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The Diagnostic Utility of Both Astrocyte Elevated Gene-1 (AEG-1) and Glypican-3 Immunohistochemical Expression in Hepatocellular Carcinoma

Mennat-Allah A Rehab, Ayman M Elsaka, Hassan Tahseen Sheir and Hend S Abo Safia*

Department of Pathology, Faculty of Medicine, Tanta University, Egypt

Abstract

Background: Hepatocellular Carcinoma (HCC) is one of the leading causes of cancer related deaths worldwide. Astrocyte Elevated Gene-1 (AEG-1) has a vital role in promoting cancer development and progression by augmenting all hallmarks of aggressive cancer. Glypican-3 (GPC3) is a heparan-sulfate cell surface proteoglycan expressed normally by fetal liver and is highly expressed in HCC. This study aimed to evaluate the diagnostic value of AEG-1 and Glypican-3 in HCC and precancerous lesions. In addition, the expression of the two markers with different grades of HCC was also evaluated.

Material and Methods: Sixty cases; 36 cases of HCC and 24 cases of precancerous lesions, were subjected to routine hematoxylin and eosin staining and immunohistochemical staining using AEG-1 and GPC3.

Results: AEG-1 was expressed in 94.4% of HCC cases and 12.5% of precancerous lesions with 94.4% sensitivity, and 87.5% specificity. GPC3 was expressed in 75% of HCC cases and 8.3% of precancerous lesions with 75% sensitivity, and 91.6% specificity. GPC3 expression has a statistically significant relation with high tumor grade. In contrast to AEG-1 that showed no statistically significant relation with the tumor grade. The combination of both markers provided better sensitivity (98.2%), and absolute specificity (100%).

Conclusion: AEG-1 showed higher sensitivity and diagnostic accuracy than GPC3, while GPC3 showed more specificity. Combined AEG-1 and GPC3 immunohistochemical panel can be used efficiently for accurate diagnosis of HCC and differentiating HCC from precancerous lesions.

Keywords: HCC; Precancerous lesions; AEG-1; GPC3

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Correspondence:
Mennat-Allah A Rehab, Department of Pathology, Tanta University, Tanta Qism 2, Second Tanta, Gharbia Governorate, Egypt,

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Abbreviations

AEG1: Astrocyte Elevated Gene-1; AJCC: American Joint Committee on Cancer; AUC: Area Under Curve; DN: Dysplastic Nodule; GPC3: Glypican-3; HBV: Hepatitis B Virus; HCC: Hepatocellular Carcinoma; HCC, NOS: Hepatocellular Carcinoma, Not Otherwise Specified; HCV: Hepatitis C Virus; H&E: Hematoxylin and Eosin; HGDN: High-Grade Dysplastic Nodule; IHC: Immunohistochemistry; LGDN: Low Grade Dysplastic Nodule; NAFLD: Non-Alcoholic Fatty Liver Disease; NPV: Negative Predictive Value; PPV: Positive Predictive Value; ROC: Receiver Operator Characteristic; SD: Standard Deviation; TNM: Tumor Node Metastasis; WHO: World Health Organization

Introduction

Liver cancer ranks the sixth most common cancer and the fourth leading cause of cancer-related death worldwide [1]. Globally, hepatocellular carcinoma is the most prevalent type of liver cancer, which makes up 80% of all primary liver cancers [2]. Among men, HCC is the fifth most frequent cancer and the second leading cause of cancer-related deaths despite being the ninth most common cancer and the fourth leading cause of death among women [3]. In Egypt, HCC occupies the fourth most common cancer and the most common cause of mortality and morbidity-related cancer [4]. Moreover, Egypt ranks the 3rd and 15th most popular country in Africa and worldwide, respectively [5].

Most HCC patients have advanced stages at the time of diagnosis and have a poor prognosis. However, if recognized at early stage, surgical resection offers a favorable prognosis, with 5-year

survival rate of more than 70%. So, surveillance programs and early diagnostic tools are needed to improve HCC survival [6].

HCC is an extremely complex condition and there are numerous contributors to its pathogenesis. Most HCC cases occur in the setting of chronic liver disease with cirrhosis being the fundamental risk factor and present in 80% to 90% of HCC patients [7]. It is predicted that one-third of cirrhotic patients will develop liver cancer during their lifetime. Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) remain the most important global risk factors for HCC [8].

However, Non-Alcoholic Fatty Liver Disease (NAFLD) is an emerging leading etiology as well [9].

Cirrhosis is believed to be a precancerous lesion associated with a high probability of developing HCC [10]. Hepatocarcinogenesis is believed to be a multistep process from cirrhosis through dysplastic nodules, including Low-Grade Dysplastic Nodules (LGDNs) and High-Grade Dysplastic Nodules (HGDNs), to early HCC and finally to advanced HCC [11].

Imaging studies are important in the identification and localization of HCC. However, accurate identification of early HCC is challenging and cannot differentiate it from other precancerous lesions. Pathological diagnosis remains the gold standard method for the identification of these lesions [12].

