Original Article

Validity and clinical impact of glucose transporter 1 expression in colorectal cancer

Ghada M. K. GabAllah, Mona Salah El-din Habib, Shimaa El-Shafey Soliman, Zienab A. Kasemy[1], Suzy F. Gohar[2]

Departments of Medical Biochemistry, [1]Public Health and Community Medicine and [2]Clinical Oncology, Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt

Abstract

Background/Aim: There is no doubt that colorectal cancer (CRC) poses a major threat to public health worldwide, and despite improvement in managements, prognosis still remains an irritating question with no definite answer. Being a fundamental player in cancer metabolism, glucose transporter 1 (GLUT1) could be utilized as a prognostic biomarker that could fuel development of new treatment strategies. The aim of this study was to assess the validity of GLUT1 expression as a prognostic biomarker and to elucidate to what extent it is immersed in poor clinical outcome among CRC patients.

Patients and Methods: GLUT1 expression in peripheral blood specimens was analyzed by quantitative real-time polymerase chain reaction in 47 CRC patients and 20 healthy controls.

Results: There was significantly elevated GLUT1 expression in peripheral blood of CRC patients than in controls ($P < 0.001$). The cutoff value of 0.605 provided 98% sensitivity and 100% specificity. There were significantly higher values of GLUT1 expression in patients under 50 years ($P = 0.003$), performance status 2 ($P = 0.009$), stage IV ($P < 0.001$), and presence of metastasis ($P < 0.001$). GLUT1 expression showed nonsignificant association with overall survival ($P = 0.068$), while tumor stage ($P = 0.01$) and metastasis ($P = 0.009$) were significantly associated with lower overall survival.

Conclusion: GLUT1 is sensitive and specific marker for CRC. It is overexpressed in young age patients, poor performance status, and stage IV patients. Although this was not statistically significant, GLUT 1 showed higher expression level in patients with lesser survival.

Keywords: Colorectal cancer, glucose transporter 1, real-time polymerase chain reaction

Address for correspondence: Dr. Suzy Fawzy Gohar, Department of Clinical Oncology, Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt.
E-mail: dr.suzygohar@gmail.com

INTRODUCTION

Colorectal cancer (CRC), the third most common cancer and the fourth leading cause of cancer-related death worldwide,[1] poses a major burden to public health.[2-5] In Egypt, CRC represents 4.2% of the total tumor burden. It is ranked fourth in females and seventh in males. According to National Cancer Institute Cairo records, it is more common in males, and the median age in Egyptian patients is about 50 years.[6] Identification of prognostic biomarkers that could fuel development of new treatment strategies would be of a particular clinical relevance.[7,8]

Reprogramed glucose metabolism is one of the cornerstones of cancer homeostasis.[9-13] In comparison to normally differentiated cells, CRC cells uptake glucose at a higher

Access this article online	
Quick Response Code:	Website: www.saudijgastro.com
	DOI: 10.4103/sjg.SJG_197_17

How to cite this article: GabAllah GM, El-din Habib MS, Soliman SE, Kasemy ZA, Gohar SF. Validity and clinical impact of glucose transporter 1 expression in colorectal cancer. Saudi J Gastroenterol 2017;23:348-56.

rate to feed the highly active aerobic glycolysis.[4,8,10,14] This enhanced uptake is mediated by glucose transporter 1 (GLUT1).[15-19]

GLUT1, encoded by the SLC2A1 gene, belongs to the facilitative superfamily of membrane integral proteins, a family of 14 members.[20-24] GLUT1 is the major glucose transporter in CRC cells.[16,25-27] It is formed of 492 amino acid residues and possesses a single site of N-linked glycosylation at N^{45}.[20,28]

Cancer cells modulate glucose uptake, the first step in glucose metabolism, by induction of GLUT1.[11] Previous studies have reported GLUT1 overexpression in various tumors;[7,8,29-45] however, there was a little consistency in results that were presented. Furthermore, considerable differences in methodological approach have prevented the reliable comparisons necessary to determine the true clinical value of GLUT1 gene expression in peripheral blood of CRC patients. In a metastatic state, each gram of tumor may shed approximately 10^6 cells into the blood vessel.[46] Being easy and safe to perform, blood tests is a good procedure.[39] In contrast, analysis of solid tumors necessitates invasive procedures that might limit patient compliance. Thus identification and validation of prognostic biomarkers of peripheral circulating cancer cells in blood specimens could be of great help in terms of patient compliance. We exploited real-time polymerase chain reaction (RT-PCR) to assess the validity of GLUT1 expression in CRC peripheral blood specimens and to explore to what extent this expression profile is related to clinical features as well as to overall survival of CRC patients.

PATIENTS AND METHODS

This case–control study was carried out on patients with histopathological proof of colorectal adenocarcinoma who attended to new cases clinic in Clinical Oncology Department, Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt. In the study period from March 2014 to October 2014, a total of 47 patients were enrolled; out of which 35 patients diagnosed with colon adenocarcinoma and 12 patients diagnosed with rectal adenocarcinoma. Twenty age and gender-matched healthy subjects were included as a control group.

A simple and clear explanation of the research objectives and procedures was provided to each of the controls in the study. All patients were subjected to full history taking (including age, gender, complaint, comorbidities, family and personal history of cancer and surgical interference), thorough clinical examination (including weight, height, performance status, local and general examination), and full investigations [body computed tomography (CT), colonoscopy, complete blood count, full kidney and liver functions].

Ethical approval was obtained from the Research Ethics Committee, Faculty of Medicine, Menoufia University, Shibin El Kom El-Kom, Egypt and informed consent was obtained from every participant. Both patients and controls were subjected to the analysis of mRNA expression levels of GLUT1 by RT-PCR.

