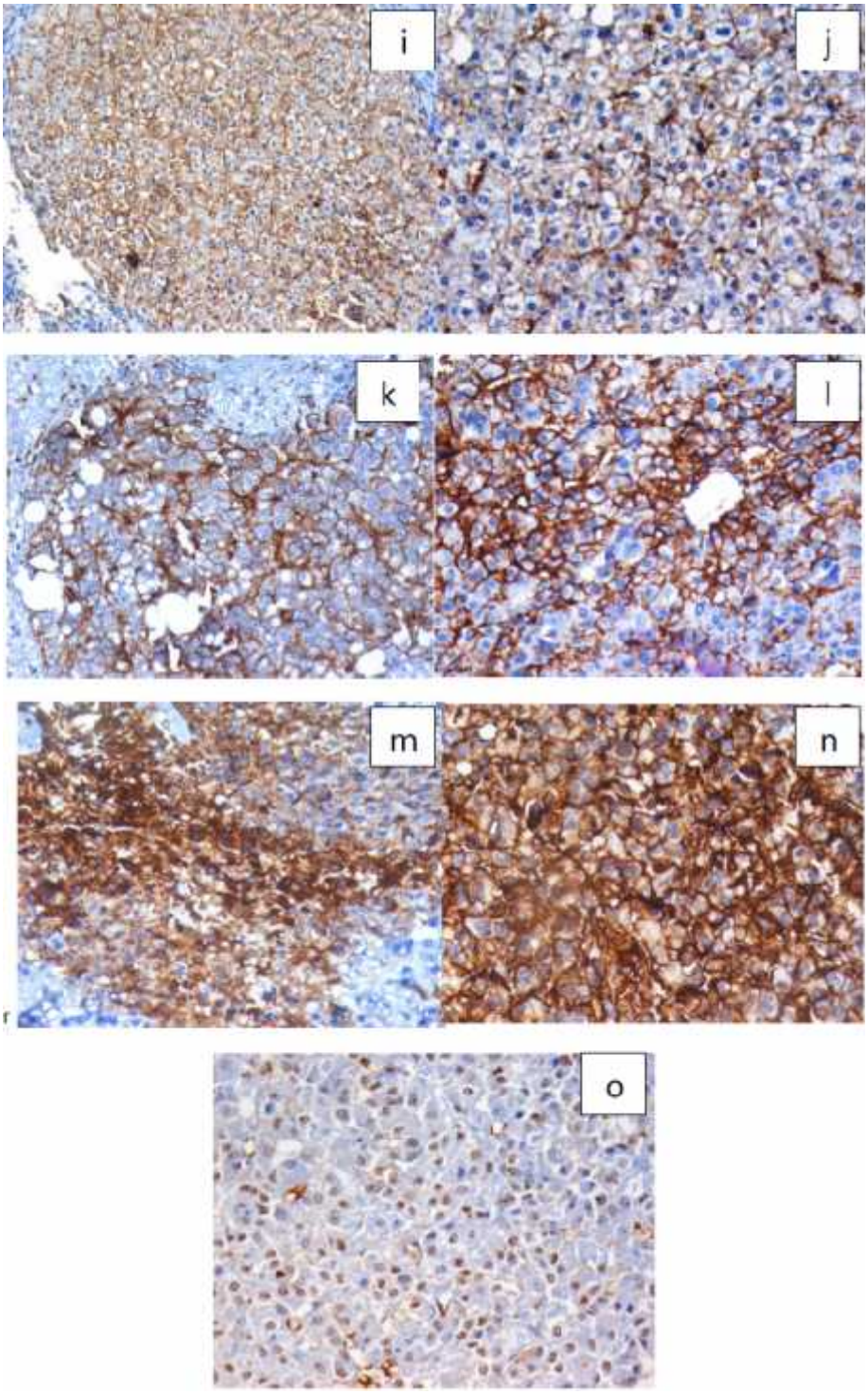


Fig.(**a**): Section in case of chronic hepatitis C, showing remarkable macrovesicular steatosis and portal lymphocytic infiltration (H&E stain, X200). Fig.(**b**): Section in case of chronic hepatitis C shows marked portal fibrosis and a cirrhotic nodule in the center. (Masson's trichrome stain,X100). Fig.(**c**): Section in Hepatocellular carcinoma (HCC) of low grade showing focal acinar formation. (H&E stain, X200). Fig.(**d**): Section in Hepatocellular carcinoma(HCC) showing clear cell pattern. (H&E stain, Fig.(**e**): Section in case of Chronic hepatitis C with cirrhosis, showing negative staining for GLUT1 in hepatocytes of the nodule (upper left) and mild patchy positivity in the nearby tissue (lower right) (Immunohistochemistry "IHC" using anti-GLUT1 monoclonal antibody and DAB, X50). Fig.(**f**): Section in case of Chronic hepatitis C with marked steatosis, showing negative staining for GLUT1 in hepatocytes (Immunohistochemistry "IHC" using anti-GLUT1 monoclonal antibody and DAB, X100). Fig.(**g**): Section in chronic hepatitis C showing mild diffuse membranous positivity for GLUT1. (IHC for GLUT1-DAB, X100) Fig.(**h**): Higher magnification from last section showing membranous positivity for GLUT1. (IHC for GLUT1-DAB, 200) Fig. (**i**): Section of liver cell dysplasia showing diffuse moderate membranous positivity for GLUT1. (IHC for GLUT1-DAB, 100) Fig. (**j**): Higher magnification from the previous section of liver cell dysplasia showing moderate membranous positivity for GLUT1. (IHC for GLUT1-DAB, 200) Fig. (**k**): Section in low grade HCC showing dense membranous positivity for GLUT1. (IHC for GLUT1-DAB, 200) Fig. (**l**): Section in grade 2 HCC showing dense membranous positivity for GLUT1. (IHC fo GLUT1-DAB, 200) Fig. (**m**): Section in grade 3 HCC showing dense membrano-cytopasmic positivity for GLUT1. (IHC for GLUT1-DAB, 100). Fig. (**n**): Higher magnification from previous section showing dense membrano-cytoplasmic positivity for GLUT1. (IHC for GLUT1-DAB, 400). Fig. (**o**): Section in low grade HCC showing nuclear positivity for GLUT1. (IHC for GLUT1-DAB, 200)