The differential diagnosis between HGDN and early HCC is extremely challenging. Histological differentiation by morphology alone is not possible most of the time and a definitive pathological criteria for differentiation between the two entities is currently lacking. In such cases, immunohistochemical study could be a potential diagnostic tool to identify and differentiate both lesions [13,14].

Glypican-3 (GPC3) is a widely used and well-established marker in HCC diagnosis. It showed negative expression in adult normal liver tissue. Many studies have shown that GPC3 is specifically expressed on the surface of most HCC cells. It was supposed that GPC3 expression was up regulated in HCC and its positive rate obviously increased following histological upgrading [15].

Although GPC3 is a sensitive and specific marker for HCC, it has a relatively limited diagnostic utility in differentiating well differentiated HCC and HGDNs as it was demonstrated that well differentiated HCC may lack GPC3 expression. However, it can also stain positively in a minority of cirrhotic nodules, active hepatitis and dysplastic nodules. Thus, the diagnosis of HCC should not be based on glypican positivity alone [16].

Existing immunomarkers for differentiation between the HGDNs and early HCC are still of limited value [17]. So, an accurate diagnostic marker for detecting early HCC is fundamentally important, since early detection of HCC remarkably improves patient survival [18].

Astrocyte Elevated Gene-1 (AEG-1), also known as Metadherin (MTDH), is a significant oncogene for HCC and is highly over expressed in patients with HCC of different etiologies by a variety of mechanisms including genomic amplification [19]. AEG-1 is essential for promoting cancer development and progression by augmenting proliferation, invasion, metastasis, angiogenesis and chemoresistance, all hallmarks of aggressive cancer [20].

However, it is important to note that further research and validation studies are still needed to establish the full diagnostic potential of AEG-1 and its application in clinical settings. Nonetheless, the emerging evidence suggests that AEG-1 holds promise as a valuable tool for distinguishing precancerous lesions from HCC, addressing the existing challenges in the literature [21,22].

The aim of the current work was to evaluate the diagnostic significance of AEG-1 and GPC3 in differentiating the cirrhotic nodules, dysplastic nodules, and hepatocellular carcinoma and also to study the immunohistochemical expression of AEG-1 and GPC3 in different grades of hepatocellular carcinoma cases.

Materials and Methods

Materials

This is a cross-sectional study that was conducted on 60 Formalin fixed paraffin embedded tissue specimens that were previously diagnosed as cirrhotic nodules, dysplastic lesions, and different grades of hepatocellular carcinoma. The included cases were retrospectively collected from the archive of the pathology department, faculty of medicine, and Tanta University and from private laboratories as well as new cases received during the period of the study from November, 2022 till November, 2023. Prior to beginning the study, approval from the research ethical committee was secured under approval code: 36117/11/227545867.

Inclusion criteria

- Patient has primary hepatocellular carcinoma with no other malignancy.
- Sufficient tissue specimens for immune staining and good quality of the blocks.
- Complete patient history and clinicopathological data including age, sex, diagnosis, histological type, and tumor grade.

Exclusion criteria

- Insufficient tissue for immunostaining or poor quality of the blocks.
- Incomplete patient history and clinicopathological data.
- Previous history of chemotherapy or radiotherapy.

Classification of the studied cases

Cases of hepatocellular carcinoma: Thirty-six cases of HCC with variable histological types and grades were classified according to the 2019 WHO classification of tumors of the digestive system [23]. The gross specimens were obtained either by Tru-cut biopsy [26 cases] and partial hepatectomy [10 cases].

Cases of precancerous lesions: Twenty-four cases of precancerous lesions were obtained; 16 cases were obtained by Tru-cut biopsy and the other 8 cases by partial hepatectomy. This group consisted of 10 cases of cirrhotic nodules without dysplasia and 14 cases of dysplastic lesions on top of cirrhosis including: Low grade dysplastic nodules (6 cases) and high-grade dysplastic nodules (8 cases).

Methods

The collection of clinicopathological data: Patients data regarding age, sex, and tumor-related characteristics (tumor size, location and multiplicity) depending on gross morphology and the accompanying pathology reports (in resection specimens) and on radiological reports for cases obtained by Tru-cut biopsy. The size of the tumor was classified into three groups; (≤ 2 cm, 2-5 cm and ≥ 5 cm) depending on the TNM staging system by the American Joint

Committee on Cancer (AJCC) 8th edition [24].

Tissue processing and staining

Hematoxylin and eosin staining and histopathological examination for hepatocellular carcinoma specimens and precancerous lesions: Hematoxylin and eosin were used to stain the paraffin blocks after they were serially sectioned (3 µm to 5 µm thick) and examined to confirm the histological diagnosis and to evaluate various histological features, although confirmatory Immunohistochemical results (IHC) were available in the reports of histologically doubtful cases.

Histological subtypes: Hepatocellular carcinoma cases were classified according to the WHO classification of 2019 of digestive system into: Hepatocellular Carcinoma, Not Otherwise Specified (HCC, NOS) (27 cases), hepatocellular carcinoma, steatohepatitic variant (4 cases), hepatocellular carcinoma, clear cell variant (2 cases), hepatocellular carcinoma, chromophobe variant (1 case). Hepatocellular carcinoma, lymphocyte-rich variant (1 case), and hepatocellular carcinoma, neutrophil-rich variant (1 case).

