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A STUDY OF GLYPICAN 3 LEVELS IN PATIENTS OF HEPATO-CELLULAR CARCINOMA

By

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ABSTRACT

Background: Although surgery is the treatment of choice of hepatocellular carcinoma, only 5-10% of their patients are candidates for surgical resection. Numerous proteins and receptors are over expressed on hepatocellular carcinoma (HCC) cell membrane including glypican3 (GPC3). It has attracted substantial attention because its expression is correlated with HCC tumorigenesis and prognosis.

Objective: To assess the diagnostic role of Glypican-3 levels in diagnosis of hepato-cellular carcinoma.

Patients and methods: This was a prospective comparative study conducted on 90 cases from the outpatient Clinic of Internal Medicine Department of Sayed Galal Hospital and Ahmed Maher Teaching Hospital. The patients of the study were classified into three equal groups: Group 1: Patients with liver cirrhosis and HCC on top (diagnosed by abdominal ultrasonography and abdominal CT with oral and IV contrast), Group 2: Patients with liver cirrhosis and no evidence of HCC, and Group 3: Patients of healthy people as a control group for comparison.

Results: Non-significant differences between groups regarding age, gender, Hb concentration, platelets (PT) INR, AST, ALT, viral markers in cirrhotics and HCC patients. GPC-3 showed higher level in cirrhotics and HCC patients with a significant over-expression in HCC. Patients with over expression of GPC-3 have significant elevation of PT, serum bilirubin. The presence of mass nodule was significantly present in HCC group, while multiple nodularity commonly present in cirrhotics but this did not deny its presence of HCC.

Conclusion: GPC-3 level is important in diagnosis and its differentiation from benign conditions.

Keywords: Glypican 3 levels, hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent and most common aggressive malignancies worldwide, with an increasing incidence globally *(Mahmoud and Mahgoub, 2020)*. HCC represents the fifth most common cancer and the second leading cause of cancer-related deaths worldwide. HCC typically develops in patients with chronic liver diseases, and cirrhosis, usually chronic hepatitis B virus (HBV) or HCV, as the strongest predisposing factors *(Tahon et al., 2019* and *Xu et al., 2019)*.

Early diagnosis of HCC and timely treatment can greatly improve life expectancy and reduce mortality. Currently, serum AFP expression is the most well-established serum biomarker for the diagnosis of HCC. However, the

diagnostic performance of AFP expression in detecting early-stage HCC is suboptimal *(Gao and Song, 2017)*. Therefore, the identification of additional effective and reliable non-invasive biomarkers for the diagnosis of early-stage HCC is of important clinical significance *(Li et al., 2019)*. The prognosis of HCC is generally poor, especially for late-stage malignancies, but a cure is possible if it is diagnosed at the early stages. In fact, 5-year survival for early stage HCC after curative treatments is as high as 70% *(Chia et al., 2019)*.

Glypican-3 (GPC3) is a member of heparin sulfate proteoglycan family, which is bound to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor *(Ghweil et al., 2018, Xu et al., 2019* and *Mahmoud & Mahgoub, 2020)*. GPC3 is widely expressed in human embryos and involved in human tissue growth. GPC3 can be detected in the fetal liver, but cannot be identified in any normal adult hepatic tissue. In recent years, GPC-3 expression levels have been found to be elevated in HCC patients, as shown by immunohistochemistry *(Li et al., 2019)*.

Glypicans play important roles in embryonic development and the regulation of cell proliferation and survival, particularly during development and malignant transformation. In HCC, GPC3 interacts with the wingless-related integration site (Wnt) ligands and stimulates cell proliferation. Cellular proliferation induced by Wnt has recently been attributed to activation of both the extracellular signal-regulated kinase (ERK) and Wnt pathways, both of which are implicated in hepato-carcinogenesis associated with HBV and HCV infections *(Tahon et al., 2018* and *Guo et al., 2020)*.

The aim of the present study was to assess the diagnostic role of Glypican-3 levels in diagnosis of hepato-cellular carcinoma.

PATIENTS AND METHODS

This was a prospective comparative study conducted on 90 cases from the outpatient Clinic of Internal Medicine Department of Sayed Galal Hospital and Ahmed Maher Teaching Hospital.

