RESEARCH ARTICLE

# Roles of Combined Glypican-3 and Glutamine Synthetase in Differential Diagnosis of Hepatocellular Lesions

**Rania Elsayed Wasfy*, Aliaa Atef Shams Eldeen**

## Abstract

**Background: Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer and thirdly leading cause of cancer-related death worldwide. The estimated risk of hepatocellular carcinoma is 15 to 20 times as high among persons infected with HCV as it is among those who are not infected, with most of the excess risk limited to those with advanced hepatic fibrosis or cirrhosis. Glypican3 (GPC3) plays a key role in relation to signaling with growth factors, regulating the proliferative activity of cancer cells. Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia in the mammalian liver. GS was suggested as a specific marker for tracing cell lineage relationships during hepatocarcinogenesis. In normal liver, GS expression is seen in pericentral hepatocytes, but not by midzonal or periportal hepatocytes. In HCC, strong and diffuse GS expression in seen in tumor cells. Results: Glypican3 immunopositvity was highly specific and sensitive indicator for hepatocellular carcinoma as well as glutamine synthetase which was found to be a sensitive and specific indicator for development of hepatocellular carcinoma when compared to cirrhosis, liver cell dyspalsia and metastatic carcinomas. Statistical analysis revealed a significant association between GPC3 and GS with tumor size (P=0.003, p=0.006, respectively). Diffuse staining significantly associated with large tumor size while, focal and mixed staining was detected more with small tumor size. Studying the relation with tumor grade also revealed significant association between diffuse GPC3 and GS staining with high tumor grade. Diffuse staining was detected in 91.7% and 100% respectively of poorly differentiated specimens and only in 33.3% and 22.2% of well differentiated specimens. Conclusions: While using GPC3 and GS to screen for premalignant hepatic lesions remains controversial, our data suggest that GPC3 and GS may be a reliable diagnostic immunomarkers to distinguish HCC from benign hepatocellular lesions. However, negative immunostaining should not exclude the diagnosis of HCC.**

**Keywords:** Glypican3 - glutamine synthetase - hepatocellular carcinoma

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## Introduction

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer, thirdly leading cancer-related death worldwide (Ismaeil et al., 2015). It has a long latency, and most patients are often diagnosed at late stages when tumors are of high grade and progress rapidly (Weng et al., 2014). Most HCCs develop in patients with a history of chronic hepatitis or cirrhosis in which there is continuous inflammation and regeneration of hepatocytes (Abouzied et al., 2015).

The estimated risk of hepatocellular carcinoma is 15 to 20 times as high among persons infected with HCV as it is among those who are not infected, with most of the excess risk limited to those with advanced hepatic fibrosis or cirrhosis. The incidence of hepatocellular carcinoma in the United States has tripled while the 5-year survival rate has remained below 12% (Donato et al., 2002).

Unfortunately, the prognosis remains unsatisfactory mainly as a result of frequent tumor recurrence and metastasis after curative resection. Consequently, it is important to identify the factors that predispose patients to tumor recurrence and death. Discovering sensitive and special prognostic factors may present opportunities for reducing the severity of this disease through early and new therapeutic interventions (Rangaswami et al., 2006).

Glypican-3 (GPC3) is one of six members of the glypican family which binds to cell membranes via a glycosylphosphatidylinositol (GPI) anchor that is present on cell membranes. Glypican3 is expressed in the fetal livers but not in adult livers (Abdelgawad et al., 2013) The functions of GPC3 are thought to include important roles in cell growth, differentiation and motility. In other words, GPC3 plays a key role in relation to signaling with growth factors, thereby regulating the proliferative activity of cancer cells (Hsu et al., 1997; Suzuki et al., 2010) have investigated genes that show increased expression in HCC and reported that the GPC3 mRNA levels were significantly higher in HCC than in normal liver tissues and non-tumorous liver tissues.

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GPC3 is involved in the regulation of a number of cell physiological and pathological processes by interaction with various ligands and receptors, including cell adhesion molecules, matrix components, growth factors, enzymes and enzyme inhibitors. GPC3 may also be involved in inhibition and regulation of the growth of the majority of mesodermal tissues and organs, however, the underlying molecular mechanisms remain unknown (Pan et al., 2013).

Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia in the mammalian liver where it has been shown to be restricted to hepatocytes surrounding the terminal hepatic venules in the murine and human liver. It is known that glutamine, the end product of GS activity, is the major energy source of tumor cells. Based on findings on experimental hepatocarcinogenesis, GS positive tumor cells are believed to be derived from GS positive hepatocytes that have been affected by carcinogens. Thus, GS was suggested as a specific marker for tracing cell lineage relationships during hepatocarcinogenesis (Christa et al., 1994; Di Tommaso et al., 2007), demonstrated the up-regulation of GS mRNA, protein, and activity in human HCC.

In normal liver, GS expression is seen in pericentral hepatocytes, but not by midzonal or periportal hepatocytes. In most hepatic adenomas, GS is negative, localized to the pericentral areas or shows patchy expression with no distinct pattern. In adenoma-like neoplasms and in HCC, strong and diffuse GS expression in seen in tumor cells (Evason et al., 2013). This work aimed to study the diagnostic role of both glypican3 and glutamine synthetase in malignant and non-malignant liver tissue. Also, to compare their expression with other clinico-pathological parameters such as age, gender, tumor size and tumor grade.

## Materials and Methods

We studied 36 specimens from patients with large regenerative cirrhotic nodules (LRNs), 18 cases of liver cell dysplasia, 32 cases of hepatocellular carcinoma and 5 cases diagnosed as metastatic carcinomas to the liver by two pathologists. Samples were collected as formalin-fixed, paraffin-embedded tissue blocks, with H&E stained slides from the archive of the pathology department of faculty of medicine, Tanta university and private labs (77) or received as fresh specimens taken by US or CT guided percutaneous liver biopsy (14). Immunohistochemistry was performed using the immunoperxoidase method on 4-m-thick sections from formalin-fixed, paraffin-embedded blocks. Pretreated sections were incubated with mouse monoclonal Glypican-3 (GPC-3) antibody (Clone 1G12, Thermo Scientific, Egypt). Tissue was scored based on the total percentage of positive cells (≤5%) = negative. Positive stains were further stratified as focal (5%-50% of cells stained) or diffuse (>50% of the cells stained) [Anatelli et al., 2008]. Rabbit polyclonal antibody to Glutamine synthetase (GS) (1:200, Thermo Scientific, Egypt) was also applied to the sections. The degree of immunostaining was scored according to the proportion of positively stained tumor cells. Positive samples showed strong cytoplasmic staining either in all (diffuse pattern) or at least 50% of the cells (mixed pattern) (Bello et al., 2010). For both antibodies, the antigen retrieval (PBS buffer; pH 7.4) was done for all sections and were incubated with the primary antibody for 2 h at room temperature. The sections were incubated with secondary antibody (HRP-Rabbit/Mouse) for 15 min at room temperature. As a negative control, a section was processed in which the primary antibody was changed by PBS. Immunohistochemical staining was evaluated independently by two pathologists.

Statistical analysis was performed by using the Kruskal Wallis test, 2-tailed Fisher exact test or the $\chi^2$ test with Yates continuity correction. A P value of less than 0.05 was considered statistically significant.

## Results

This study included 91 cases of liver specimens, which were divided into (32 cases of hepatocellular carcinoma, 36 cases of cirrhosis, 18 cases of liver cell dysplasia and 5 cases were metastatic carcinomas of various origins to the liver).

The age of patients with hepatocellular carcinoma ranges from 45 to 77 with a mean of 62.75±8.08, for cirrhosis the age ranges from 40 to 67 with a mean of 57.72±8.64 while the mean age for dyspalsia 61.33±9.91 was and for metastatic cases was 64.00±8.51 (Table 3).

Cases of hepatocellular carcinoma included various sizes (T1, T2 and T3) and various grades (well, moderate and poorly differentiated). Most of the cases (15 cases) obtained large size in the category of T3, twelve cases were in T2 category and only 5 cases were of T1 size.