Grading: Hepatocellular carcinoma cases were graded according to the 2019 WHO classification into: Well, differentiated, moderately differentiated and poorly differentiated tumors [23].

Staging: Pathological staging of the studied cases was performed according to TNM staging by the American Joint Committee on Cancer (AJCC) 8th edition [24].

Primary antibodies used Astrocyte Elevated Gene-1 (AEG-1): A rabbit polyclonal antibody against human MTDH (NP_848927.2); 1:1000. Breast carcinoma was served as positive control. Glypican-3 antibody (GPC3): A mouse monoclonal antibody against human Glypican-3 (Clone 1G12, CA; 1:50), placental tissue was served as positive control.

Methodology for immunohistochemical staining: On positively charged slides, sections cut at 3 µm from formalin-fixed paraffin immersed blocks. Slides were relocated into the Autostainer Link 48 device (Dako, Santa Clara, USA: Agilent Technologies Inc.). High pH EnVision™ FLEX Target Retrieval Solution (Dako, Agilent Technologies Inc, Santa Clara, USA) was used. AEG-1 and Glypican-3 antibodies were used for immunostaining. The Dako EnVision™ FLEX detection system was then utilized according to standard protocol times as follows: Peroxidase blocking reagent: 10 min; primary antibodies: 20 min to 30 min; detection system: 20 min; chromogen (DAB; Diaminobenzidine): 10 min. At the end of the staining run, slides were poured with distilled water and after that, a one-minute counterstain using Mayer's hematoxylin was applied. Subsequently, tap water was used to wash the slides and Canada balsam was used to cover the slides. Negative control for each marker was carried out by using the phosphate buffered saline instead of the primary antibody [25].

Interpretation of immunohistochemical results: The immunostaining evaluation was performed in a minimum of 10 randomly selected high-power fields (400x) containing representative sections of the tumor using Leica DM500 microscope with built-in Leica ICC50 digital camera (Leica microsystems, Wetzlar, Germany).

Interpretation of AEG-1 expression and evaluation of the diagnostic value of AEG-1: The stain was considered positive for AEG-1 when showing membranous and/or cytoplasmic staining. The percentage of positive tumor cells (%) was considered regardless of the staining intensity.

ROC curve analysis was performed to identify the best cut-off point for the diagnosis of hepatocellular carcinoma. At each percentage, the sensitivity and specificity of AEG-1 were plotted thus generating the ROC curve. The percentage located closest to the point with both maximum sensitivity and specificity, the point (0.0, 1.0) on the curve was selected as the cut-off point. At this point, the greatest numbers of cases were correctly classified as hepatocellular carcinoma or precancerous lesions.

Interpretation of GPC3 expression and evaluation of the diagnostic value of GPC3: The stain was considered positive for GPC3 when showing membranous and/or cytoplasmic staining. The percentage of positive tumor cells (%) was considered regardless of the staining intensity. The stain was considered positive for Glypican-3 when expressed in a cytoplasmic and/or membranous pattern, and at least 5% of tumor cells were positive [26].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software version. 25 was utilized to tabulate and statistically analyze the obtained data. Categorical variables were expressed as frequencies whereas numerical variables were expressed as mean ± SD. Accuracy, specificity, sensitivity, positive and negative predictive values were employed to evaluate diagnostic values of the tested markers [27,28]. Receiver Operator Characteristic (ROC) curve to determine the best cut-off point for AEG-1 by evaluating the diagnostic values of different percentages of AEG-1 expression. Area Under the ROC Curves (AUC) of each marker was determined which is a widely used predictor of the accuracy of a diagnostic test [29]. An effective way to enhance the diagnostic accuracy is the combination of multiple markers: To combine the two markers (AEG-1, and GPC3); the best linear coefficient that maximized the AUC for these combinations was determined. HCC was considered positive for the combination if any of the involved markers showed positivity. Chi-square tests were used to analyze the relation between AEG-1, GPC3 expression and the tumor grade, Significance was adopted at $p<0.05$ [30].

Results

Clinicopathological data

This study is conducted on 60 cases, 36 of them were hepatocellular carcinoma, 26 cases (72.2%) were obtained by Tru-cut biopsy and 10 cases (27.8%) were obtained by partial hepatectomy; the clinicopathological features are demonstrated in Table 1.

Immunohistochemical results

Expression of AEG-1 and GPC3 in HCC specimens: AEG-1 was detected as a brownish cytoplasmic and/or membranous staining in 34 cases, representing 94.4% of HCC specimens. Whereas, GPC3 expression, it was detected as a brownish cytoplasmic and/or membranous staining in 27 (75%) of HCC specimens (Table 2).

Expression of AEG-1 and GPC3 in hepatocellular carcinoma according to the tumor grade: As for AEG-1 expression in different grades of HCC specimens, there is no statistically significant relation between AEG-1 expression and tumor grade. Majority of well differentiated and moderately differentiated HCC cases, as well as all poorly differentiated cases showed positive AEG-1 expression.

Regarding GPC3 expression in different grades of HCC specimen, a statistically significant relation between positive GPC3 expression

Table 1: Clinicopathological data in the studied hepatocellular carcinoma cases.