liver cell dysplasia and malignant [HCC] [Fig. 1 c,d]. We found that 50% of peri-malignant tissue biopsies were positive for GLUT1 expression, while dysplasia showed 83.3% positivity and HCC biopsies showed 85.7% positivity.

Most cases of peri-malignant group showed mild and moderate intensity of GLUT1 expression [Fig. 1e,g,h]. On the other hand, most cases of HCC showed moderate and marked intensity of GLUT1 expression [Fig. 1k-n]. Cases of dysplasia showed mild and moderate intensity of GLUT1 expression [Fig. 1i,j]. Pearson Chi-Square showed non-significant difference between studied groups [Table 1].

As regard the percentage of GLUT1 expression in different studied groups, we found that HCC cases showed the highest percentage of cellular expression of GLUT1, with significant difference with both the peri-malignant and the dysplastic liver tissue sections with significant difference between groups [p<0.001] [Graph 1]

We found also that positive and negative cases of GLUT1 expression were equally distributed in cirrhotic and fibrotic lesions of peri-malignant liver tissue.[p>0.05] [Fig. 1e-h].

Table 1 GLUT1 Expression in different examined lesions

			GLUT1 Expression		INTENSITY Score			Total
			negative	positive	+1	+2	+3	
Lesion	Peri-malignant	Count	21_a	21_b	21_a	15_a	6_a	42
		%	50.0%	50.0%	50.0%	35.7%	14.3%	100.0%
	dysplasia	Count	1_a	5_a	3_a	3_a	0_a	6
		%	16.7%	83.3%	50.0%	50.0%	0.0%	100.0%
	HCC	Count	6_a	36_b	12_a	$15_{a,b}$	15_b	42
		%	14.3%	85.7%	28.6%	35.7%	35.7%	100.0%
	Total	Count	28	62	36	33	21	90
		%	31.1%	68.9%	40.0%	36.7%	23.3%	100.0%

Each subscript letter denotes a subset of POSITIVE/NEGATIVE categories whose column proportions do not differ significantly from each other at the .05 level.