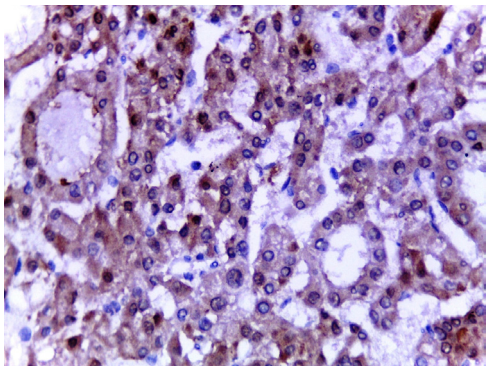

**Figure 1. Well Differentiated HCC with Diffuse Cytoplasmic Staining of GPC3 (X400)**

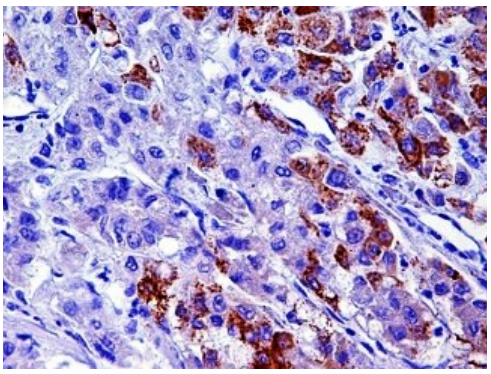

**Figure 2. Moderately Differentiated HCC with Focal Cytoplasmic Staining of GPC3 (X400)**

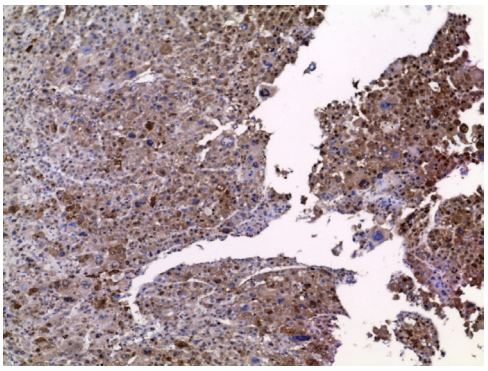

**Figure 3. Poorly Differentiated HCC with Diffuse Cytoplasmic Staining of GPC3 (X100)**

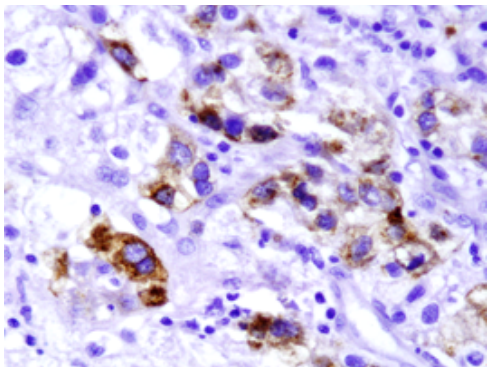

**Figure 4. Liver cell dysplasia with Focal Cytoplasmic Staining for GPC3 (X400)**

**Table 1. The Relation between GPC3 Expression with tumor Size and Grade**

| | | GPC3 | | | FE test |
|---|---|---|---|---|---|
| | | Focal positive | Diffuse positive | -ve | (P value) |
| HCC | | | | | |
| Size | T1 n=5 | 2 (40.0) | 1 (20.0) | 2 (40.0) | 15.7 |
| | T2 n=12 | 5 (41.7) | 7 (58.3) | 0 (0.0) | -0.003 |
| | T3 n=15 | 2 (13.3) | 13 (86.7) | 0 (0.0) | |
| | Well n=9 | 5 (55.6) | 3 (33.3) | 1 (1.1) | 10.98 |
| Grade | Moderate n=11 | 3 (27.3) | 7 (63.6) | 1 (9.1) | -0.02 |
| | Poor n=12 | 1 (8.3) | 11 (91.7) | 0 (0.0) | |

**Table 2. The Relation between GS Expression with Tumor Size and Grade**

| | | GS | | | FE |
|---|---|---|---|---|---|
| | | Mixed | Diffuse | Negative | (p value) |
| HCC | | | | | |
| size | T1 n=5 | 2 (40.0) | 0 (0.0) | 3 (60.0) | 12.11 |
| | T2 n=12 | 2 (16.7) | 8 (66.7) | 2 (16.7) | -0.006 |
| | T3 n=15 | 4 (26.7) | 11 (73.3) | 0 (0.0) | |
| | Well n=9 | 3 (33.3) | 2 (22.2) | 4 (44.4) | 16.46 |
| grade | Moderate n=11 | 5 (45.5) | 5 (45.5) | 1 (9.1) | (<0.001) |
| | Poor n=12 | 0 (0.0) | 12 (100.0) | 0 (0.0) | |

Regarding tumor grade, 9 cases were well differentiated, 11 cases were moderately differentiated and 12 cases were poorly differentiated.