Parameter		No. of case (n= 36)	%
Age	Mean age of 62.03 ± 8.49 years		
Sex	Male	25	69.4
	Female	11	30.6
Tumor Focality	Solitary	26	72.2
	Multifocal	10	27.8
Tumor Size (cm)	≤ 2	11	30.6
	2-5	17	47.2
	≥ 5	8	22.2
Histopathological type	Not otherwise specified	27	75
	Steatohepatitic variant	4	11.1
	Clear cell variant	2	5.5
	Chromophobe variant	1	2.8
	Lymphocyte rich variant	1	2.8
	Neutrophil rich variant	1	2.8
Vascular invasion	Present	20	55.5
	Absent	16	44.5
Perineural invasion	Present	5	13.9
	Absent	29	86.1
Histopathological grade	Well differentiated	13	36.1
	Moderately differentiated	11	30.6
	Poorly differentiated	12	33.3
(T) stage	T1a	9	25
	T1b	10	27.8
	T2	15	41.7
	T3	2	5.5
	T4	0	0

Table 2: Expression of AEG1 and GPC3 in hepatocellular carcinoma specimens.

Marker expression (N=36)	Positive		Negative	
	No.	%	No.	%
AEG-1	34	94.4	2	5.6
GPC3	27	75	9	25

AEG-1: Astrocyte Elevated Gene-1; GPC3: Glypican 3

and high tumor grade was noted. 100% of poorly differentiated HCC and 81.8% of moderately differentiated HCC cases were positive for GPC3. While in well differentiated HCC cases, less than half of cases representing 46.2% were positive for GPC3 (Table 3 and Figure 1).

Expression of AEG-1 and GPC3 in precancerous lesions: Among the studied precancerous lesions, the majority of cases (21 cases representing 87.5%) showed negative AEG-1 expression while three cases (12.5%) displayed a brownish cytoplasmic and/or membranous staining distributed as all cases of cirrhotic nodules showed negative AEG-1 expression while 21.4% of the Dysplastic Nodules (DN) were positive for AEG-1. AEG-1 expressions were detected in 33.3% of HGDNs and 12.5% of LGDNs (Table 4 and Figure 2).

Analysis of the diagnostic significant of AEG-1 and GPC3

Validity of AEG-1 in the diagnosis of HCC: ROC curve was performed to identify the optimal cut-off value of AEG-1 expression that could best identify hepatocellular carcinoma which was 40% (Figure 3 and Table 5). ROC curve analysis demonstrated 75% sensitivity, 91.7% specificity, and 0.872 AUC. GPC3 had 93.1% positive predictive value, 70.9% negative predictive value and diagnostic accuracy of 81.6%.

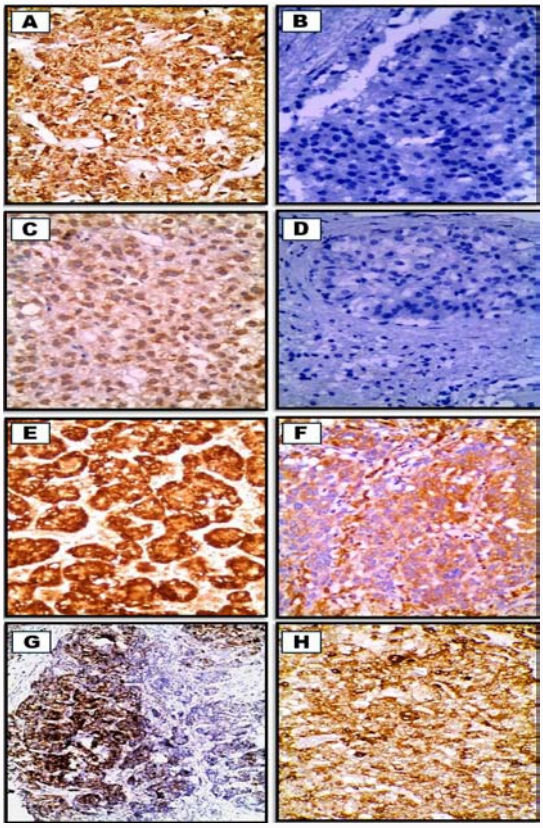

Figure 1: Immunohistochemical expression of AEG-1 and GPC3 in HCC cases (400x). (A)Well differentiated HCC cases and (C) showed positive AEG-1 expression, while (B) and (D) showed negative GPC3 expression. Moderately differentiated HCC (E) showed positive AEG-1 expression and (F) showed positive GPC3 expression. Poorly differentiated HCC (G) showed positive AEG-1 and (H) showed positive GPC3 as well.

Validity of GPC3 in HCC diagnosis: ROC curve for GPC3 was performed and demonstrated 75% sensitivity, 91.7% specificity, and 0.872 AUC. GPC3 had 93.1% positive predictive value, 70.9% negative predictive value and diagnostic accuracy of 81.6% (Figure 4).