Pearson Chi-Square test shows a significant difference between examined groups [p<0.01]

Most studied lesions showed patchy distribution [66.7%], however, peri-malignant tissues showed also a reasonable percentage of central distribution [21.4%], with significant difference between groups [p<0.05] [Table 2] [Fig 1,e].

There was no significant difference in the distribution of examined peri-malignant tissue as regards the intensity of GLUT1 expression between the stages of fibrosis and cirrhosis [p>0.05].[Table 4].

Table 2 Distribution of GLUT1 positivity in examined cases

			DISTRIBUTION				Total
			acinar	central	diffuse	patchy	
Lesions	Peri-malignant	Count	$0_{a,b}$	9_b	3_a	$30_{a,b}$	42
		%	0.0%	21.4%	7.1%	71.4%	100.0%
	dysplasia	Count	0_a	0_a	0_a	6_a	6
		%	0.0%	0.0%	0.0%	100.0%	100.0%
	HCC	Count	$3_{a,b,c}$	3_c	12_b	$24_{a,c}$	42
		%	7.1%	7.1%	28.6%	57.1%	100.0%
	Total	Count	3	12	15	60	90
		%	3.3%	13.3%	16.7%	66.7%	100.0%

Each subscript letter denotes a subset of DISTRIBUTION categories whose column proportions do not differ significantly from each other at the .05 level.

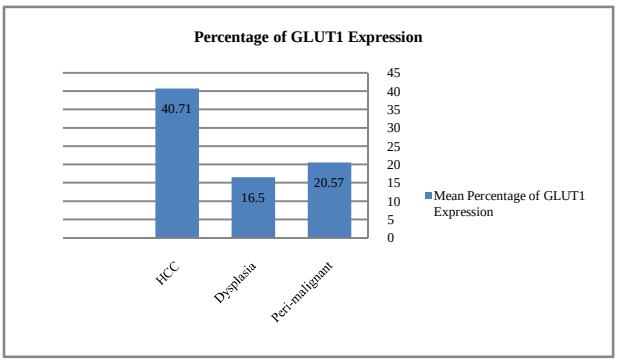
All examined lesions showed mostly membranous localization of GLUT1 expression [Fig. 1g-l]. Peri-malignant tissue showed in addition some cases with nucleo-cytoplasmic and perinuclear expression. HCC cases showed also smaller percentage of variable combinations of cellular localization concerning GLUT1 expression [Fig. 1m-o]. The difference between groups is statistically significant [p<0.001] [Table 3].

There was a significant inverse correlation between both the percentage and intensity of GLUT1expression with the percentage of steatosis in peri-malignant tissue [p<0.01 and p<0.05 respectively]. However, there was no obvious correlation between both parameters of GLUT1 expression and both of the hepatitis activity index or the stage of fibrosis [Table 5].

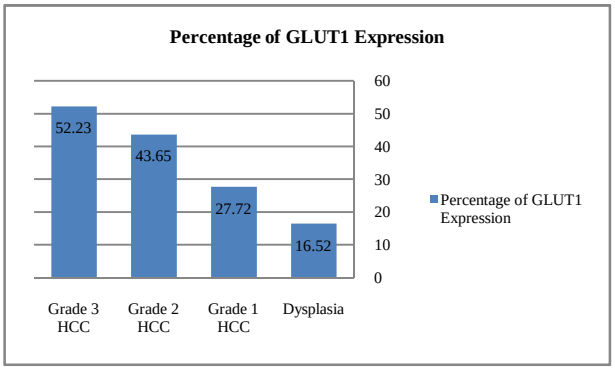
Table 3 Differences in cellular localization of GLUT1 expression in studied lesions