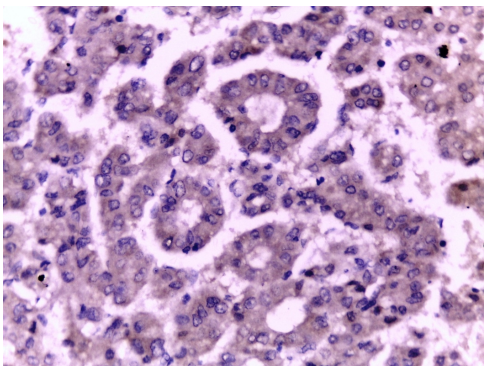

**Figure 5. Well Differentiated HCC with Diffuse Cytoplasmic Staining for Glutamine Synthetase (X200)**

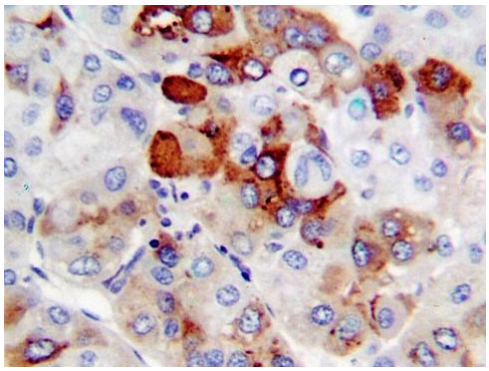

**Figure 6. Moderately Differentiated HCC with Mixed Cytoplasmic Staining for Glutamine Synthetase (X400)**

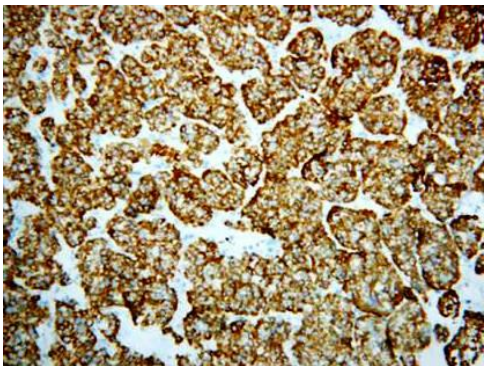

**Figure 7. Moderately Differentiated HCC with Diffuse Cytoplasmic Staining for Glutamine Synthetase (X200)**

*Glypican3 results*

Glypican3 was detected as a cytoplasmic staining of the tumor cells in 30 (93.8%) out of 32 specimens of hepatocellular carcinoma. In only 2 specimens of cirrhosis (5%), 5 specimen of liver cell dysplasia (28%) (Figures 1-4) and only one specimen of metastatic carcinoma (20%) and the remaining 4 cases were negative (Figure 9).

Statistical analysis revealed a significant association between GPC3 and tumor size (P=0.003). Diffuse staining was significantly associated with large tumor size while, focal staining was detected more with small tumor size (Table 1).

Studying the relation with tumor grade also revealed significant association between diffuse GPC3 staining and high tumor grade. Diffuse staining was detected in 91.7% of poorly differentiated specimens and only in 33.3% of well differentiated specimens (Table 1).

**Table 3. GPC3 and GS Expression in HCC, Dyspalsia, Cirrhosis and Other Metastasis**

| | | HCC (n=32) | | Dysplasia (n=18) | | Cirrhosis (n=36) | | Metastasis (n=5) | | Fisher's Exact test | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | | |
| Age (mean ± SD) | | 62.75 ± 8.08 | | 61.33 ± 9.91 | | 57.72 ± 8.64 | | 64.00 ± 8.51 | | 6.20* | 0.1 |
| Gender: | Male: | 21 | 65.60% | 12 | 66.70% | 26 | 72.20% | 3 | 60 | 0.54 | 0.9 |
| | Female: | 11 | 34.40% | 6 | 33.30% | 10 | 27.80% | 2 | 40 | | |
| GPC3: | +ve: | 30 | 93.80% | 5 | 27.80% | 2 | 5.60% | 1 | 20 | 57.39 | <0.001 |
| | -ve: | 2 | 6.20% | 13 | 72.20% | 34 | 94.40% | 4 | 80 | | |
| GS: | +ve: | 27 | 84.40% | 6 | 33.30% | 6 | 16.70% | 0 | 0.00% | 32.66 | <0.001 |
| | -ve: | 5 | 15.60% | 12 | 66.70% | 30 | 83.30% | 5 | 100% | | |

*Kruskal Wallis test

**Table 4. Sensitivity and Specificity of GPC3 and GS**

| | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| GPC3 | 94% | 86% | 79% | 96% | 89% |
| GS | 84% | 80% | 69% | 90% | 81% |

*Glutamine synthetase results*

Glutamine synthetase was detected by cytoplasmic staining of the tumor cells. Twenty seven specimens (84.4%) out of 32 cases of hepatocellular carcinoma were positive to GS, while only five specimens were negative. Six specimens of dysplasia and also six specimens of cirrhosis were positive to GS. All of metastatic carcinoma specimens were negative to GS (Figures 5-7) (Table 3).