The patients of the study were classified into three equal groups: Group 1: Patients with liver cirrhosis and HCC on top (diagnosed by abdominal ultrasonography and abdominal CT with oral and IV contrast), **Group 2:** Patients with liver cirrhosis and no evidence of HCC, and **Group 3:** Patients of healthy people as a control group for comparison.

Exclusion criteria: History of any chronic infections, patients who have any malignancies: cancer lung, cancer breast, cancer prostate, …..etc, patients with chronic kidney disease, diabetic patients, patients who received any treatment for liver cancer, alcoholics, patients who have auto immune diseases patients who suffer of severe burns, and patients who refuse to be included in this thesis.

A written consent from every patient about the nature and the characters of the study were taken.

All subjects of the study were subjected to the following:

- Detailed history taking with special emphasis on age, sex, history of

alcohol and drug use; previous history of chronic liver disease.

- Full clinical examination including:
- General examination: weight loss, ecterus, examination of lymph nodes sites.
- Local abdominal examination: commenting on mass, ascites, dilated veins, lower limb edema.
- Investigations:

I. Blood chemistry and markers:

1. Complete blood picture (C.B.C):

- Hemoglobin (g/dL)
- White blood cells (x103/ul)
- Platelets (x103/ul)

2. Fasting blood sugar and 2 hours post-prandial (mg/dL)
3. CRP
4. Alpha fetoprotein "□-FP"
5. Glypican 3 levels in the blood
6. Liver function tests:

- Serum alanine aminotransferase (ALT) (IU/L).
- Serum aspartate aminotransferase (AST) (IU/L).
- Serum alkaline phosphatase (ALP) (U/L).
- Serum total and direct bilirubin (mg/dl).
- Serum albumin (g/dl).
- Prothrombin time and the international normalization ratio (PT, INR).
- glutamyle transferase "GGT".

7. Renal function tests:

- Serum creatinine (mg/dl).
- Blood urea

8. Viral markers:

- HBsAg by using 3rd generation enzyme linked immunosorbent assay technique (ELISA).
- Anti-HCV-Ab by using 3rd generation enzyme linked immunosorbent assay technique (ELISA).
- ANA

II. Imaging:

1. Real time abdominal ultrasound and abdominal CT with oral or intravenous contrast dye was done for all patients and control included in the study for the evaluation of:
2. Liver: size, border, parenchymal echotexture, hepatic veins, biliary radicals, common bile duct and focal lesions and masses.
3. Portal vein: Caliber, patency by color Doppler.
4. Spleen: Size, splenic vein diameter and collaterals.
5. Ascites: Present or not.

Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean±standard deviation (S.D), median, IQR and range. Chi square test (X2), or Fisher's exact test (FET) were used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at

P □0.05. Difference among 3 independent means were analyzed using Kruskal Wallis test (KW) for non-parametric variables. Significant KW tests was followed by post hoc multiple comparisons using Bonferroni test to detect the significant pairs. Spearman's correlation coefficient (rho) was used to assess correlations. ROC curve analysis was constructed to assess the performance of real time in prediction of fibrosis among patients' group. P value < 0.05 was considered significant.

RESULTS

The mean age of patients of group I was 47.63±13.54 years while in group II it was 47.4±10.84 years and 48.56±11.09 years and the statistical analysis revealed that there was no difference between groups of the study regarding age (P = 0.922). There were 16 (53.33%), 16 (53.33%), and 12 (40%) females among HCC, cirrhotic liver, and healthy control groups respectively and the statistical analysis revealed that there was no difference between groups regarding gender (P = 0.0490) **(Table 1)**.