Gene expression analysis of GLUT1

RNA isolation

Total RNA was extracted from whole blood (collected in ethylenediamine tetraacetic acid tube) by GeneJET Whole Blood RNA Purification Mini Kit (Thermo Scientific), according to the manufacturers' protocol. RNA samples were stored at –20°C until analysis. The concentration of RNA was determined by measuring its absorbance at 260 nm (A260). Absorbance readings should be >0.15 to ensure significance. The ratio between the absorbance value at 260 and 280 nm (A260:A280) gives an estimate of RNA purity. (A260:A280) ratio >1.6 was accepted. Two-step RT-PCR was done as follows: for reverse transcription step, a reverse transcriptase kit (SensiFAST cDNA synthesis kit, Bioline Reagents Ltd, UK) was used for complementary DNA (cDNA) synthesis on 2720 thermal cycler (Singapore). For cDNA synthesis, RNA (10 μl) was reverse transcribed in a final volume of 20 μl containing 1 μl of reverse transcriptase enzyme, 4 μl of 5x TransAmp buffer, and 5 μl of DNase/RNase free water. The samples were incubated at 25°C for 10 min (primer annealing), and 42°C for 15 min (reverse transcription). Reverse transcriptase was then inactivated by heating at 85°C for 5 min. All products were stored at −20°C till the next step. For cDNA amplification, a relative quantitation of GLUT1 mRNA expression normalized to the endogenous reference gene β-actin was performed by RT-PCR reverse transcription, using the 2x SensiFAST™ SYBR® Lo ROX Kit (Bioline Reagents Ltd. located in Humber Road, The Edge Business Centre, Unit 16, London, NW2 6EWUnited Kingdom), on Applied Biosystems 7500 RTPCR system. GLUT1 primers were: 5'-CAACTGGACCTCAAATTTCATTGTGGG-3' (forward) and 5'-CGGGTGTCTTATCACTTTGGCTGG-3' (reverse).[47] β-actin was used as an endogenous reference with primers: 5'-AGTTGCGTTACACCCTTTCTTG-3' (forward) and 5'-TCACCTTCACCGTTCCAGTTT-3' (reverse). Specificity of the primers was verified using Primer BLAST program provided by NCBI. The PCR reaction mixture

(final volume, 25 μl) contained 12.5 μl of 2x SensiFAST™ SYBR® Lo ROX Master Mix, 1 μl of each primer (Sigma), 5.5 μl of DNase/RNase free water, and 5 μl of cDNA. Thermocycling conditions were 10 min at 95°C, followed by 45 cycles at 95°C for 15 s, and 60°C for 1 min. For relative quantification of the results obtained by RT-PCR, the comparative cycle threshold (Ct) method was used. Analysis was performed using Applied Biosystems 7500, software version 2.0.1.

Patients were followed up for at least 24 months by CT scan for abdomen and pelvis (searching for liver metastases), tumor markers (CA19-9 and CEA) every 3 months, and annual colonoscopy for cases of colon cancer, whereas rectal cancer patients were followed up by magnetic resonance imaging of pelvis (searching for local recurrence), tumor markers (CA19-9 and CEA) every 3 months, and annual colonoscopy.

Time to progression was calculated for all patients as the length of time from the date of start of treatment until the disease starts to progress in the form of local recurrence, newly developed metastases (for patients with localized disease), or increase in size and/or number of metastases in patients with primary metastatic disease. Overall survival was calculated as the length of time from the date of start of treatment until date of patient's death.

Statistical analysis

The Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA) was used in data analysis. Descriptive statistics were used to present the distribution of demographic and clinical characteristics. The Chi-square and Fisher's exact tests were used for qualitative data. *t*-test, Mann–Whitney, and Kruskal–Wallis tests were used to test the difference in quantitative data. The odds ratio (OR) and 95% confidence intervals (CI) were calculated. *P* value <0.05 was considered to be statistically significant. Response to treatment was assessed according to revised RECIST guideline (version 1.1).[48] Receiver operating characteristics (ROC) curve was used to assess sensitivity, specificity, and to determine cutoff point. Survival was analyzed using the Kaplan–Meier curve.

RESULTS

As shown in Table 1, out of the 47 patients, 35 (74.5%) patients were diagnosed with colon adenocarcinoma, while only 12 (25.5%) patients were diagnosed with rectal adenocarcinoma. The mean age of patients was 50.25 ± 10.79 years. The patients under 50 years constituted 53.1% of all CRC patients. There were 20 (42.6%) males

Table 1: Descriptive statistics of the different parameters in (CRC) patients

	CRC patients (*N*=47)
Age (years)	
X±SD	50.25±10.79
Range	26-75
BMI (kg/m^2)	
X±SD	25.98±5.22
Range	17.8-40
Age (years)	No (%)
≤50	25 (53.1)
>50	22 (46.9)
Gender	
Male	20 (42.6)
Female	27 (57.4)
Diagnosis regarding tumor location	
Cancer colon	35 (74.5)
Cancer rectum	12 (25.5)
The Presenting Symptoms	
Abdominal pain	24 (51.1)
Vomiting	1 (2.1)
Constipation	4 (8.5)
Intestinal obstruction	4 (8.5)
Intestinal perforation	2 (4.3)
Melena	1 (2.1)
Bleeding per rectum	9 (19.1)
Diarrhea	2 (4.3)
Performance status	
0	29 (61.7)
1	15 (31.9)
2	3 (6.4)
Smoking	
Yes	8 (17.0)
No	39 (83.0)
Co-morbidities	
Negative	27 (57.4)
Hypertension	8 (17.0)
Hepatitis C	6 (12.8)
DM & HTN	2 (4.3)
Liver cirrhosis	2 (4.3)
HTN & Hepatitis C	1 (2.1)
DM & HTN & Hepatitis C	1 (2.1)
Tumor location	
Right colon	17 (36.2)
Left colon	15 (31.9)
Transverse colon	3 (6.4)
Upper rectum	7 (14.9)
Lower rectum	5 (10.6)
Tumor stage at presentation	
Stage II	7 (14.9)
Stage III	19 (40.4)
Stage IV	21 (44.7)
Metastasis	
Positive	21 (44.7)
Negative	26 (55.3)
Metastatic sites	
Liver	6 (28.6)
Peritoneal metastasis	7 (33.3)
Lung	5 (23.8)
Liver + lung	2 (9.5)
Liver + abdominal lymph node	1 (4.8)
Tumor Grade	
Well differentiated	2 (4.3)
Moderately differentiated	34 (72.3)
Poorly differentiated	11 (23.4)
Signet ring differentiation	