Validity of combined AEG-1 and GPC3 in the diagnosis of hepatocellular carcinoma: Combination of different markers together may increase their validity. To recognize the importance of this combination in the diagnosis of HCC, ROC curves were plotted to detect the sensitivity, specificity and AUC of this combination. Combining AEG-1 with GPC3 provided better sensitivity (97.2%), specificity of 100%) and larger AUC (1.000) (Table 6 and Figure 5).

Discussion

Liver cancer is a significant factor in the worldwide cancer burden. Incidence rates have risen in many countries in recent decades. Globally, HCC is the fundamental histologic type of liver cancer, representing about 80% of all primary liver cancer cases [31].

Liver cancer ranks as the sixth most common cancer and the fourth leading cause of cancer-related deaths worldwide [32]. According to 2020 Global Cancer Statistics, the number of newly diagnosed HCC cases is 905,677 accounting for 4.7% of all cancer

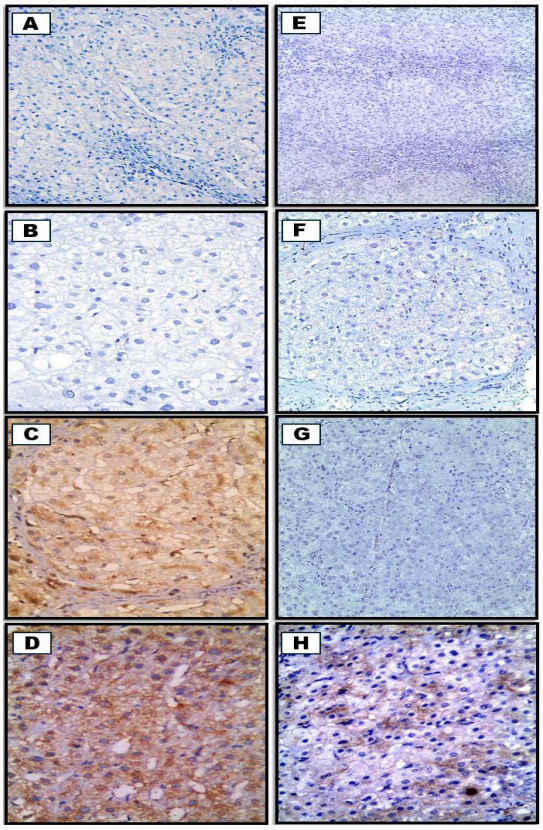

Figure 2: Immunohistochemical expression of AEG-1 and GPC3 in precancerous lesions; (A) cirrhotic nodule showed negative AEG1. (B) LGDN showed negative AEG-1 expression. (C) LGDN showed positive AEG-1 expression. (D) HGDN showed positive AEG-1 expression. (E) Cirrhotic nodule showed negative GPC3. (F) LGDN showing negative GPC3 expression. (G) HGDN showed positive GPC3 expression. (H) HGDN showed positive GPC3 expression.

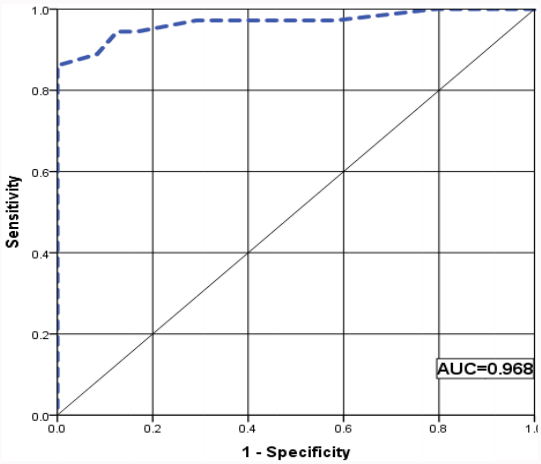

Figure 3: ROC curve for AEG-1 expression. (AUC: Area Under the Curve).

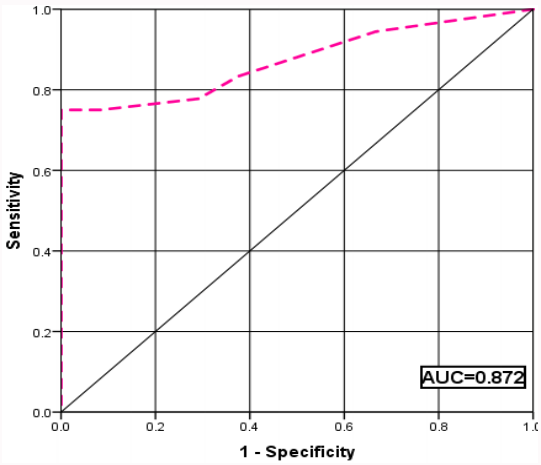

Figure 4: ROC curve for GPC3 expression. (AUC: Area Under the Curve).

cases and the number of new deaths is 830,180 accounting for 8.3% of all cancer cases [33].

Liver cirrhosis is an established risk factor for HCC; 80% to 90% of HCCs develop from cirrhosis. Viral hepatitis is considered the fundamental risk factor. Globally, HCV infection is the leading cause of cirrhosis (93%) while HBV accounts for 88% of all cirrhosis-related HCC [34]. However, In Egypt, a decline in the prevalence of HBV and HCV infection was reported over the past two decades due to the successful strategy of nationwide vaccination and successful antiviral therapy respectively [35]. Although, HCC risk is not completely eliminated, and HCC continues to prevail due to underlying cirrhosis and other risk factors including; non-alcoholic fatty liver disease and alcohol abuse [36].