Studying the relation between Glutamine synthetase immunoexpression and tumor grade, a highly significant association was found between diffuse expression and increasing tumor grade. Similarly, comparing Glutamine synthetase expression with different tumor sizes revealed a highly significant association between the increase in tumor size and the diffuse staining of tumor cells (Table 2).

Concerning the age and gender, no statistically significant relation was found between glypican3 and GS expression on one hand and patient's age or gender on the other hand, in all studied cases.

Regarding statistical relations, Glypican3 immunopositvity was highly specific and sensitive indicator for hepatocellular carcinoma as well as Glutamine synthetase which was found to be a sensitive and specific indicator for development of hepatocellular carcinoma when compared to cirrhosis, liver cell dyspalsia and metastatic carcinoma to the liver (Table 4).

## Discussion

Distinguishing hepatocellular carcinoma especially the well differentiated case from normal, cirrhotic liver tissue, liver cell dysplasia and liver metastatic carcinomas may be very difficult in some cases, particularly in small needle core biopsies. In the present study, we analyzed the expression of GPC3 in a human liver lesions including cirrhotic large regenerative nodules (LRNs), liver cell dysplasia, HCC and metastatic carcinomas. GPC3 revealed a high frequency of expression (93.8%) in HCC compared to the low frequency found in the rest of the studied liver lesions. Statistical analysis also revealed significant positive association between diffuse GPC3 staining with high tumor grade and large tumor size. While, there was no significant relation to the age or gender. The specificity and sensitivity of GPC3 were 86% and 94% respectively. This high rate and specificity of expression of GPC3 in HCC was in agreement with several earlier studies that have documented the GPC3 expression in various liver lesions. GPC3 appears not to inhibit HCC, but to promote it. In HCC, GPC3 acts as an oncofetal protein promoting cell growth, differentiation and tumor formation not as a tumor suppressor gene as it is in other organs. This may explain the increase of GPC3 expression with the higher histologic HCC grade.

This was supported by Yan et al. (2011) who reached similar results when they noticed a high incidence of GPC3 expression (492/757; 65%) in HCC, whereas intrahepatic cholangiocarcinomas, adenocarcinomas, and benign liver lesions displayed rare positive cases. There were significant correlations between GPC3 expression and clinicopathologic characteristics, especially histologic grade.

Wang et al. (2012), concentrated on the relation between GPC3 staining and the tumor size. They found that the sensitivity and specificity of GPC3 single staining for all HCC nodules were 80.3% and 98%, respectively, while for nodules 3 cm or smaller, the values were 83% and 98%, suggesting that GPC3 staining helps achieve an accurate diagnosis. They also found that GPC3 expression in benign nodules 3 cm or smaller, was very low. The positive rate in high grade dysplasia was only 3.2%. These results revealed the value of GPC3 in differentiating between malignant nodules and high grade dysplasia. Moreover, in addition to the previous findings, they also observed GPC3 staining to be more accurate in HCC nodules 3 cm or smaller than its use in all HCC nodules, further revealing its diagnostic value in small HCC lesions.

In approval to the previous results, Di Tommaso et al., 2007, investigated the expression of GPC3 in 52 surgically removed non-malignant liver nodules (LRNs, liver cell dysplasia) and 53 HCCs (10 early, 22 grade 1, and 21 grade 2-3) and found that the sensitivity and specificity of GPC3 was 69% and 91% respectively.

In addition, Coston et al., 2008, as well, studied the expression of Glypican-3 (GPC3) in 107 cases of HCC, 19 cases of hepatic adenomas (HA), 16 cases of focal nodular hyperplasia (FNH) and 225 cases of nonhepatic human tumors with epithelial differentiation. Ninety-four of 107 cases (88%) of HCC showed focal or diffuse cytoplasmic GPC3 staining, whereas all HA and FNH cases were GPC3-negative, and only 7 of 225 cases (3%) of nonhepatic tumors with epithelial differentiation expressed GPC3. The sensitivity and specificity of GPC3 for HCC was 88% and 97%, respectively.