Table (1): The age and sex distribution among the studied groups (number of each group=30)

Groups	Age (Years) Mean ±SD	P-Value			
Group I Group II Group II	47.63±13.54 47.4±10.84 48.56±11.09	0.922			
	Sex	Females		Males	
		No	%	No	%
Group I		16	53.3%	14	46.7%
Group II		16	53.3%	14	46.7%
Group III		12	40%	18	60%

Regarding laboratory assessment, the statistical analysis revealed that there was no statistically significant difference between HCC, cirrhotic liver, and healthy control groups regarding the levels of haemoglobin concentration with mean of 13.23±1.83 g/dL, 1.83±1.64 g/dL and 13.17±2.19 g/dL respectively. In addition, the mean levels of PT showed a statistically significant difference between such groups (P = 0.013). However, on Post-hoc test, this significant was only obtained by comparison of patients within cirrhotic group (81.1±21.21) and healthy individuals (93.6±7.2). The mean levels of INR revealed no statistically significant difference between the three groups (P = 0.31) **(Table 2)**.

Table (2): Blood profile among the included participants

Variables and Groups	Mean±SD	P-Value	Post Hoc Test
Hemoglobin **Group I** **Group II** **Group III**	**13.23±1.83** **1.83±1.64** **13.17±2.19**	**0.530**	**NA**
PT: **Group I** **Group II** **Group III**	**83.17±19.59** **81.1±21.21** **93.6±7.2**	**0.013**	**Group I Vs Group II (P = 0.88)** **Group I Vs Group III (P = 0.054)** **Group II Vs Group III (P = 0.016)**
INR: **Group I** **Group II** **Group III**	**1.005±0.02** **0.99±0.004** **1.003±0.018**	**0.31**	**NA**

As regarding liver assessment, there was no statistically significant difference (P = 0.99) regarding the median levels of AST among HCC, 27.5 (10-76), cirrhotic liver, 27 (9-200), and healthy control group, 31 (14-117). Similar to that, there was no statistically significant difference between the three groups regarding the median levels of ALT (P = 0.96). Besides that, despite being relatively high among HCC group (34.05±11.79), there was no statistically significant difference (P = 0.094) between such group and cirrhotic liver (32.72±6.7), and normal healthy control group (29.303±6.1). On the contrary, there was a statistically significant difference between the three groups regarding the mean levels of total bilirubin (P = 0.037). However, this significant difference was only revealed between cirrhotic liver patients (0.77±0.16) and healthy control group (0.65±0.19) (P = 0.028). Of note, the median levels of Glypican-3 were considerably high (P < 0.001) among patients with HCC, 670 (220-900), relative to patients with liver cirrhosis, 158 (10-233), and healthy control group, 5.5 (0-52) **(Table 3)**.

Table (3): Liver functions and Glypican-3 profile among the included participants

Groups and Variables	Mean ±SD (Median-range)	P-Value	Post Hoc Test
AST: **Group I** **Group II** **Group III**	 **27.5 (10-76)** **27 (9-200)** **31 (14-117)**	**0.99**	**NA**
ALT: **Group I** **Group II** **Group III**	 **31(13-77)** **30 (12-181)** **31 (8-115)**	**0.96**	**NA**
Serum albumin: **Group I** **Group II** **Group III**	 **4.18±0.345** **4.16±0.42** **4.17±0.3**	**0.970**	**NA**
GGT: **Group I** **Group II** **Group III**	 **34.05±11.79** **32.72±6.7** **29.303±6.1**	**0.094**	**NA**
Serum Bilirubin: **Group I** **Group II** **Group III**	 **0.721±0.18** **0.77±0.16** **0.65±0.19**	**0.037**	**Group I Vs Group II (P = 0.465)** **Group I Vs Group III (P = 0.33)** **Group II Vs Group III (P = 0.028)**
Glypican 3: **Group I** **Group II** **Group III**	 **670 (220-900)** **158 (10-233)** **5.5 (0-52)**	**<0.001**	**Group I Vs Group II (P < 0.001)** **Group I Vs Group III (P < 0.001)** **Group II Vs Group III (P < 0.001)**

There were 14 (46.6%) and 8 (26.6%) patients with positive HBsAg among HCC and liver cirrhosis patients, respectively (P = 0.09). There was an equal proportion of patients with positive HCV-Ab, 17 (56.6%), among HCC and liver cirrhosis groups (P = 1.0) **(Table 4)**.