Hepatocarcinogenesis is suggested to be a multistep process from cirrhosis through dysplastic nodules including LGDNs and HGDNs to early HCC and finally advanced HCC. It has been proposed that DN in the cirrhotic liver is a high-risk precancerous lesion that would eventually lead to HCC [37].

Approximately 75% of HCC patients came with advanced unresectable disease with severely disturbed liver profile. So, surveillance of the at-risk cirrhotic population and early detection of HCC could increase the treatment options by ≥ 50% and improve the outcome as well as the 5-year survival rate [38].

Differentiating between HGDN and well differentiated HCC is extremely challenging. Histological differentiation by morphology alone is not possible most of the time and a definitive pathological differentiation between the two groups are currently lacking [39].

Available immunohistochemical markers have limited diagnostic sensitivity and specificity. Therefore, there is continuous interest in the identification of newer immunomarkers and combinations of immunohistochemical markers to achieve higher sensitivity and specificity to differentiate between HGDNs and well-differentiated HCC [40].

Astrocyte Elevated Gene-1 is considered a new marker that has a critical role in the initiation and progression of cancer. AEG-1 stimulates cancer development and progression by enhancing proliferation, invasion, metastasis, angiogenesis and chemoresistance, all hallmarks of aggressive cancer [41].

In normal liver tissue, AEG-1 expression is typically low or absent. AEG-1 is down regulated in cirrhosis and dysplastic nodules suggesting a distinctive molecular signature that differentiates them from HCC. Conversely, the up regulation of AEG-1 expression in HCC indicates its involvement in hepatocarcinogenesis and makes it a valuable indicator of malignant transformation [21].

Table 3: Expression of AEG-1 and GPC3 in hepatocellular carcinoma according to the tumor grade.

(n=36 specimens)	Cases No.	AEG1				GPC3			
		Positive		Negative		Positive		Negative	
	N	No.	%	No.	%	No.	%	No.	%
Well differentiated	13	12	92.3	1	7.7	6	46.2	7	53.8
Moderately differentiated	11	10	90.9	1	9.1	9	81.8	2	18.2
Poorly differentiated	12	12	100	0	0	12	100	0	0
x^2	24.083					35.106			
P-value	0.457					0.038*			

Table 4: Expression of AEG1 and GPC3 in precancerous lesions.

n=24 specimens	Cases No.	AEG1				GPC3			
		Positive		Negative		Positive		Negative	
	N	No.	%	No.	%	No.	%	No.	%
Liver cirrhosis	10	0	0	10	100	1	10	9	90
Dysplastic lesions	14	3	21.4	11	78.6	1	7.1	13	92.9
LGDNs	8	1	12.5	7	87.5	0	0	8	100
HGDNs	6	2	33.3	4	66.7	1	16.6	5	83.4

LGDNs: Low Grade Dysplastic Nodule; HGDNs: High Grade Dysplastic Nodule; AEG-1: Astrocyte Elevated Gene1; GPC3: Glypican 3; n: number

It is worth mentioning that there is very limited literature data to evaluate the diagnostic role of AEG-1 in hepatocellular carcinoma and precancerous lesions.

For such reasons, this study aimed to investigate the immunohistochemical expression of AEG-1 and GPC3 in hepatocellular carcinoma and precancerous lesions including cirrhotic nodules and dysplastic nodules. The diagnostic value of AEG-1 and GPC3 alone and the double combinations of them were also evaluated. In addition, the expression of the two markers with different grades of HCC.

This study is conducted on 60 cases of hepatic focal lesions, grouped as 36 cases of HCC which were divided as: 13 cases (36.1%) of well differentiated HCC, 11 cases (30.6 %) of moderately differentiated HCC and 12 cases (33.3%) of poorly differentiated HCC. In addition to, 24 cases of precancerous lesions which were subdivided into 10 cirrhotic nodules (41.7%) and 14 dysplastic nodules (58.3%) including 8 cases of LGDNs and 6 cases HGDNs.

Analyzing AEG-1 expression in HCC cases and precancerous lesions, the current study demonstrated that AEG-1 was detected as a brownish cytoplasmic and/or membranous staining in the majority of cases (34 HCC cases; 94.4%) out of 36 cases. In contrast to precancerous lesions, three cases (12.5%) out of 24 were AEG-1 positive; 2 cases were HGDNs (representing 33.3% of HGDNs) and only one case of LGDN (representing 12.5% of LGDNs).

This study was the first to perform ROC curve analysis and setting a suggested optimal cut off value (40%) for expression of AEG-1 that achieved the highest sensitivity and specificity for distinguishing hepatocellular carcinoma from precancerous lesions.

AEG-1 provided 94.4% sensitivity, 87.5% specificity, and 0.968 areas under curve. AEG-1 had a 91.8% positive predictive value, 91.3% negative predictive value and a diagnostic accuracy of 91.6%.