Contd...

Table 1: Contd...

	CRC patients (*N*=47)
Yes	1 (2.1)
No	46 (97.9)
Mucoid differentiation	
Mucinous	10 (21.3)
Non-mucinous	37 (78.7)
Surgery	
No	19 (40.4)
Right hemicolectomy	11 (23.4)
Left hemicolectomy	5 (10.6)
Tumor mass excision	6 (12.8)
Low anterior resection	4 (8.5)
Total colectomy	1 (2.1)
Transverse colectomy	1 (2.1)
CA 19.9	
Normal	38 (80.9)
Elevated	9 (19.1)
CEA	
Normal	40 (89.6)
Elevated	7 (10.4)
Patient Fate at the end of follow up period	
Died	25 (53.2)
Alive	22 (46.8)
Time to progression	
<6 month	8 (17.0)
6-12 month	20 (42.6)
>12 month	19 (40.4)
Treatment related toxicities	
No	29 (61.7)
Heamatological	9 (19.1)
Diarrhea	3 (6.4)
Vomiting	1 (2.1)
Heamatological , diarrhea and vomiting	2 (4.3)
Cardiac toxicity	2 (4.3)
Heamatological toxicity and diarrhea	1 (2.1)

BMI: Body mass index; DM: Diabetes mellitus; HTN: Hypertension

versus 27 (57.4%) females. The mean ± SD of BMI was 25.98 ± 5.22 within the patients. Abdominal pain and bleeding per rectum were the most common presenting symptoms (51.1 and 19.1%, respectively). Out of all patients, 26 patients (61.7%) were of performance status 0, 15 patients (31.9%) were of performance status 1, and only 3 patients (6.4%) with performance status 2. Also most of patients were nonsmokers (83.0%) and had no comorbidities (57.4%).

Regarding the location of tumor, the most common site was the right colon (36.2%) followed by left colon, upper rectum (tumor lies >9 cm up to 19 cm from anal verge, i.e. in the upper two-thirds of the rectum), lower rectum (tumor lies within 9 cm from the anal verge, i.e. in the lower third of the rectum), and transverse colon (31.9, 14.9% and 10.6, 6.4%, respectively).

Most patients were in stage III and IV representing 40.4 and 44.7%, respectively. Interestingly, the liver was the most frequently involved site either alone or in association with other involved sites. The majority of patients had moderately or poorly differentiated histologic tumor types (72.3 and 23.4%, respectively); only one patient (2.1%) had signet ring differentiation, while 37 (78.7%) patients showed non-mucinous differentiation.

Twenty-eight patients representing 59.6% were able to undergo surgery as primary treatment modality. During follow-up most patients completed their treatment schedule (either with chemotherapy or concomitant chemo and radiotherapy) with no treatment-related toxicities (61.7%). At the end of follow-up period, 25 (53.2%) patients died, while 22 (46.8%) survived with overall survival rate of 46.8%.

Based on RT-PCR data, there was significantly elevated GLUT1 expression in peripheral blood of the 47 CRC patients in comparison to the 20 healthy controls ($P < 0.001$) [Table 2 and Figure 1].

To evaluate the diagnostic power of the quantitative GLUT1 assay to discriminate CRC from healthy individuals, ROC curve analysis was performed. The cutoff value of 0.605 provided 98% sensitivity and 100% specificity (area under the curve was 0.98, suggestive of a high discrimination power, positive predictive value 100%, negative predictive value 95%) [Figure 2 and Table 3].

The association between GLUT1 expression and different clinico-pathologic prognostic parameters is shown in Table 4 in which there was statistically significant relation between GLUT1 expression and age, performance status, tumor stage and metastasis with higher values in patients under 50 years ($P = 0.003$), performance status 2 ($P = 0.009$), stage IV ($P < 0.001$), and presence of metastasis ($P < 0.001$). There was no significant difference in GLUT1 expression regarding gender ($P = 0.788$), tumor location ($P = 0.372$), tumor differentiation ($P = 0.878$), and initial tumor markers level [Table 4 and Figure 3].

Moreover, there was an elevated GLUT1 expression level in died patients compared to survived ones, but this finding did not reach a significant level ($P = 0.068$) [Table 4 and Figure 4]. There was significant correlation between GLUT1 expression and both of age ($P = 0.017$) and tumor stage ($P \leq 0.001$), while there was no significant correlation between GLUT1 expression and either BMI ($P = 0.074$), grade ($P = 0.710$), performance status ($P = 0.425$), or overall survival ($P = 0.128$) [Table 5].