			Cellular Localization*				
			C/M/N	M	M/C	N	Total
Lesions	Peri-malignant	Count	$3_{a,b}$	36_b	0_a	$3_{a,b}$	42
		%	7.1%	85.7%	0.0%	7.1%	100.0%
	dysplasia	Count	0_a	6_a	0_a	0_a	6
		%	0.0%	100.0%	0.0%	0.0%	100.0%
	HCC	Count	$9_{a,b}$	15_b	12_a	$6_{a,b}$	42
			21.4%	35.7%	28.6%	14.3%	100.0%
	Total	Count	12	57	12	9	90
		%	13.3%	63.3%	13.3%	10.0%	100.0%

*[C: cytoplasmic, M: membranous N:nuclear]



Graph 1 Difference in cellular percentage of GLUT1 expression in examined lesions:



Graph 3 Difference in percentage of GLUT1 expression between dysplasia and different grades of HCC in liver tissue:

Table 4 Distribution of positive and negative cases of GLUT1 expression in relation to the stage of fibrosis in peri-malignant tissue

			GLUT1 Expression		INTENSITY of GLUT1 expression			Total
			negative	positive	+1	+2	+3	
Stage	Cirrhosis	Count	15_a	15_a	15_a	12_a	3_a	30
		%	50.0%	50.0%	50.0%	40.0%	10.0%	100.0%
	Fibrosis	Count	6_a	6_a	6_a	3_a	3_a	12
		%	50.0%	50.0%	50.0%	25.0%	25.0%	100.0%
Total		Count	21	21	21	15	6	42
		%	50.0%	50.0%	50.0%	35.7%	14.3%	100.0%

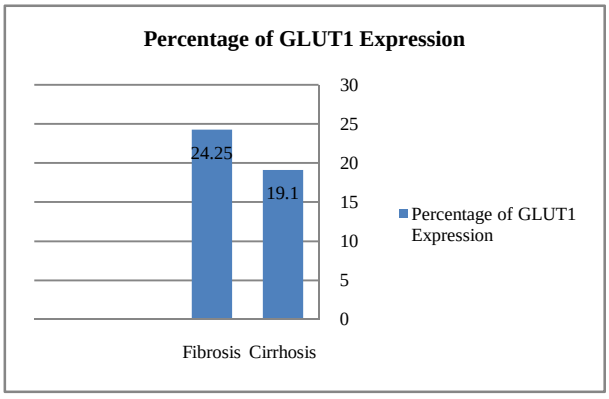
Each subscript letter denotes a subset of POSNEG categories whose column proportions do not differ significantly from each other at the .05 level.

Table 5 Correlation between scores of GLUT1 expression and Hepatitis parameters in peri-malignant liver tissue sections:

Parameter		HAI	STAGE	STEATOSIS
INTENSITY	Correlation Coefficient	-.146	.004	-.461**
	Sig. [2-tailed]	.358	.980	.002
	N	42	42	42
PERCENTAGE	Correlation Coefficient	-.269	.015	-.350*
	Sig. [2-tailed]	.084	.926	.023
	N	42	42	42

**. Correlation is significant at the 0.01 level [2-tailed].
*. Correlation is significant at the 0.05 level [2-tailed].

The fibrotic group showed non-significantly higher percentage of GLUT1 expression than the cirrhotic group. [p>0.05] [Graph 2].



Graph 2 Difference in percentage of GLUT1 expression between the fibrotic and cirrhotic groups of peri-malignant liver tissue:

There was a progressive increase in the percentage of cellular expression of GLUT1 from dysplasia to malignancy; being the lowest in dysplasia and highest in grade 3 HCC with significant difference between groups by ANOVA [p<0.01]. [Graph 3].

We have studied also the sensitivity and specificity of GLUT1 as regard detection of malignancy in peri-malignant and HCC cases. We found that the sensitivity was 87.5% and specificity was 66.67%.

DISCUSSION

HCV is a major global cause of liver disease [24]. Egypt has the highest burden of the HCV in the world with prevalence rate as high as14.7%. In Egypt, liver cancer forms 1.68% of the total malignancies. HCC constitutes 70.48% of all liver tumors among Egyptians [11].

Hypoxia is a hallmark of various cancers and is often associated with disease progression. Tumors respond to hypoxic conditions by activating genes that regulate glycolysis and glucose transport including GLUT1 [25].