These results resembled those found by Yip et al. (2011), who stated that GPC3 protein was positive in 72% HCC (257/357), but negative in the rest 374 of non-HCC cases, including cholangiocarcinoma, HCC adjacent hepatic tissue including cirrhosis, hemangioma adjacent hepatic tissues and metastatic carcinomas. GPC3 positive percentage was significantly correlated with histological grading of HCC ($P<0.01$), highest in grade 3 (77.1%, 64/83) followed by grade 2 (73.3%, 187/255), grade 1 (6/12) and grade 4 (0).

According to Libberecht et al., 2006, GPC3 expression was much higher in HCCs than in cirrhosis and other types of focal lesions, indicating that the transition from premalignant lesions to HCC is associated with a sharp increase of GPC3 expression in a majority of cases. The sensitivity and specificity of a positive GPC3-staining for the diagnosis of HCC was 0.77 and 0.96, respectively, in resected cases, and 0.83 and 1, respectively, for needle biopsies. Moreover, Shafizadeh et al. (2008), performed immunohistochemistry for glypican-3 on 80 resection cases of hepatocellular lesions to examine the utility of glypican-3 immunohistochemistry in hepatocellular carcinoma at two ends of the differentiation spectrum. Glypican-3 was expressed in 46 (79%) hepatocellular carcinomas (56, 83 and 89% of well, moderately and poorly differentiated respectively). All hepatic adenomas and macroregenerative nodules were negative, and three (43%) high grade dysplastic nodules were positive. Focal staining was seen in regenerative nodules in four (11%) cirrhosis cases. They also found Glypican-3 to be significantly more sensitive than Hep Par 1 for diagnosis of poorly differentiated hepatocellular carcinomas (89 *vs* 63%).

Interestingly, Anatelli et al. (2008), examined 120 liver needle biopsy specimens, including 46 from cirrhotic livers and 74 hepatocellular carcinomas (HCCs), for expression of GPC3. The results showed strong cytoplasmic and membranous staining in 36 HCCs (49%), among which 20 cases (56%) showed diffuse immunoreactivity. None of the 46 cirrhotic livers exhibited positive GPC3 immunostaining. The non-neoplastic liver tissues (cirrhotic or non-cirrhotic) that were present in the majority of the HCC cases were also completely negative for GPC3 expression. These data demonstrate that GPC3 is a reliable immunohistochemical marker for the diagnosis of HCC on needle biopsy specimens when positive.

In 2003, Capurro et al. (2003) assessed GPC3 in liver tissue sections by immunohistochemistry and in serum by enzyme-linked immunosorbent assay. They showed that GPC3 is expressed in 72% of HCCs (21 of 29), whereas it is not detectable in hepatocytes from normal liver and benign liver diseases. Consistent with this, GPC3 was undetectable in the serum of healthy donors and patients with hepatitis, but its levels were significantly increased in 18 of 34 patients (53%)with HCC. In addition, only 1 of 20 patients with hepatitis plus liver cirrhosis displayed elevated levels of serum GPC3.

Yamauchi et al. (2005) also observed diffusely positive staining of GPC3 in malignant hepatocytes in hepatocellular carcinomas (84%) but GPC3 expression was independent of the differentiation and size of the hepatocellular carcinoma. On the other hand, there was only weak and focal staining in low-grade (2/8) and high-grade dysplastic nodules (6/8). GPC3 immunoreactivity was detected in only one of 23 metastatic lesions of colorectal carcinoma. Besides, Zou et al., 2010, suggested that GPC3 is not only a diagnostic and prognostic marker in hepatocellular carcinoma, but also is expected to be an ideal target for the therapy of hepatocellular carcinoma. This was also stated by Kandil and Cooper (2009) as they suggested that GPC3 is a reliable marker for hepatocellular carcinoma. The sensitivity and specificity exceeds both alpha-fetoprotein and hepatocyte-paraffin1.

Recently, Zaakook et al. (2013), performed GPC3 immunostaining on HCC and metastatic carcinomas samples taken by fine needle aspiration cytology and cell blocks. 95.2% of HCC cases expressed GPC3. Poorly differentiated cases showed the highest GPC3 sensitivity (100%), followed by moderately differentiated cases (96.5%), and while well differentiated cases expressed GPC3 in 90% of cases. 83.3% of metastatic carcinomas were negative for GPC3. In this study, sensitivity of GPC-3 in HCC was 95.2%, specificity was 83.3%, positive and negative predictive values were 93% and 88.2% respectively, and total accuracy was 91.7%.