Regarding survival analysis, the median overall survival was 24 months [Table 6]. By univariate analysis, tumor stage ($P = 0.01$) and metastasis ($P = 0.009$) were

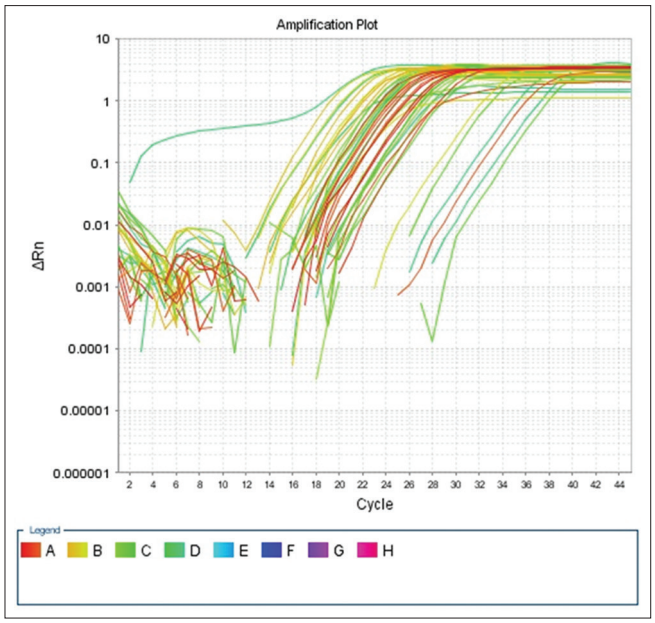

Figure 1: Amplification plot of GLUT1 expression (ΔRn vs. Cycle)

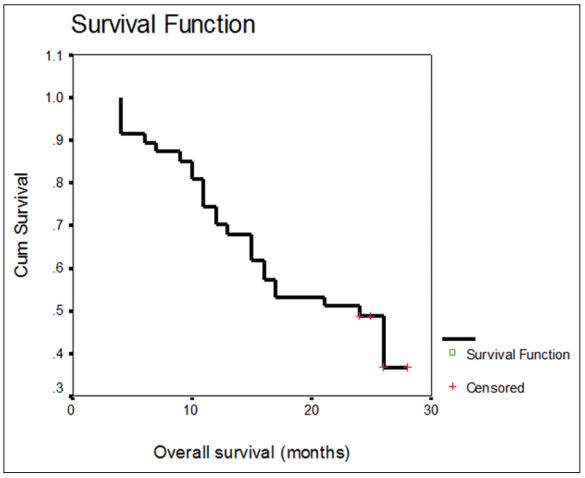

Figure 3: Overall survival among the studied cases

Table 2: GLUT1 expression among the studied subjects

	The studied subjects		U test	*P*
	CRC patients *N*=47	Control *N*=20		
GLUT1				
X±SD	1.87±1.11	0.28±0.16	6.25	<0.001
Range	0.14-6.8	0.02-0.51		

X: mean; SD: Standard deviation, U: Mann Whitney test

significantly associated with lower overall survival [Table 7 and Figures 5,6], while other factors such as age, gender, performance status, tumor differentiation were nonsignificant predictive factors [Table 7].

DISCUSSION

In order to proliferate, enhanced glycolytic profile is a constitutive tumor survival response.[11,49] GLUT1 plays

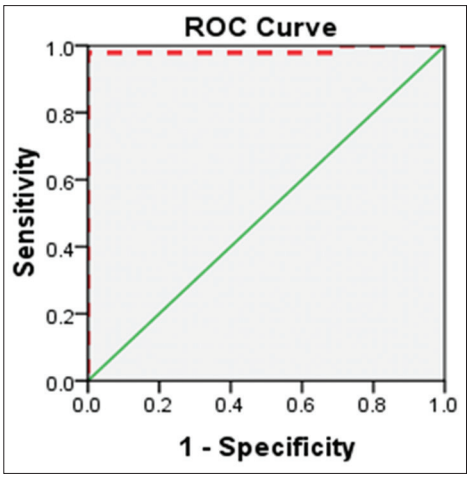

Figure 2: ROC curve of GLUT1 to differentiate between cases and control

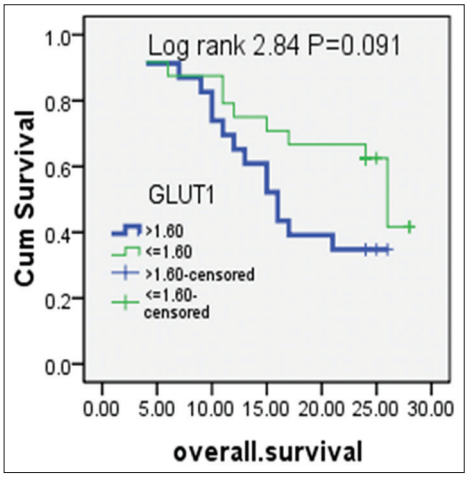

Figure 4: GLUT1 and survival analysis

a fundamental role in cancer metabolism. [4,50] Expressing high levels of the GLUT1 is a cancer tool to resist the harsh tumor microenvironment.[4,10,14,39]

Accumulating evidence has demonstrated GLUT1 overexpression in a wide variety of tumors obtained with different methodologies.[25] However, there are few data confirming this metabolic phenotype by sensitive and relatively an easy method. Accordingly, we exploited RT-PCR technique to clarify the validity and prognostic significance of GLUT1 expression in peripheral blood of CRC patients and its association with patient outcome and overall survival.