In our studied groups, GLUT1 was expressed mainly as membranous staining; however, membrano-cytoplasmic, nucleo-cytoplasmic and nuclear stainings were also seen. This finding is consistent with data reported by Mano *et al* [26]; Izuishi *et al* [27] and Amann *et al* [28] who found strong membranous stain in their HCC cases, and with findings of Roh *et al* [29] who found variable cytoplasmic GLUT1 expression in HCC. Additionally, Taganaka and Frommer [30] explained GLUT1cytoplasmic expression as GLUTs mediate endoplasmic reticulum glucose transport en route to the plasma membrane.

Predominance of membranous staining in benign lesions is related to hypoxia, which is often associated with disease progression and has been shown to result in translocation of GLUT1 to the plasma membrane as well as activation of preexisting GLUT1 in the plasma membrane [31].

To our knowledge nuclear location of GLUT1 has not been reported previously except in a study conducted by Pantaleon

et al [32] who reported that GLUT1 was observed in nuclei of polyspermic oocytes in their studied experiments on mice. The significance of this nuclear expression of GLUT1 needs to be further studied.

In accordance with Czech *et al* [33] and Smith [34], our study showed that immunopositivity was more frequently overexpressed in dysplastic and HCC groups [83.3% and 85.7% respectively] compared to peri-malignant group [50%]. The expression of GLUT1 in a significant number of HCCs and its lower expression in corresponding benign hepatic tissue indicate that this transporter probably plays an important role in the uptake of glucose by HCC cells, being the main energetic source of these neoplastic growths. On the contrary to our results, Amann *et al* [28] reported that GLUT1 was detectable in only 13.2% of the HCC tissues, and in none of the noncancerous liver tissues.

In peri-malignant group, we found GLUT1 expression in half of cirrhotic and fibrotic lesions. GLUT1 expression in these lesions can be related to role played by hypoxia in the pathogenesis of HCV and in turn hypoxia results in an increased transcription of the GLUT1 gene [35, 36].

Our data demonstrated that intensity of GLUT1 expression increased in HCC group compared to dysplasia and peri-malignant groups. This is in agreement with Izuishi *et al* [27] who observed a strong staining in poorly differentiated [grade 3] hepatocellular carcinoma. In peri-malignant group, no correlation was found between intensity of GLUT1expression and both of the hepatitis activity index or the stage of fibrosis.

Percentage of positive hepatocytes for GLUT1 was significantly high in HCC group compared to peri-malignant and dysplasia groups. In addition, this percentage progressively increases with raising grades of HCC. This goes with Gaten by *et al* [37] who reported a correlation between GLUT1 expression and tumor grade in breast cancer cases, and also Krzeslak *et al* [38] found a significantly higher GLUT1 expression in higher grades of breast and endometrial carcinomas than in grade 1. In peri-malignant group, no correlation was found between percentage of GLUT1 expression and both of the hepatitis activity index or the stage of fibrosis.

Concerning pattern of GLUT1 distribution we detect patchy distribution in most our studied lesions. HCC group showed in addition diffuse staining in some cases, however, peri-malignant group showed some cases with central distribution mainly in cirrhotic lesions. Patchy distribution can be explained by hypoxia which is a hallmark of cancer and chronic liver disease; in hypoxic cell environment, there is enhanced expression of GLUT1 [36,39,40]. Additionally, pericentral hepatocytes are more susceptible to hypoxia and also are responsible for glucose uptake. This can explain central distribution of GLUT1 [41].

Many patients with chronic HCV are noted to have a degree of steatosis. Although Czech *et al* [20] reported increased GLUT1 expression in cases of nonalcoholic fatty liver disease of various degrees, we found a significant inverse correlation between percentage of steatosis in peri-malignant tissue and both percentage of GLUT1 expression and intensity of GLUT1. This difference could be related to different etiologic factors of steatosis, being caused by HCV infection in our cases.

CONCLUSION AND RECOMMENDATIONS

GLUT1 lower expression in fibrotic and cirrhotic peri-malignant tissue and its higher expression in dysplastic lesions and sustained expression in hepatocellular carcinoma indicates that changes in GLUT1 levels represent early events during the development of hepatocellular carcinoma. So GLUT1 can be a reliable marker in the diagnosis of premalignant lesions associated with HCV infection, and usage of antagonists to GLUT1 can regulate tumor metabolism and inhibit the progression of chronic liver disease to hepatocellular carcinoma.