In agreement with our results, despite using different analytical methods Cao et al. [42] reported positive AEG-1 expression in 34 HCC specimens (91.8%) out of 37 cases and in 6 out of 37 dysplastic nodules and adjacent non-tumorous tissue (16.2%). His results reported 92% sensitivity and 83.7% specificity, 85% PPV, 91.2% NPV and 87.8% diagnostic accuracy. This was in concordance with Yoo et al. [43] who studied the expression of AEG-1 in HCC cases only and reported expression in 93.6%.

On the other hand, Zhu et al. [44] and Yung et al. [45] recorded lower values of positive AEG-1 expression in 54.2%, 67% respectively

Table 5: Measuring AEG1 expression cut-off point.

Test Result Variable(s): AEG-1		
Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
2.00	1.000	1.000
4.00	1.000	0.958
5.50	1.000	0.875
8.00	1.000	0.792
11.00	0.972	0.583
13.50	0.972	0.417
15.50	0.972	0.333
18.00	0.972	0.292
22.50	0.944	0.167
40.00	0.944	0.125
57.50	0.889	0.083
62.50	0.861	0.000
66.50	0.778	0.000
69.00	0.750	0.000
71.00	0.556	0.000
73.50	0.528	0.000
77.50	0.417	0.000
82.50	0.167	0.000
85.50	0.056	0.000
88.00	0.028	0.000

Table 6: Combination of AEG-1 and GPC3 in HCC diagnosis.

Marker	Sensitivity %	Specificity %	PPV %	NPV%	Diagnostic accuracy %	AUC
AEG-1	94.4	87.5	91.8	91.3	91.7	0.968
GPC3	75	91.7	93.1	70.9	81.7	0.872
AEG-1+ and/or GPC3+	97.2	100%	100	96	98.3	1.000

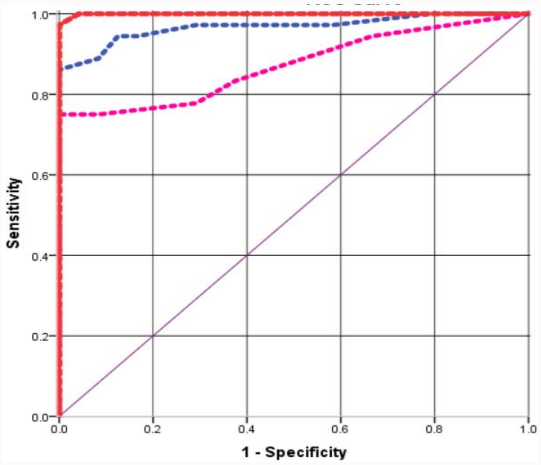

Figure 5: ROC curve for combination of AEG-1 and GPC3 validity in the diagnosis of hepatocellular carcinoma.

of their HCC studied cases. These discrepancies in the results can be attributed to the different methodology, tissue processing and different antibodies used.

From the result of this study, it was found that AEG-1 provided high sensitivity and diagnostic accuracy in the detection of HCC and exclusion of precancerous lesions. Thus, it can be considered as a potential diagnostic marker for HCC.

Glypican-3 is a well-established and widely used marker for hepatocellular carcinoma alone or in combination with other markers as a part of different panels for HCC diagnosis. It is expressed normally by fetal liver and is highly expressed in HCC and certain other human cancers. GPC3 undergo transcriptional regulation *via* epigenetic alterations including both DNA methylation and histone modification [26]. Several studies have shown that the expression of GPC3 regulates tumor proliferation and progression through Wnt signaling cascade.

Evaluating GPC3 expression in the studied HCC cases and pre-cancerous lesions, GPC3 was detected as a brownish cytoplasmic and/or membranous staining in 27 HCC cases (75%) out of 36 cases and in only two (8.3%) of precancerous lesions out of 24 cases including only one case of cirrhotic nodule (representing 10% of cirrhotic nodules) and only one case of HGDN as well (representing 16.6% of HGDNs).

Xu et al. [46] in a larger scale study conducted on 111 cases of HCC reported a very close percentage of positive GPC3 expression which was 75.7% of HCC cases.

ROC curve analysis for GPC3 revealed 75% sensitivity, 91.7% specificity, and 0.872 AUC. GPC3 had 93.1% PPV, 70.9% NPV and diagnostic accuracy of 81.6%. This was consistent with other researchers that noticed nearly the same results; Tomasso et al. [47] reported GPC3 sensitivity of 73.6%, 96.2% specificity, 95.12% PPV and 78.13% NPV. Although, Mohamed et al. [48] reported GPC3 sensitivity of 80%, 82.5% specificity, 83.3% PPV and 79.2% NPV.

However, there is wide variability in GPC3 sensitivity ranging from 54.1% up to 86.4% and specificity ranging from 79% to 100% [47-54]. This variability can be attributed to difference in case selection, different tumor grades, methodology and antibodies used.

Focusing on precancerous lesions, some authors have demonstrated positive GPC3 staining in cirrhotic nodules ranging from none [55] to 11% [56,57]. LGDNs showed positive staining in 8% [58] while HGDNs showed positive staining in 7% [59] up to 22% [58].