In this prospective study, patients under 50 years constituted 53.1% of all CRC patients. This is higher than previous a study in Egypt in which the incidence of patients under 50 years was 32%,[8] indicating that CRC incidence in patients under 50 years in Egypt is increasing. The current data showed a higher female prevalence (57.4%). This is slightly

Table 3: Validity of GLUT1 expression in diagnosis of malignant transformation

AUC	*P*	95% CI	Cutoff point	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)
0.98	0.000	0.96-1.02	0.605	98%	100%	100%	95%

Table 4: GLUT1 expression in relation to different clinicopathologic prognostic variables

	GLUT1 Mean±SD	Mann-Whitney Test	*P*
Age			
≤50	2.27±1.27	2.94	0.003*
>50	1.40±0.64		
Sex			
Male	2.0±1.45	0.26	0.788
Female	1.76±0.76		
Smoking			
Yes	1.73±0.77	0.22	0.821
No	1.89±1.16		
Co-morbidities			
Negative	1.61±0.87	1.95	0.051
Positive	2.21±1.30		
Diagnosis	1.89±1.10	1.12	0.261
Cancer colon			
Cancer rectum	1.77±1.16		
Performance status			
0	1.33±0.68	9.46#	0.009*
1	2.05±1.22		
2	2.73±0.41		
Tumor location			
Right colon	1.92±0.77		
Left colon	1.85±1.46	4.25#	0.372
Transverse colon	2.0±0.88		
Upper rectum	2.02±1.30		
Lower rectum	1.42±0.94		
Tumor stage			
Stage II	1.06±0.65	15.28#	<0.001*
Stage III	1.48±0.44		
Stage IV	2.48±1.32		
Metastasis			
Positive	2.28±0.78	3.94	<0.001*
Negative	1.52±1.22		
Tumor Grade			
Well differentiated	1.45±0.35	0.26#	0.878
Moderately differentiated	1.92±1.21		
Poorly differentiated	1.74±0.81		
Signet Ring differentiation			
Yes	2.40		
No	1.85±1.11	-	-
Mucoid differentiation			
Mucinous	1.83±1.35	0.47	0.481
Non-mucinous	1.87±1.04		
CA.19.9			
Normal	1.88±1.11	0.32	0.745
Elevated	1.81±1.13		
CEA			
Normal	1.89±1.15	0.34	0.731
Elevated	1.68±0.79		
Fate			
Death	2.08±1.25	1.82	0.068
Survival	1.61±0.87		
Time to progression			
<6 month	1.81±0.66	0.98#	0.610
6-12 month	2.0±1.35		
>12 month	1.74±0.99		

#Kruskal-Wallis, *<0.05 is considered significant

different from Rashed *et al.*[8] and Eisa studies[51] (40 and 41.6%, respectively).

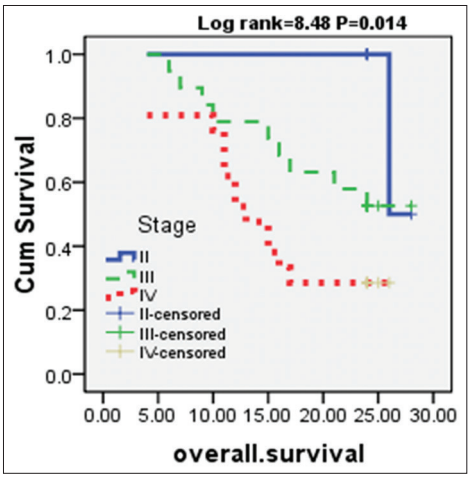

Figure 5: Tumor stage and survival analysis

According to our results, the most common site was the right colon, while the rectum represented 25.5%. This is similar to an earlier Egyptian publication where the rectum constituted 27%.[52] Conversely, in a study by Eisa, rectal carcinoma constituted 42.7%.[51]

The present findings revealed that most patients were in stage III and IV (40.4 and 44.7%, respectively) and 44.7% of all patients had metastatic lesions. In concordance with our results, Eisa observed that young patients had more advanced stage at presentation and explained this either due to the aggressive behavior of the tumor itself or delay in diagnosis.[51]

In this study there was a significant elevated GLUT1 expression in peripheral blood of CRC patients in comparison to controls. Interestingly, the discriminative power of GLUT1 expression was 98% sensitivity and 100% specificity. This exceeds what was documented in a previous report about other serological markers used in CRC such as CEA and CA19-9 where their sensitivity was only 30% and 18%, respectively.[53] This confirmed that GLUT1 is a reliable diagnostic marker for tumorigenesis and may be used as an indicator of possible malignant transformation in high-risk patients such as those with multiple polyposis and ulcerative colitis.

CRC, as many solid tumors, experiences hypoxic microenvironment.[40,54,55] Hypoxia-inducible factor-1, when stabilized by hypoxia, upregulates several glycolytic genes to promote survival under these tough environments.[56] One of these genes is GLUT1 gene.[39,57-60] It is likely that CRC

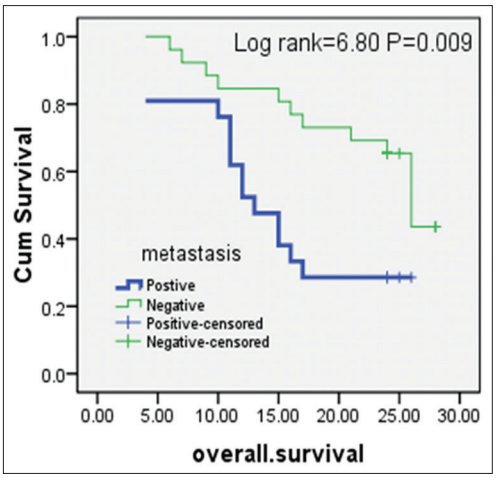

Figure 6: Metastasis and survival analysis

cells upregulate GLUT1, thus increasing glucose uptake[4] to feed enhanced glycolysis as they require high energy levels to proliferate.[25,55,61] Moreover, GLUT1 expression is suppressed by p53, an important tumor suppressor in cancer.[62] The alteration in p53 expression may explain GLUT1 overexpression observed in many cancer types, as well as their enhanced glucose metabolism and their higher energy consumption.[16]

Similar to the current observation, Chung *et al.*[39] found that GLUT1 mRNA was increased in the peripheral blood of stage II and III CRC patients as compared to stage I patients, suggesting that GLUT1 may be a stage-related marker that could be determined by a noninvasive method.