Acknowledgment

Authors of this paper are greatly thankful to Mrs. Nadia Abdullah; histopathology technician at the pathology department, Theodor Bilharz Research Institute, for her help in routine and immunohistochemical techniques.

Conflict of Interest

No conflicts of interest.

References

World Health Organization [WHO]. Hepatitis C Fact Sheet. In: WHO [ed.], WHO Fact Sheet 2006. Geneva: WHO [Updated July 2016].

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380:2095-2128

El-Serag HB [2012]: Epidemiology of Viral Hepatitis and Hepatocellular Carcinoma. Gastroenterology 2012; 142[6]: 1264–1273.

Wilder J., Patel K. A review of the natural history of chronic hepatitis C infection. *N Am J Med Sci* 2014; 7: 1–7

Maan R and van der Meer AJ. Recent advances in managing chronic HCV infection: focus on therapy in patients with severe liver disease. Version 1. F1000Res; 5: F1000 Faculty. 2016; Rev-367.

GLOBOCAN. Database [version 1.2]. Online analysis. Available at http://globocan.iarc. fr/accessed on 26 Oct 2012] 2012.

Yu K, Schomisch SJ, Chandramouli V and Lee Z. Hexokinase and glucose-6-phosphatase activity in woodchuck model of hepatitis virus-induced hepatocellular carcinoma. Comp Biochem Physiol C Toxicol Pharmacol 2006; 143: 225-231.

Whittaker S, Marais, R and Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene 2010; 29: 4989-5005.

Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. Infect Dis 2013; 24: 13:288.

Baghdady I, EI-Kaffrawy N, Abd EI-Atti E, Abd EI-Bary N, Saber M. Study of the risk factors for hepatocellular carcinoma: effect of their synergism. *Journal of American Science* 2013; 9[4]: 211-217.

Mokhtar N, Gouda I, Adel I. Cancer pathology registry 2003-2004 and time trend analysis. In: Mokhtar N, Gouda I, Adel I, eds. Malignant digestive system tumors. Cairo: Elsheraa Press 2007; 55–67.

Abdel-Atti, E. HCC Burden in Egypt. Gastroenterol Hepatol 2015; 2[3]: 00045

Kallinowski E, Schienger KH, Kunkel S. Blood flow, metabolism, cellular microenvironment and growth rate of human tumor xenography. Cancer Res 1989; 49:3759-64

Airley RE, Mobasheri A. Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. Chemotherapy 2007; 53:233–256

Medina RA, Owen GI Glucose transporters: expression, regulation and cancer. Biol Res 2002; 35:9–26

Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut-1 mRNA by hypoxia inducible factor-1. *J Biol Chem* 2001; 276: 9519-25.

Kasai D, Adachi T, Deng L, Nagano-Fujii M, Sada K, Ikeda M, Kato N, Ide YH, Shoji I, Hotta H. HCV replication suppresses cellular glucose uptake through down-regulation of cell surface expression of glucose transporters. *J Hepatol* 2007; 50: 883-894

Abdul Rashid MM, Toh1TB, Silva A, Abdullah LN, Ho CM, Ho D, *et al*. Identification and optimization of combinatorial glucose metabolism inhibitors in hepatocellular carcinomas. *Journal of laboratory automation* 2015; 20[4]: 423–437

Deng D, Xu C, Sun P, Wu J, Yan C, Hu M, *et al*. Crystal structure of the human glucose transporter GLUT1. Nature 2014; 510[7503]: 121-125

Czech B, Valletta D, Saugspier M, Weiss TS, Dorn C, Bosserhoff A, *et al*. Increased expression of the glucose transporter GLUT1 in liver fibrosis. Z Gastroenterol 2012; 50:1-6

Cooper R, Sarioglu S, Sökmen S, Füzün M, Küpelioglu A, Valentine A, *et al*. Glucose transporter- 1 [GLUT-1]: a potential marker of prognosis in rectal carcinoma. *Br J Cancer* 2003; 89: 870-876.