Table (4): HBsAg and HCV-Ab patterns among patients of the studied groups (Group I and Group II)

Groups / Variables	Group I		Group II		P-Value
	No.	%	No.	%	
HBsAg	**14**	**46.6%**	**8**	**26.6%**	**0.108**
HCV-Ab	**17**	**56.6%**	**17**	**56.6%**	**1**

Based on abdominal ultrasonography, 7(233%) patients within HCC had a hepatic mass, whereas 12(40%), and 11(36.6%) patients had multiple and single modules, respectively, among patients with HCC. There were 14(46.6%) patients within liver cirrhotic group had hepatic nodularity whereby the remaining 16(53.3%) patients had normal liver architecture (P <0.001). These findings were also confirmed based on CT-Abdomen **(Table 5)**.

Table (5): Abdominal ultrasonography evaluation of the included patients

Groups / Variables	Group I		Group II		Group III		P-Value
	N	(%)	N	(%)	N	(%)	
Normal	**0**	**(0.0%)**	**16**	**(53.3%)**	**30**	**(100%)**	**<0.001**
Mass	**7**	**(23.3%)**	**0**	**(0.0%)**	**0**	**(0.0%)**	
Multi-nodules	**12**	**(40%)**	**0**	**(0.0%)**	**0**	**(0.0%)**	
Single nodule	**11**	**(36.6%)**	**0**	**(0.0%)**	**0**	**(0.0%)**	
Nodularity	**0**	**(0.0%)**	**14**	**(46.6%)**	**0**	**(0.0%)**	

There was a statistically significant positive correlation between the findings of abdominal US (r = 0.62, P <0.001), and Glypican-3 levels. Apart from this, there was a statistically significant positive correlation between patients with hepatic multi-nodular lesions and Glypican-3 levels (r = 0.446, P <0.001). On the contrary, there was no statistically significant correlation between patients' age (r = -0.040, P = 0.7), AST levels (r = 0.003, P = 0.981), ALT levels (r = 0.049, P = 0.64), serum albumin (r = -0.090, P = 0.401), GGT (r = 0.160, P = 0.13), total bilirubin (r = 0.137, P = 0.19), and Glypican-3 levels **(Table 6)**.

Table (6): Correlation between patients' characteristics and Glypican-3 levels

Variables	Correlation	P-Value
Age	**Correlation Coefficient**	**-0.040**
	Sig. (2-tailed)	**0.707**
AST	**Correlation Coefficient**	**0.003**
	Sig. (2-tailed)	**0.981**
ALT	**Correlation Coefficient**	**0.049**
	Sig. (2-tailed)	**0.648**
Serum albumen	**Correlation Coefficient**	**-0.090**
	Sig. (2-tailed)	**0.401**
GGT	**Correlation Coefficient**	**0.160**
	Sig. (2-tailed)	**0.133**
Bilirubin	**Correlation Coefficient**	**0.137**
	Sig. (2-tailed)	**0.198**
US/Abdomen	**Correlation Coefficient**	**0.628****
	Sig. (2-tailed)	**<0.001**

Serum Glypican3 Level for the Diagnosis of HCC:

According to the ROC curve and the AUC analysis, Glypican-3 showed a high diagnostic capability in the detection of HCC with AUCs of 0.999 (95 % CI 0.958 to 1, P <0.0001), subsequently, at the optimal cut off value of 201, Glypican-3 achieved sensitivity and specificity of 85%, and 82.3%, respectively **(Table 7)**.

Table (7): Diagnostic ability of Glypican-3 in the prediction of Hepatocellular carcinoma

Variable	Value
Cut-off value	**201**
Sensitivity	**85%**
Specificity	**82.3%**
Likelihood ratio (+ve)	**30**
Likelihood ratio (-ve)	**0**
PPV	**76.9%**
NPV	**100%**
AUC	**0.999**
95% CI	**0.958-1.000**
P value	**<0.0001**

DISCUSSION

Our results revealed that there was no difference between the studied groups regarding age, sex. *Li et al. (2019)* in their study, revealed that there was no significance difference between healthy control, hepatic cirrhosis and HCC regarding age and sex which run in lines with our study. *Tahon et al. (2018)* found that the age in HCC group was higher than in cirrhotic patients or healthy controls and also, there was a male predominance in their study which conflicting with what we found in our study. *Mahmoud and Mahgoub (2020)* found that there was no difference between groups regarding age which was in agreement with our results BUT with a male predominance which disagree with our results.