This study confirmed the existence of significantly higher values of GLUT1 expression in patients under 50 years, performance status 2, stage IV, and presence of metastasis. This reflected the close relation between GLUT1 overexpression and poor clinicopathologic factors representing a warning sign of aggressive tumor behavior. High levels of GLUT1 expression make cancer cells resistant to a hypoglycemic environment and have the propensity to survive, proliferate, and metastasize.[14] This means that these patients will benefit from GLUT1 inhibitors. Similar to our finding, Younes *et al.*, Haber *et al.*, Sakashita *et al.*, and Chung *et al.* noticed that induction of GLUT1 is significantly associated with lymph node metastasis and poor prognosis in CRC.[30,31,35,39]

Previous studies based on immune-histochemical detection of GLUT1 also showed that GLUT1 overexpression was an indicator of poor prognostic parameters in CRC.[4,7,8,31,32,35]

This study showed that there was a lack of significant association between GLUT1 expression and gender, tumor site, and tumor differentiation. Similarly, Younes *et al.*[30] and Haber *et al.*[31] reported that there was no correlation between GLUT1 expression and histologic differentiation. Moreover, there was no significant difference in GLUT1 expression regarding the initial level of CA19-9 and CEA.

Table 5: Correlation between marker results and some chosen parameters

	GLUT1	
	r	*P*
Age	0.348	0.017*
BMI	0.263	0.074
Stage	0.581	<0.001*
Grade	0.056	0.710
Performance status	-0.119	0.425
Overall survival	-0.225	0.128

*<0.05 is considered significant

Table 6: Mean and median overall survival among the CRC patients

Overall survival	Months
Mean	
Value	19.5
SE	1.29
95%CI	16.98-22.02
Median	
Value	24.0
SE	3.51
95%CI	17.11-30.89

Currently, there was significant correlation between GLUT1 expression and both of age and tumor stage, while there was no statistically significant relation between GLUT1 expression and overall survival. Consistently, the pooled data gathered by Yang *et al* demonstrated that GLUT1 still had no significant association with overall survival irrespective of tumor location, cancer type, and treatment.[55] On the contrary, Jun *et al.*[7] documented that patients with GLUT1 expression demonstrated poor overall survival and disease-free survival.

In the present study, tumor stage and presence of metastases had a statistically significant relation with overall survival, with the least survival in advanced stage and metastatic disease. In agreement with this, Eisa stated that stage at presentation, lymph node involvement, and performance status are predictors for overall survival in young CRC patients.[51]

CONCLUSION

Taken as a whole, these results support the fundamental role played by GLUT1 in tumor growth and progression, making it a potential biomarker of tumor detection and patient prognosis. Also, this study sheds light on exploitation of this technique on peripheral blood samples maximizing

Table 7: Univariate analysis for factors affecting overall survival among the CRC patients

	Overall survival			Log Rank	*P*
	Mean	SE	95% CI		
Age					
≤50	21.56	3.17	15.35-27.76	1.11	0.29
>50	19.02	1.39	16.30-29.08		
Sex					
Male	21.70	2.0	17.78-25.62	2.87	0.09
Female	18.05	1.62	14.88-21.22		
Performance status					
0	18.55	1.69	15.24-21.86	2.86	0.24
1	22.0	2.15	17.78-26.22		
2	15.33	0.33	14.68-15.99		
Site					
Right colon	15.24	1.91	11.49-18.98	6.0	0.20
Left colon	22.53	2.16	18.31-26.76		
Transverse	21.67	2.72	16.33-27		
Upper rectum	19.43	3.61	12.36-26.5		
Lower rectum	16.60	3.26	13.21-25.99		
Tumor stage					
Stage II	27.0	0.71	25.61-28.39	8.48	0.01
Stage III	21.32	1.87	17.65-24.98		
Stage IV	15.0	1.72	11.62-18.38		
Tumor Grade					
Well	18.0	4.24	9.68-26.32		
Moderate	21.04	1.43	18.25-23.84	4.35	0.11
undifferentiated	13.73	2.17	9.47-17.99		
Metastasis					
Negative	22.68	1.47	19.79–25.57	6.80	0.009
Positive	15.0	1.92	11.62-18.38		
Signet Ring Differentiation					
Yes	15.0	0.0	15.0-15.0	0.65	0.42
No	19.6	1.31	17.03-22.17		
Mucoid Differentiation					
Yes	21.5	1.53	18.5-24.5	1.12	0.29
No	18.55	1.50	15.61-21.5		
CA.19.9					
Normal	19.53	1.41	16.76-22.3	0.0	0.95
Elevated	18.56	3.0	12.68-24.43		
CEA					
Normal	19.74	1.34	17.11-22.37	0.09	0.58
Elevated	17.0	3.56	10.02-23.98		