Regarding our results of glutamine synthetase immunostaining, 84% of studied HCC cases showed positive expression for GS, meanwhile, only 28% of liver cell dysplasia and 13.9% of LRNs were positive for GS immunostaining. All cases of metastatic carcinomas were negative for GS. We noticed a highly significant association between diffuse GPC3 expression and increasing tumor grade and tumor size as well. The age and gender had no significant statistical relation with GS expression. The specificity and sensitivity of GS were 80% and 84% respectively.

The reason for GS overexpression in HCC cases is not clear, but it is likely that these tumors have abnormalities in the Wnt signaling pathway which plays an important role in cell adhesion and cell proliferation. Beta-catenin, a key component of this pathway is predominantly bound to cell membranes in normal cells. Mutations in beta-catenin or abnormalities in other components of this pathway can lead to nuclear translocation of beta-catenin and activation of several transcription factors leading to increased expression of several genes that play a key role in cell proliferation including Glutamine synthetase. Consequently, with increased rate of cell proliferatin in the higher HCC grades, as well as, the larger tumors, the more diffuse and strong the immunoexpression of GS will be.

In consistency with our results, when Long et al. (2011), assessed GS tissue expression and serum level in HCC cases, they showed that GS was expressed in 70% of HCC patient. The v2 tests showed significant difference between HCC samples and non-tumor tissues. The serum GS levels were increased in cases of HCC.

In 2010, Bello et al. (2010) found that 91 patients (43.9%) had GS-positive HCCs by immunostaining. These tumors had larger size and characteristic histology (low grade, pseudoacini, hydropic changes, bile staining, lack of steatosis, and fibrosis). In addition, Tremosini et al. (2012), investigated the diagnostic utility of GS in

diagnosis of HCC. The sensitivity and specificity of GS to be 50% and 90% respectively.

Di Tommaso et al. (2007), stated that GS immunoreactivity was seen in a majority of HCCs (37 of 53 cases, 69.81%). In non-malignant nodules, GS overexpression was only seen in 3 liver cell dysplasia (13.64%). In these cases, GS over-expression was restricted to 11.5%-50% hepatocytes (Figure 3C), whereas in HCC the majority of cases (28 of 53, 52.83%), including HCC (60%), showed diffuse immunostaining. However, they noted that GS immunoreactivity did not correlate with tumor dedifferentiation or with other clinico-pathological features (age, sex, aetiology of cirrhosis, and tumor size). Overall, the sensitivity and specificity of GS for HCC detection were 69.81% and 94.23% respectively.

In contrast to our results, a recent study by Lagana et al. (2013), studied the usefulness of using a panel of immunostains including GPC3 and GS. They observed GS reactivity in 31 of 41 intrahepatic cholangiocarcinoma (76%), with the median amount of staining being 65% of tumor cells. GS reactivity was present in 17 of 24 tumors metastatic to liver (71%), with the median amount of staining being 50% of tumor cells. They suggested that, of the panel of immunostains currently commonly used to distinguish hepatocellular carcinoma from dysplastic hepatocytic nodules, only GPC-3 did not react frequently with metastatic tumors and intrahepatic cholangiocarcinoma, although there was staining in 2 metastatic tumors.

In conclusio, our results support the use of a diagnostic panel of both markers (GPC3 and GS) in the diagnostic workout of hepatocellular lesions uncertain for malignancy rather than individual markers. The adopted panel, because of its ability to identify HCC especially the well differentiated ones, may prove very useful not only for diagnostic purposes but also in bringing conformity to investigative aspects of this important field.

## References


Anatelli F, Chuang ST, Yang XJ, Wang HL (2008). Value of Glypican 3 immunostaining in the diagnosis of hepatocellular carcinoma on needle biopsy. *Am J Clin Pathol*, **130**, 219-23.

Abdelgawad IA, Mossallam GI, Radwan NH, Elzawahry HM, Elhifnawy NM (2013). Can Glypican 3 be diagnostic for early hepatocellular carcinoma among egyptian patients? *Asian Pac J Cancer Prev*, **14**, 7345-9.

Abouzied MM, Eltahir HM, Fawzy MA, et al (2015). Estimation of leucine aminopeptidase and 5-Nucleotidase increases alpha-fetoprotein sensitivity in human hepatocellular carcinoma cases. *Asian Pac J Cancer Prev*, **16**, 959-63.

Bello BD, Rosa L, Campanini N, et al (2010). Glutamine synthetase immunostaining correlates with pathologic features of hepatocellular carcinoma and better survival after radiofrequency thermal ablation. *Clin Cancer Res*, **16**, 2157.

Capurro M, Wanless IR, Sherman M, et al (2003). Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology*, **125**, 89-97.