In our study, there were no difference between the studied groups regarding hemoglobin concentration and INR while there was a significant increase in the PT in HCC group than other groups. *Badr et al. (2014)* found that GPC-3 was significantly elevated in HCC patients than cirrhotic patients and normal controls which contradicting with our results. *Tahon et al. (2018)* found that there was no difference between HCC and cirrhosis regarding hemoglobin concentration which run in lines with our results but INR was increased in HCC than hepatic cirrhosis or healthy controls disagree with our study. *Li et al. (2019)* revealed that there was no significance difference between the studied groups regarding haemoglobin concentration which run in lines with our study. *Mahmoud and Mahgoub (2020)* found that there was a significant elevation in INR in HCC and liver cirrhosis than in normal controls without significant difference between HCC and liver cirrhosis which disagree with our study. Liver enzymes in our study showed no difference between the three groups. *Mahmoud and Mahgoub (2020)* found that liver enzymes significantly elevated in cases of HCC and

hepatic cirrhosis than in normal controls which conflicting with our results. *Tahon et al. (2018)* found that there was significant increase in the liver enzymes in HCC group than in cirrhotic patients or healthy controls which disagree with our study.

Our results revealed significant increase of glypican-3 in HCC and liver cirrhosis over normal controls with significant increase in HCC. *Jia et al. (2016)* found there was no significant difference in the serum level of GPC3 between HCC and LC patients, indicating that GPC3 is not efficiently valid as a HCC serum biomarker which disagrees with our study. *Tahon et al. (2018)* found that there was a linear correlation between glypican-3 and the occurrence of HCC and hepatic cirrhosis which was in agreement with our results. *Xu et al. (2019)* concluded that glypican-3 alone can be used aloe for diagnosis of HCC from liver cirrhisis which contradecting with our results. *Mahmoud and Mahgoub (2020)* found that there was a significant elevation in the serum GPC-3 in HCC and liver cirrhosis than in normal controls in addition to significant elevation in HCC than hepatic cirrhosis which run in line with what we found in our study.

In our study, there was no difference between groups of the study regarding HCV and HBV infections. *Mahmoud and Mahgoub (2020)* found that there was no difference between HCC and hepatic cirrhotic patients regarding HCV or HBV infections which was in agreement with our results.

The use of U/S revealed that the presence of nodularity whether single of multiple were significant in HCC than other groups and there was a positive correlation between the findings of abdominal U/S and Glypican-3 levels. *Kansagara et al. (2014)* discuss a meta-analysis of 19 studies evaluating the accuracy of USG for HCC surveillance, the pooled data showed that USG had a sensitivity of 94% for identifying HCC at all stages and 63% for detecting HCC at an early stage but the meta-analysis emphasized that the results cannot differential between HCC and cirrhosis which run in lines with our results. *Ghweil et al. (2018)* concluded that the use of U/S was very useful in diagnosis of nodularity in cases of malignant liver disease which run in line with what we found in our study.

In our study the presence of gypican-3 has a high percentage of specificity and sensitivity with a very high NPV in absence of gypican-3.

Ghweil et al. (2018) when analyses the ROC curve for GPC3 in HCC found it an excellent HCC predictor with AUROC of 0.928, and the relevant cut off value of GPC3 for HCC detection was 3.15 ng/ml with 82% sensitivity and 95% specificity which run in lines with our results.

Tahon et al. (2018) found that ROC curve was used for the best cutoff point of GPC3 and at a cutoff value of 1.5 ng/ml has higher sensitivity (82.5%) which was in agreement with our study.

Li et al. (2019) revealed that for cases of HCC the AUC of GPC3 was 0.909 and the optimum cutoff of GPC3 was 1.75 ng/ml with a sensitivity of 78.72%, and a specificity of 87.86% which run in lines with our study.

Mahmoud and Mahgoub (2020) found in their study when using ROC for differentiation between the studied groups regarding glypican-3 that AUC for GPC3 was 0.993 at cut off 2.72 ng/ml which run in lines with our results.

CONCLUSION

GPC-3 level is important in diagnosis and its differentiation from benign conditions.