patient compliance. However, approval of application of this technique on peripheral blood samples of CRC patient needs further research allowing easy, sensitive, and most importantly, repeated detection of GLUT1 for early detection and proper therapeutic interventions. Overall, GLUT1 should be targeted in addition to other traditional therapeutic lines.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Villalobos C, Sobradillo D, Hernández-Morales M, Núñez L. Calcium remodeling in colorectal cancer. Biochim Biophys Acta 2017;186:843-9.
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017;66:683-91.
3. Chen XS, Li LY, Guan YD, Yang JM, Cheng Y. Anticancer strategies based on the metabolic profile of tumor cells: Therapeutic targeting of the Warburg effect. Acta Pharmacol Sin 2016;37:1013-9.
4. Martins SF, Amorim R, Viana-Pereira M, Pinheiro C, Costa RF, Silva P, *et al.* Significance of glycolytic metabolism-related protein expression in colorectal cancer, lymph node and hepatic metastasis. BMC Cancer 2016;16:535.
5. Moridikia A, Mirzaei H, Sahebkar A, Salimian J. MicroRNAs: Potential candidates for diagnosis and treatment of colorectal cancer. J Cell Physiol 2017.
6. El- bolkainy N, Nouh A, El-bolkainy T, Farahat I, Badawy O. Pathology of cancer. 4th ed. Cairo, Egypt: Cairo press; 2013.
7. Jun YJ, Jang SM, Han HL, Lee KH, Jang KS, Paik SS. Clinicopathologic significance of GLUT1 expression and its correlation with Apaf-1 in colorectal adenocarcinomas. World J Gastroenterol 2011;17:1866-73.
8. Rashed HE, Ahmed SA, Abdelgawad M. Clinicopathologic significance of galectin-3 and glucose transporter 1 expressions in colorectal cancer. Life Sci J 2015;12 (1):9-11. (ISSN: 1097-8135). http://www.lifesciencesite.com
9. Shibuya K, Okada M, Suzuki S, Seino M, Seino S, Takeda H, *et al.* Targeting the facilitative glucose transporter GLUT1 inhibits the self-renewal and tumor-initiating capacity of cancer stem cells. Oncotarget 2015;6:651-61.
10. Fang S, Fang X. Advances in glucose metabolism research in colorectal cancer. Biomed Rep 2016;5:289-95.
11. Hay N. Reprogramming glucose metabolism in cancer: Can it be exploited for cancer therapy? Nat Rev Cancer 2016;16:635-49.
12. Li C, Zhang G, Zhao L, Ma Z, Chen H. Metabolic reprogramming in cancer cells: Glycolysis, glutaminolysis, and Bcl-2 proteins as novel therapeutic targets for cancer. World J Surg Oncol 2016;14:15.
13. Yu L, Chen X, Wang L, Chen S. The sweet trap in tumors: Aerobic glycolysis and potential targets for therapy. Oncotarget 2016;7:38908-26.
14. Saigusa S, Toiyama Y, Tanaka K, Okugawa Y, Fujikawa H, Matsushita K, *et al.* Prognostic significance of glucose transporter-1 (GLUT1) gene expression in rectal cancer after preoperative chemoradiotherapy. Surg Today 2012;42:460-9.
15. Hauptmann S, Grünewald V, Molls D, Schmitt WD, Köbel M, Kriese K, *et al.* Glucose transporter GLUT1 in colorectal adenocarcinoma cell lines is inversely correlated with tumour cell proliferation. Anticancer Res 2005;25:3431-6.
16. Calvo MB, Figueroa A, Pulido EG, Campelo RG, Aparicio LA. Potential role of sugar transporters in cancer and their relationship with anticancer therapy. Int J Endocrinol 2010;2010.
17. Thorens B, Mueckler M. Glucose transporters in the 21st Century. Am J Physiol Endocrinol Metab 2010;298:E141-5.
18. 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;18:721-8.
19. Barron CC, Bilan PJ, Tsakiridis T, Tsiani E. Facilitative glucose transporters: Implications for cancer detection, prognosis and treatment. Metabolism 2016;65:124-39.
20. Asano T, Katagiri H, Takata K, Lin JL, Ishihara H, Inukai K, *et al.* The role of N-glycosylation of GLUT1 for glucose transport activity. J Biol Chem 1991;266:24632-6.
21. Joost HG, Bell GI, Best JD, Birnbaum MJ, Charron MJ, Chen YT, *et al.* Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endocrinol Metab 2002;282:E974-6.
22. Uldry M, Thorens B. The SLC2 family of facilitated hexose and polyol transporters. Pflugers Arch 2004;447:480-9.
23. Suganuma N, Segade F, Matsuzu K, Bowden DW. Differential expression of facilitative glucose transporters in normal and tumor kidney tissues. BJU Int 2007;99:1143-9.

24. Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. IUBMB life 2010;62:315-33.
25. Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. Mol Aspects Med 2013;34:121-38.
26. Patra KC, Hay N. Hexokinase 2 as oncotarget. Oncotarget 2013;4:1862-3.
27. Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, *et al*. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. Cancer Cell 2013;24:213-28.
28. Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, *et al*. Sequence and structure of a human glucose transporter. Science 1985;229:941-5.
29. Brown RS, Wahl RL. Overexpression of Glut-1 glucose transporter in human breast cancer. An immunohistochemical study. Cancer 1993;72:2979-85.
30. Younes M, Lechago LV, Lechago J. Overexpression of the human erythrocyte glucose transporter occurs as a late event in human colorectal carcinogenesis and is associated with an increased incidence of lymph node metastases. Clin Cancer Res 1996;2:1151-4.
31. Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz SH, Bodian C, *et al*. GLUT1 glucose transporter expression in colorectal carcinoma: A marker for poor prognosis. Cancer 1998;83:34-40.
32. Wang BY, Kalir T, Sabo E, Sherman DE, Cohen C, Burstein DE. Immunohistochemical staining of GLUT1 in benign, hyperplastic, and malignant endometrial epithelia. Cancer 2000;88:2774-81.
33. Cantuaria G, Fagotti A, Ferrandina G, Magalhaes A, Nadji M, Angioli R, *et al*. GLUT-1 expression in ovarian carcinoma: Association with survival and response to chemotherapy. Cancer 2001;92:1144-50.
34. Furudoi A, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, *et al*. Clinical significance of human erythrocyte glucose transporter 1 expression at the deepest invasive site of advanced colorectal carcinoma. Oncology 2001;60:162-9.
35. Sakashita M, Aoyama N, Minami R, Maekawa S, Kuroda K, Shirasaka D, *et al*. Glut1 expression in T1 and T2 stage colorectal carcinomas: Its relationship to clinicopathological features. Eur J Cancer 2001;37:204-9.
36. Kunkel M, Reichert TE, Benz P, Lehr HA, Jeong JH, Wieand S, *et al*. Overexpression of Glut-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma. Cancer 2003;97:1015-24.
37. Yuan HZ, Zhan BL, Zhang GL. Expression of Glut1 and MVD in colorectal carcinoma and its relationship with liver metastasis. China Cancer 2003;59-61.
38. Casneuf VF, Casneuf VF, Fonteyne P, Van Damme N, Demetter P, Pauwels P, *et al*. Expression of SGLT1, Bcl-2 and p53 in primary pancreatic cancer related to survival. Cancer Invest 2008;26:852-9.
39. Chung FY, Huang MY, Yeh CS, Chang HJ, Cheng TL, Yen LC, *et al*. GLUT1 gene is a potential hypoxic marker in colorectal cancer patients. BMC Cancer 2009;9:241.
40. Carvalho KC, Cunha IW, Rocha RM, Ayala FR, Cajaíba MM, Begnami MD, *et al*. GLUT1 expression in malignant tumors and its use as an immunodiagnostic marker. Clinics (Sao Paulo) 2011;66:965-72.
41. Hong R, Lim SC. ^{18}F -fluoro-2-deoxyglucose uptake on PET CT and glucose transporter 1 expression in colorectal adenocarcinoma. World J Gastroenterol 2012;18:168-74.
42. Lastraioli E, Bencini L, Bianchini E, Romoli MR, Crociani O, Giommoni E, *et al*. hERG1 channels and Glut-1 as independent prognostic indicators of worse outcome in stage i and ii colorectal cancer: A pilot study. Transl Oncol 2012;5:105-12.
43. Sasaki H, Shitara M, Yokota K, Hikosaka Y, Moriyama S, Yano M, *et al*. Overexpression of GLUT1 correlates with K-ras mutations in lung carcinomas. Mol Med Rep 2012;5:599-602.
44. Maki Y, Soh J, Ichimura K, Shien K, Furukawa M, Muraoka T, *et al*. Impact of GLUT1 and Ki-67 expression on early stage lung adenocarcinoma diagnosed according to a new international multidisciplinary classification. Oncol Rep 2013;29:133-40.
45. Szablewski L. Expression of glucose transporters in cancers. Biochim Biophys Acta 2013;1835:164-9.
46. Chang YS, di Tomaso E, McDonald DM, Jones R, Jain RK, Munn LL. Mosaic blood vessels in tumors: Frequency of cancer cells in contact with flowing blood. Proc Natl Acad Sci USA 2000;97:14608-13.
47. Fleming JB, Shen GL, Holloway SE, Davis M, Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: Justification for K-ras-directed therapy. Mol Cancer Res 2005;3:413-23.
48. Eisenhauer E, Therasse P, Bogaerts J, Schwartzd L, Sargente D, Fordf R, *et al*. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
49. Hutton JE, Wang X, Zimmerman LJ, Slebos RJ, Trenary IA, Young JD, *et al*. Oncogenic KRAS and BRAF drive metabolic reprogramming in colorectal cancer. Mol Cell Proteomics 2016;15:2924-38.
50. de Wit M, Jimenez CR, Carvalho B, Belien JA, Delis-van Diemen PM, Mongera S, *et al*. Cell surface proteomics identifies glucose transporter type 1 and prion protein as candidate biomarkers for colorectal adenoma-to-carcinoma progression. Gut 2012;61:855-64.
51. Eisa H. Colorectal cancer in Upper Egypt. Does age make a difference in survival? Med J Cairo University 2010;78:145-50.
52. El-Bolkainy N, Nouh MA, El-Bolkainy TN. Topographic pathology of cancer, 3rd ed. NCI Cairo; 2006. p. 33-9.
53. Herszenyi L, Farinati F, Cardin R, Istvan G, Molnar LD, Hritz I, *et al*. Tumor marker utility and prognostic relevance of cathepsin B, cathepsin L, urokinase-type plasminogen activator, plasminogen activator inhibitor type-1, CEA and CA 19-9 in colorectal cancer. BMC Cancer 2008;8:194.
54. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144:646-74.
55. Yang J, Wen J, Tian T, Lu Z, Wang Y, Wang Z, *et al*. GLUT-1 overexpression as an unfavorable prognostic biomarker in patients with colorectal cancer. Oncotarget 2017;8:11788-96.
56. Korkeila E, Jaakkola PM, Syrjänen K, Pyrhönen S, Sundström J. Pronounced tumour regression after radiotherapy is associated with negative/weak glucose transporter-1 expression in rectal cancer. Anticancer Res 2011;31:311-5.
57. Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 2001;276:9519-25.
58. Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells: Identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem 2001;276:43407-12.
59. Chiche J, Brahimi-Horn MC, Pouyssegur J. Tumour hypoxia induces a metabolic shift causing acidosis: A common feature in cancer. J Cell Mol Med 2010;14:771-94.
60. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular datasets. Nucleic Acids Res 2012;40:D109-14.
61. Bannasch D, Safra N, Young A, Karmi N, Schaible RS, Ling GV. Mutations in the SLC2A9 gene cause hyperuricosuria and hyperuricemia in the dog. PLoS Genet 2008;4:e1000246.
62. Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res 2004;64:2627-33.