On the other side Yamauchi et al. [60] reported positivity in one out of three cases (33%) of cirrhotic nodules and two out of eight (25%) cases of LGDNs and six out of eight (75%) of HGDNs. Also, Gong et al. [61] found that 5.5% LGDN and 50% HGDN were positive for GPC3. This variability could be explained by differences in the used anti-GPC3 antibody and immunohistochemical technique; this provides a reasonable explanation for why they could not discriminate well differentiated HCCs from LGDNs and HGDNs.

Analyzing these findings, the current study showed that GPC3 provided low sensitivity in the detection of HCC and high specificity in the exclusion of precancerous lesions.

The present study investigated the expression of AEG-1 and GPC3 in different grades of HCC. The expression of AEG-1 in well, moderate, and poorly differentiated HCC were 92.3%, 90.9% and 100% respectively. Expression of AEG-1 gradually increased with the tumor grade but there is no statistically significant relation between AEG-1 expression and high tumor grade (p value =0.457).

On the contrary, Jung et al. [45] reported a statistically significant relation between AEG-1 and high tumor grade (p value =0.009). This discrepancy could arise from the fact that their study was focusing on the usage of AEG-1 as a prognostic factor for HCC and they grouped the cases into low expression group and high expression group by assessing the percentage of positively stained immunoreactive cells and staining intensity. Gong et al. [62] also reported a statistically significant relation between AEG-1 and high tumor grade (p value =0.020). This could be attributed to the difference in case selection since he focused only on HBV-related HCC cases.

The expression of GPC3 in well, moderately, and poorly differentiated HCC was 46.2%, 81.8% and 100% respectively, showing increased expression with high tumor grade with a statistically significant relation between GPC3 expression and high tumor grade (p value =0.038).

This could be explained that GPC3 serves as an oncofetal protein enhancing cell growth, differentiation and tumor formation. This could be the reason why GPC3 expression rises with increasing HCC grade. In agreement with this finding, Wasfy et al. [57] reported a statistically significant relation between GPC3 expression and high tumor grade, 91.7% of poorly differentiated and 33.3% of well differentiated HCC cases. This was also in line with Elzeftawy et al. [63] who reported a statistically significant relation between high tumor grade and GPC3 expression (41.2% of well differentiated,

39.5% of moderately differentiated and 63.6% of poorly differentiated HCC cases).

However, many studies showed that the expression of GPC3 was lower in well-differentiated HCC (50% to 72.7%) than that in moderately or poorly differentiated HCC (83% to 89%) but with no statistically significant relation [56,59,64].

That was not in line with, Wang et al. [54] who reported opposing results that GPC3 showed no obvious difference in the expression between different grades of HCC. The expression of GPC3 in well, moderately and poorly differentiated HCC was 62.50%, 73.68% and 65.00% respectively. Moreover, Yamauchi et al. [60] suggested that GPC3 is a good marker for the identification of well-differentiated HCC hence he reported expression in 78% of cases.

Considering the validity of the examined markers individually, this study found that AEG1 was more sensitive than GPC3 in the detection of HCC. However, GPC3 was more specific than AEG1 in the exclusion of precancerous lesions. These results were in accordance with those of Cao et al. [42].

It should be mentioned that the validity of different markers in the identification of HCC could be improved by their combination. Therefore, this work was extended to study the validity of AEG1 in combination with GPC3 in the diagnosis of HCC.

Double combination of AEG-1 and GPC3 in the current study provided better sensitivity (98.2%), specificity (100%) and a larger AUC (1.000) compared to AEG-1 alone (94.4% sensitivity, 87.5% specificity, 0.968 AUC) and GPC3 alone (75% sensitivity, 91.7% specificity, AUC 0.872).

In comparison to other commonly used combinations, according to Abdelrahman et al. [64] combining GPC3, Arginase and HepPar-1 provide 87.5% sensitivity, 78.1% specificity, 80% PPV and 86.2% NPV. While combining Arginase with GPC3 provide 87.5% sensitivity, 87.5% specificity, 87.5% PPV and 87.5% NPV. In addition, Tommaso et al. [47] demonstrated that using HSP70, GS and GPC3 provide 90.63% sensitivity, 72.73% specificity, 82.86% PPV and 84.21% NPV.

Therefore, the current work suggested that the double combination of AEG-1 and GPC3 was a promising combination serving the highest sensitivity and specificity in the detection of HCC cases and differentiating it from precancerous lesions.

Conclusion

In conclusion, AEG-1 can be used as an accurate diagnostic marker in HCC. It has high sensitivity in the detection of HCC and high specificity in excluding precancerous lesions. Forty percent is the ideal AEG-1 cut-off value, providing the maximum sensitivity and specificity for differentiating between HCC and precancerous lesions. GPC3 is more specific for excluding the precancerous lesions but less sensitive than AEG-1 in detecting HCC. GPC3 showed a statistically significant relation with high tumor grade, being less expressed in well differentiated compared to poorly differentiated HCC. In contrast to AEG-1 that showed no statistically significant relation with the tumor grade. AEG-1 and GPC-3 are useful combination offering higher sensitivity and specificity so the application of both markers in clinical practice will achieve better results.

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