Christa L, Simon MT, Flinois JP, et al (1994). Overexpression of glutamine synthetase in human primary liver cancer. *Gastroenterology*, **106**, 1312-20.

Coston WM, Loera S, Lau SK, et al (2008). Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. *Am J Surg Pathol*, **32**, 433-44.

Di Tommaso L, Franchi G, Park YN, et al (2007). Diagnostic value of HSP70, Glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology*, **45**, 725-34.

Donato F, Tagger A, Gelatti U, et al (2002). Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol*, **155**, 323-31.

Evason K, Grenert JP, Ferrell LD, Kakar S (2013). Immunohistochemical classification of hepatic adenomas and atypical hepatocellular neoplasms: correlation with clinicopathologic characteristics and chromosomal abnormalities. *Mod Pathol*, **44**, 750-8.

Hsu HC, Cheng W, Lai PL (1997). Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. *Cancer Res*, **57**, 5179-84.

Ismail S, Mayah W, Battia HE, et al (2015). Plasma nuclear factor kappa b and serum peroxiredoxin 3 in early diagnosis of hepatocellular carcinoma. *Asian Pac J Cancer Prev*, **16**, 1657-66.

Kandil DH, Cooper K (2009). Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. *Adv Anat Pathol*, **16**, 125-9.

Lagana SM, Moreira RK, Remotti HE, Bao F (2013). Glutamine synthetase, heat shock protein-70, and glypican-3 in intrahepatic cholangiocarcinoma and tumors metastatic to liver. *Appl Immunohistochem Mol Morphol*, **21**, 254-7.

Libbrecht L, Severi T, Cassiman D, et al (2006). Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol*, **30**, 1405-11.

Long J, Wang H, Lang ZW, et al (2011). Expression level of glutamine synthetase is increased in hepatocellular carcinoma and liver tissue with cirrhosis and chronic hepatitis B. *Hepatol Int*, **5**, 698-706.

Pan Z, Chen C, Long H, et al (2013). Overexpression of gpc3 inhibits hepatocellular carcinoma cell proliferation and invasion through induction of apoptosis. *Mol Medicine Reports*, **7**, 969-974.

Rangaswami H, Bulbule A, Kundu GC (2006). Osteopontin: Role in cell signaling and cancer progression. *Trends Cell Biol*, **16**, 79-87.

Shafizadeh N, Ferrell LD, Kakar S (2008). Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol*, **21**, 1011-8.

Suzuki M, Sugimoto K, Tanaka J, et al (2010). Up-regulation of glypican-3 in human Hepatocellular carcinoma. *Anti Cancer Res*, **30**, 5055-62.

Tremosini S, Forner A, Boix L, et al (2012). Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. *Gut*, **61**, 1481-7.

Wang F, Jing X, Wang T, et al (2012). Differential diagnostic value of GPC3-CD34 combined staining in small liver nodules with diameter less than 3 cm. *Am J Clin Pathol*, **137**, 937-45.

Weng LL, Xiang JF, Lin JB, et al (2014). Asparagus polysaccharide and gum with hepatic artery embolization induces tumor growth and inhibits angiogenesis in an orthotopic hepatocellular carcinoma model. *Asian Pac J Cancer Prev*, **15**, 10949-55.

Yamauchi N, Watanabe A, Hishinuma M, et al (2005). The glypican 3 oncofetal protein is a promising diagnostic

marker for hepatocellular carcinoma. *Modern Pathology*, **18**, 1591-8.

Yan B, Wei JJ, Qian YM, et al (2011). Expression and clinicopathologic significance of glypican 3 in hepatocellular carcinoma. *An Diagnostic Pathol*, **15**, 162-9.

Yip YC, Wang FH, Vong HT, Zhang M, Wen JM (2011). Significance of glypican-3 immunohistochemistry in diagnosis of hepatocellular carcinoma. *Zhonghua Bing Li Xue Za Zhi*, **40**, 626-9.

Zaakook M, Ayoub M, Abu Sinna E, El-Sheikh S (2013). Role of glypican-3 immunocytochemistry in differentiating hepatocellular carcinoma from metastatic carcinoma of the liver utilizing fine needle aspiration cytology. *J Egyptian Nat Cancer Inst*, **25**, 173-180.

Zou ZQ, Ding YP, Long B, et al (2010). Gpc-3 is a notable diagnostic, prognostic and a latent targeted therapy marker in hepatocellular carcinoma. *Hepatogastroenterology*, **57**, 102-3, 1285-90.