French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsies in patients with chronic hepatitis C. Hepatology 1994; 20: 15-20.

Theise ND, Park YN, Curado MP, Sakamoto M, Franceschi S, Torbenson M, *et al*. Hepatocellular carcinoma. WHO Classification of tumours of the Digestive System, 4th Edition, International Agency for Research on Cancer, Lyon. 2010:205–216

Gomaa AI, Hashim MS, Waked I. Comparing staging systems for predicting prognosis and survival in patients with hepatocellular carcinoma in Egypt. PLoS One 2014; 9:e90929.

Chiche J, Brahimi-Horn MC, Pouysse´gur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. *J Cell Mol Med* 2010; 14:771-94

Mano Y, Ishima S, Kubo Y, Tanaka Y, Motomura T, Toshima T, Shirabe K, Baba S, Maehara Y, Oda Y. Correlation Between Biological Marker Expression and Fluorine-18 Fluorodeoxyglucose Uptake in Hepatocellular Carcinoma. *Am J Clin Pathol* 2014; 142:391-397.

Izuishi K, Yamamoto Y, Mori H, Kameyama R, Fujihara S, Masaki T, *et al*. Molecular mechanisms of [18F] fluorodeoxyglucose accumulation in liver cancer. Oncology reports 2014; 31:701-706

Amann T, Maegdefrau U, Hartmann A, Agaimy A, Marienhagen J, Weiss TS, *et al*. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am J Pathol* 2009; 174: 1544–1552.

Roh MS, Jeong JS, Kim YH, Kim MC, Hong SH. Diagnostic utility of GLUT1 in the differential diagnosis of liver carcinomas. Hepatogastroenterology 2004; 51: 1315-1318

Takanaga H, Frommer WB. Facilitative plasma membrane transporters function during ER transit. FASEB J 2010; 24:2849-58

Zhang JZ, Behrooz A, Ismail-Beigi F. Regulation of glucose transport by hypoxia. *Am J Kidney Dis* 1999; 34:189–202.

Pantaleon M, Ryan JP, Gil M, Kaye PL [2001]: An Unusual Subcellular Localization of GLUT1 and Link with Metabolism in Oocytes and Preimplantation Mouse Embryos. Biology of Reproduction 2001; 64[4]: 1247-1254

Czech B, Valletta D, Saugspier M, Müller M, Bosserhoff A, Hellerbrand C. Effect of increased glucose transporter 1 [GLUT1] expression in activated hepatic stellate cells, Z Gastroentero 2013; 51, 1:10

Smith TA. Facilitative glucose transporter expression in human cancer tissue. Br J Biomed Sci. 1999; 56:285–92.

Xie H, Song J, Liu K, Ji H, Shen H, Hu S, Yang G, *et al*. The expression of hypoxia-inducible factor-1alpha in hepatitis B virus-related hepatocellular carcinoma. Correlation with patients' prognosis and hepatitis B virus x protein. Digestive diseases and sciences 2008; 53:3225–3233.

Czech B, Valletta D, Müller M, Bosserhoff A, Hellerbrand C. Expression and function of glucose transporter 1 [GLUT1] expression in activated hepatic stellate cells. Z Gastroentero 2014; 52:1-15

Gatenby RA, Smallbone K, Maini PK, Rose F, Averill J, Nagle RB, *et al*. Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. Br J Cancer 2007; 97:646-53

Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, *et al*. Expression of GLUT1 and GLUT3 Glucose Transporters in Endometrial and Breast Cancers. Pathol Oncol Res 2012; 18:721–728

Cannito S, Paternostro C, Busletta C, Bocca C, Colombatto S, Miglietta A, *et al*. Hypoxia, hypoxia-inducible factors and fibrogenesis in chronic liver diseases. Histol Histopathol 2014; 29: 33-44

Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324:1029-33

Broughan TA, Naukam R, Tan C, Van CJ, wiele D, Refai H, *et al*. Effects of Hepatic Zonal Oxygen Levels on Hepatocyte Stress Responses. *J Surgical research* 2008; 145:150-160

****