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دراسة مستوى الجليبيكان 3 في مرضى سرطان الكبد

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خلفيـــة البحـــث: علـــى الـــرغم مـــن أن العـــلاج الجراحـــى هـــو العـــلاج المفضـــل لســـرطان الخلايـــا الكبديـــة إلا أن 5-10% فقـــط مـــن الحـــالات تعـــد مـــن الفئـــات الصـــالحة للجراحـــة. وتوجـــد العديـــد مـــن البروتينـــات والمســـتقبلات علـــى جـــدار خلايـــا الكبـــد الســـرطانية مشـــتملة الجليبيكـــان-3 ممـــا وجـــه الإنتبـــاه إلـــى علاقـــة الوجـــود المتزايـــد لهـــذه المســـتقبلات وحدوث سرطان الخلايا الكبدية ومايُنتج عنها.

الهـــدف مـــن البحـــث: تقيـــيم الـــدور التشخيصـــى لمســـتوى الجليبيكـــان-3 فـــى تشـــخيص سرطانات الخلايا الكبدية.

المرضـــي وطـــرق البحـــث: أجريـــت هـــذه الدراســـة علـــى 90 مـــن حـــالات العيـــادات الخارجيـــة لقســـم الأمـــراض الباطنـــة بمستشـــفى ســـيد جـــلال ومستشـــفى أحمـــد مـــاهر التعليمـــى. وقـــد تـــم تقســـيم المرضـــى إلـــي 3 مجموعـــات متســـاوية: المجموعـــة الأولـــى حـــالات ســـرطانات الكبـــد الناتجـــة عـــن تليـــف الكبـــد, والمجموعـــة الثانيـــة ضـــمت حـــالات ســـرطانات الكبـــد مـــن دون تليـــف الكبـــد, بينمـــا كانـــت حـــالات المجموعـــة الثالثـــة مجموعـــة ضابطة مقارنة.

نتـــائج البحـــث: لايوجـــد فـــروق ذات دلالـــة إحصـــائية بـــين المجموعـــات الـــثلاث فيمـــا يخـــتص بـــالعمر، والجـــنس، ونســـبة هيموجلـــوبين الـــدم، المعامـــل الـــدولى للمعـــايرة، ومســـتوى إنزيمـــات الكبـــد، والمؤشـــرات الحيويـــة للفيروســـات فـــى مرضـــى تليـــف الكبـــد وحـــالات ســـرطانات الخلايـــا الكبديـــة. وقـــد وجـــد أن الجليبيكـــان-3 بصـــورة إحصـــائية فارقـــة فـــى حـــالات ســـرطان الخلايـــا الكبديـــة عنـــه فـــى بـــاقى المجموعـــات علـــى الـــرغم مـــن وجـــوده بصـــورة ليســـت فارقـــة إحصـــائياً عـــن وجـــوده فـــى حـــالات ســـرطان الخلايـــا الكبديـــة, كمـــا أن الحـــالات التـــى وجـــد بهـــا زيـــادة فـــى الجليبيكـــان-3 وجـــد بهـــا زيـــادة فارقـــة

إحصـــائياً لـــزمن البروثـــرومبين والبليـــروبين. إضـــافة إلـــى ماســـبق, فقـــد أثبتـــت الدراســـة أن وجـــود كتلـــة ورميـــة أو عقـــدة ورميـــة كبيـــرة وجـــدت بصـــورة إحصـــائية فـــى حـــالات ســـرطان الخلايـــا الكبديـــة، بينمـــا العقـــد الورميـــة الصـــغير يوجـــد بصـــورة إحصـــائية فـــى حـــالات تليـــف الكبـــد علـــى الـــرغم مـــن أن هـــذا لاينفـــى وجـــود ســـرطان الخلايـــا الكبديـــة فـــى مثل هذه الحالات.

الاســـتنتاج: مســـتوى الجليبيكـــان-3 فـــى دم المرضـــى يعـــد مؤشـــراً مهمـــا فـــى تشـــخيص حالات سرطان الكبد وكذا تفريقها من العقد الخلوية الورمية الحميدة بالكبد.

الكلمات الدالة: مستوى الجليبيكان 3 , سرطانات الخلايا الكبدية.